Diarrhetic Shellfish Toxins in blue mussels (*Mytilus edulis*) in Sweden – experiences from monitoring of the toxins and *Dinophysis* spp., the causative organisms

Bengt Karlson (1), Marie Johansen(1) and Malin Persson (2)
(1) Swedish Meteorological and Hydrological Institute, Oceanographic unit,Västra Frölunda, Sweden;
(2) National Food Agency, Uddevalla, Sweden,. Presenter contact details: bengt.karlson@smhi.se, Phone +46 31 751 8958

Summary
Dinophysis is a dinoflagellate genus that has toxin producing members. This group of toxins, named Diarrhetic Shelllfish Toxins (DST), include ocadaic acic, DTX1, DTX2 and its esters (DTX3). The toxins may accumulate in filter feeding organisms such as mussels. This is a problem when *Dinophysis* spp. occurs in areas where mussels are harvested for human consumption. The European Union has strict regulation about control of toxins in bivalves and of monitoring of biotoxin producing phytoplankton in areas where bivalves are harvested. The Skagerrak is located adjacent to the North Sea. The majority of the Swedish mussel harvesting occur on the Swedish coast of the Skagerrak. Monitoring of ocadaic acid using high performance liquid chromatography started in 1988 and monitoring of phytoplankton, including harmful algae started approximately at the same time. Monitoring has continued since then and methods have been improved over time. The 25 years of data from the area show that the inter-annual variability of DST concentrations in blue mussels is substantial. Some years DST concentrations are below the regulatory limit all year while other years DST-concentrations remain high for several months. The area is a mosaic of bays and fjords and the spatial variation may also be high. Results of monitoring and effects of the aqua culture industry will be discussed.

Introduction
Harvesting of wild and farmed bivalves is a large industry worldwide. The value of bivalves for human consumption was recognized already in about a hundred years ago (Field, 1922). Also the problem with toxins was recognized early but the cause was unknown. Today certain dinoflagellates and a few diatoms are known to be the causative organisms. Diarrhetic Shellfish Poisoning (DSP) is one of the syndromes. It was not until 1980 that dinoflagellates from the genus *Dinophysis* were identified as the causative organisms. (Yasumoto et al., 1980). A review by Reguera et al. (2014) describes current knowledge on the Dinophysis toxins and the causative organisms. It is worth noting that members of *Dinophysis* are mixotrophic and need food when cultured (Parke et al. 2006. In Sweden the farming of mussels started in the 1970's along the Skagerrak coast. In 1988 regular analyses of DST started but also mouse bioassays have been used. DSP occur some years but is almost absent other years (Karlson et al. 2007).

Materials and Methods
Fig. 1 show the area investigated. Phytoplankton was sampled in national and regional monitoring programs. Sampling frequency was usually monthly but in some cases more frequent. In general a tube was used to collect a depth integrated water sample from 0-10 m. Samples were preserved with acid Lugol's solution and analyzed microscopically for species composition and abundance of phytoplankton using the Utermöhl method. Data was downloaded from the Swedish National Oceanographic Data Centre at SMHI. http://www.smhi.se/. In addition data from biweekly phytoplankton sampling at some of the mussel harvesting sites was also used. Farmed and wild blue mussels (*Mytilus edulis*) were collected from bands/ropes and from the sea floor respectively. A method based on High Performance Liquid Chromatography (HPLC) was used for analysis of Dinophysis Shellfish Toxins (DST, ocadaic acid, DTX1 and DTX2) from 1988 to early 2000's. From early 2000's to 2013 a method based on liquid chromatography and mass spectrometry was used. In addition mouse bioassays were used until 30 June 2011. The data from mouse bioassays are not presented here. On 1 July 2011 liquid chromatography combined with mass spectrometric detection became the official method in Sweden.

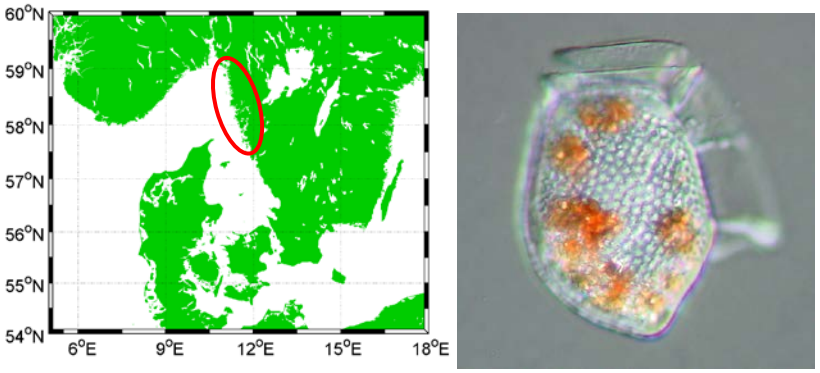

Figure. 1 Left: The map indicates the location of the area investigated encircled in red. Right *Dinophysis acuta*, one of the organisms producing Diarrhetic Shellfish Toxins. Photo by B. Karlson.

Results and Discussion

An example of data is shown in Figs. 2 and 3. The inter-annual variability in toxin content in blue mussels is very large. The data from 2006 to 2013 indicate a correlation between the occurrence of *D. acuta* (Fig. 1) and DST in blue mussels. Analyses of the larger data set may reveal more correlations.

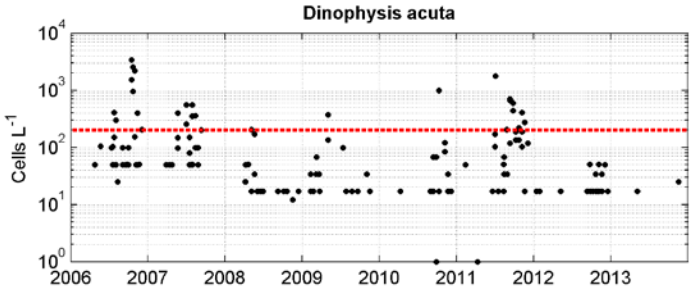

Figure. 2 The abundance of *Dinophysis acuta* near some of the mussel harvesting sites 2006-2013.

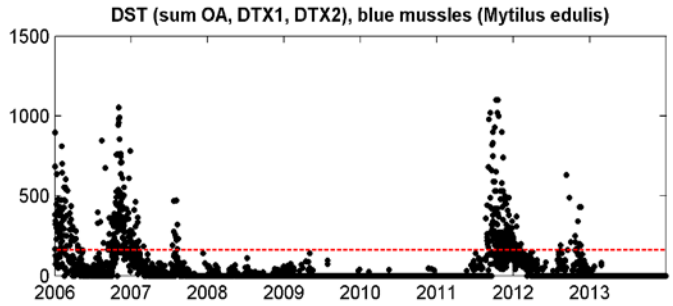

Figure. 3 The concentrations of Diarrehtic Shellfish toxins (DST) in blue mussels along the Swedish Skagerrak coast 2006-2013. Red line indicate the regulatory limit of 160 µg per kg mussel meat.

References

Field, I.A. 1922. Biology and economic value of the sea mussel *Mytilus edulis*. Bull. Bur. Fish., Washington, 38, 128-29.

Karlson, B., Rehnstam-Holm, A-S. Loo,L-O. 2007. Temporal and spatial distribution of diarrhetic shellfish toxins in blue mussels, *Mytilus edulis* (L.), at the Swedish West Coast, NE Atlantic, years 1988-2005, Reports Oceanography, SMHI, pp. 48.

Lindahl, O., Lundve, B. and Johansen M. 2007. Toxicity of *Dinophysis* spp in relations to population density and environmental conditions on the Swedish west coast, Harmful Algae, pp 218-231.

Reguera, B., Riobó P., Rodríguez, F., Díaz P.A., Pizarro, G., Paz, B., Franco, J.M., Blanco, J. 2014. *Dinophysis* Toxins: Causative Organisms, Distribution and Fate in Shellfish, Mar. Drugs 2014, 12, 394-461.

Park, M.G.; Kim, S.; Kim, H.S.; Myung, G.; Kang, Y.G.; Yih, W. First successful culture of the marine dinoflagellate *Dinophysis acuminata*. Aquat. Microb. Ecol. 2006, 45, 101–106.

Yasumoto, T.; Sugawara, W.; Fukuyo, Y.; Oguri, H.; Igarashi, T.; Fujita, N. 1980. Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning in the Tohoku district. Bull. Jpn. Soc. Sci. Fish, 46, 1405–1411.