No.93(March, 2018)

A-553 HIROSHI SHIMADA

Biological Oceanographic Study on Method for Predicting the Occurrence of Paralytic Shellfish Toxin along the Okhotsk Sea Coast off Hokkaido

The scallop fishery along the Okhotsk Sea coast of Hokkaido is known as "sowing culture", harvesting after three years of scallop seed sowing on the fisheries grounds, and is one of the largest bivalve fisheries (catch slightly less than 300 thousand tons per year) in the world. However, the scallop fishery has experienced economic damage due to incidental occurrence of paralytic shellfish toxin accumulation by shellfish (PST) caused by the toxic dinoflagellate *Alexandrium tamarense* in summer once every several years. An intensive PST occurrence recorded in summer 2002 stopped the scallop fishing for more than one month, leading to a steep fall in the scallop market value and serious economic losses. To minimize economic damage due to PST occurrence caused by *A. tamarense*, the transportation mechanism of water mass contaminated *A. tamarense* from the oceanic area to the scallop fishing ground along the coastal area was revealed and a method for predicting the occurrence of PST was established in the present study.

Seasonal changes in occurrences of vegetative cells of *A. tamarense* and PST toxicity were researched along the coast of Hokkaido during 2005－2006. Vegetative cells occurred in the Okhotsk Sea and the Pacific coast (cold current area affected by the East Sakhalin Current and the Oyashio) but not detected in the Sea of Japan and the Tsugaru Strait (warm current area affected by the Tsushima Warm Current and the Tsugaru Warm Current). Occurrences of PST over the quarantine level (4 MU g-1 scallop whole meat) were recorded in Funka Bay during blooms of *A. tamarense* (＞102 cells L-1) were observed.

Horizontal distribution of resting cysts of toxic *Alexandrium* spp. was investigated around Hokkaido during 1999－2000. Resting cysts of *A. tamarense* were widely distributed in the Okhotsk Sea and the Pacific Ocean. Regarding the relationship between past PST occurrences and cyst abundance in the sediment of each area, positive correlations were found between the frequencies of PST occurrence years and the cyst abundances and between the annual maximum PST toxicities and the cyst abundances. Therefore the cyst abundance implies important information about the past PST occurrences (frequency and magnitude) of each area. The occurrences of

vegetative cells of *A. tamarense* during 2005－2006 (above mentioned) were considered to reflect the cyst abundance of each area. However the occurrences of vegetative cells and cyst abundance in the Okhotsk Sea off Hokkaido, showed that despite the low occurrences of vegetative cells in the coastal area, large cyst abundances were found on the continental shelf from Hokkaido to Sakhalin. Thus the oceanic area of the Okhotsk Sea off Hokkaido was considered to have a high potential for initiation of *A. tamarense* blooms.

Spatial distribution of vegetative cells of *A. tamarense* was examined in the Okhotsk Sea off Hokkaido in summer during 2002－2007. The vegetative cells frequently occurred in the surface low salinity water (LSW, salinity < 32.5) in the oceanic area and rarely appeared in the Soya Warm Current water (SWC, salinity > 33.6) along the coastal area and the dichothermal water (DTW, temperature < 2 oC) below 30 m deep in the oceanic area. Nutrient concentrations were respectively higher in the DTW than the LSW and the SWC. Despite the low nutrient concentrations of the LSW, *A. tamarense* can be considered to utilize nutrients originating from the DTW just below the LSW due to the effects of diel vertical migration. A continuous diatom bloom was observed along the front area between the LSW and the SWC where a belt－shaped upwelling area occurred with higher nutrient concentrations. Bloom of *A. tamarense* tended to be found in the LSW just outside the front area, because of interspecific competition with the diatom bloom. Lower DIP concentration of the SWC is supposed to restrict formation of a *A. tamarense* bloom in addition to the absence of the DTW with higher DIP concentration below the SWC. Therefore environmental conditions of the SWC were concluded to be severe for bloom formation of *A. tamarense*. Regarding the interannual relationship between the abundance of *A. tamarense* in summer and the relative frequency of each water mass in spring and summer, the abundance tended to be higher in years when higher frequencies were recorded of the SWC in spring and of the LSW in summer. The results suggest that warming by the SWC in spring prompts germination of *A. tamarense* cysts in the sediment and domination of the LSW in summer gives optimum medium for bloom formation of *A. tamarense*.

Toxin profiles of 103 culture strains of *A. tamarense* isolated from sediment or seawater samples collected from the coast of Hokkaido and Aniva Bay (southern Sakhalin) were analyzed using HPLC during 2005－2009. As a result of cluster analysis of the toxin profiles of culture strains, 101 culture strains were classified in the same cluster, producing C－toxin －2, gonyautoxin－4, gonyautoxin－3 and neosaxitoxin as dominant toxin components excluding two culture strains. The toxin profiles of the 101

culture strains were almost the same as past reports on toxin profiles of *A. tamarense* from Japan and Sakhalin. Cellular toxin contents of culture strains varied from 1 to 1128 fmol cell-1, and were inversely proportional to cell densities. As a result of the estimations if a bloom of *A. tamarense* (cellular toxin content: 103 fmol cell-1, cell density: 102 cells L-1) was fed on by scallops (filtration rate: 102 L day-1, accumulation ratio of toxin: 35 %), toxification rate of scallop is calculated as 0.4 MU g-1 digestive diverticula day-1. Result of the estimation suggests that scallop become toxic over the self－imposed quarantine level (20 MU g-1 digestive diverticula) after 50 days from the initial occurrence of a *A. tamarense* bloom, scientifically proving the empirical data, "Bloom of *A. tamarense* exceeding ca. 102 cells L-1 causes shellfish toxification over the quarantine level".

To clarify the transportation mechanism of *A. tamarense* in the Okhotk Sea coast off Hokkaido, area－wide sampling in the oceanic area, time－series monitoring in the coastal area and current velocity measurements of the SWC using ADCP were conducted in 2004, 2007 and 2008. These surveys were organized based on the hypothetical scenario, "PST occurrence is caused by the inflow of LSW contaminated with *A. tamarense* to the scallop fishing ground at the temporal weakening of SWC indexed by the decrease of the sea－level difference (SLD) between Wakkanai and Abashiri". It was revealed that *A. tamarense* blooms appeared in the coastal fishing ground simultaneously with the weakening of SWC indexed by the SLD. Retrospective analysis on time－series relationship between the weakening of SWC and the PST toxicity in PST occurrence years also elucidated that the toxicity increased just after the weakening of SWC. Therefore the hypothetical scenario was verified, and a method for predicting the occurrence of PST was constructed as follows;

(1) Sampling in the oceanic area in June (before PST occurrence) and July (during annual peak of PST occurrence) to monitor horizontal distribution of *A. tamarense*.

(2) Monitoring of the weakening of SWC indexed by the SLD using internet.

(3) If *A. tamarense* bloom (> 102 cells L-1) has been found in the oceanic area, and the weakening of SWC is observed, warning of potential PST occurrence within a few weeks should be provided.

Semi－realtime data of the prediction method is available to the public for controlling shipping plan of scallop since 2009. The prediction method gives high cost effectiveness since the essential part of prediction can be simply constructed with the twice a year sampling in the oceanic area and the monitoring of SLD.

The present study provides important information about feeding environment of main fishery resources, not only scallop but also fish such as salmon, since the high contrast structure and dynamics of water masses has been revealed by biological oceanographic studies in the Okhotsk Sea off Hokkaido, focusing on *A. tamarense* as a biological tracer. Recently, it had been reported that warm—water, non—armored flagellates causing harmful red tides have been detected for the first time in the coast of the Sea of Japan and the Tsugaru Strait of Hokkaido. Once a harmful red tide appears in the scallop fishing ground, serious damage may occur to the scallop fishery. Therefore monitoring of non—armored red tide flagellates is necessary to start in addition to armored harmful dinoflagellates such as *A. tamarense* in northern Japan, since the northward expansion of warm—water harmful flagellates is increasingly possible due to ocean warming in the future.

A-554 KENJI SAKAGUCHI, YUTARO SUZUKI, YASUFUMI HADA, HIROSHI ASAMI AND TAKAHIRO TAKASHIMA

Changes in body size with decrease of biomass of Arabesque greenling *Pleurogrammus azonus* in the sea off northern Hokkaido and its influence on spawning stock biomass

Changes in body weight and maturation rate of 1-year-old Arabesque greenling, *Pleurogrammus azonus*, in the Sea of Japan and Sea of Okhotsk off northern Hokkaido (seas off northern Hokkaido) with decrease in biomass were examined to improve the method for estimating spawning stock biomass (SSB). The body weight of 0-4-year-old fish increases as the recruitment of 0-year-old individuals decreased after 2009 year-class. With the low number of recruited individuals since 2009 year-class, the maturation rate of 1-year-old fish almost reached the upper limit (1.0). The SSB in 2010-2016 calculated using estimated body weight and maturation rate was 1.27-1.46 times as high as that calculated using constant body weight and maturation rate. Therefore, the method for estimating the SSB of *Pleurogrammus azonus* was suggested to be improved by considering changes in body size and maturation rate.

A-555 ARE-LANG CHEN, HIDEO YOSHIDA and YASUNORI SAKURAI

Normal embryonic-hatching process of saffron cod(*Eleginus gracilis*)

Embryonic development and hatching process of saffron cod (*Eleginus gracilis*) were studied in their artificially fertilized eggs at water temperatures and salinity conditions. Eggs were incubated in plastic containers placed in water tanks with natural running water. Eggs failed to develop at temperature above 6℃ and salinity below 8.2 in salinity. at all examined conditions. Deformed development and low hatching rate were observed at 4℃ and 13～20 in salinity. These results indicate that the optimum temperature for normal embryonic development and hatching could be below 2℃ with 20~25 in salinity. Embryonic development was divided into 7 stages from gastrula to 50% hatching at 2℃. At 2℃, hatched larvae's yolk was completely absorbed in two weeks. Most of the hatched larvae were normally developed and a few individuals opened their mouth just after hatching. It was confirmed that saffron cod attain advanced morphogenesis at hatching. Despite of normal development of eggs, their hatching rates were extremely low around 30% with gradually increasing water temperature. Natural water temperature and salinity levels in Lake Akkeshi during winter to spring period showed that environmental conditions were extremely stable until melting ice.

A-556 ARE-LANG CHEN, HIDEO YOSHIDA and YASUNORI SAKURAI

Effects of various temperature-salinity conditions on post embryonic-hatching process of Saffron cod (*Eleginus gracilis*)

The post embryonic-hatching process of saffron cod *Eleginus gracilis* was examined at 28 different temperature-salinity conditions ranging from -1.3 to 3.3℃ and from 10 to 33‰. Our results indicate that the relationship between temperature and the number of days to 50% hatching was exponential. The hatching process observed at constant conditions (< 0℃, 32~35‰) as well as increasing temperatures (1.3~3.3℃) or decreasing salinities (10~20‰) indicate that the advanced developmental stages were acclimated to higher temperatures rather than salinity dilution. Complete yolk absorption of hatched larva occurred within 19 days at 1℃ and 13 days at 3℃. Artificially fertilized eggs incubated in suspended basket and transplantation in Lake Saroma, developed up to stage Ⅳ～Ⅴ in 43～51 days in the beginning of March, and up to stage Ⅴ in 66 days by late March. Natural water temperature gradually increased from below zero and abruptly

reached around 3℃ in the middle of April. Their normal hatching period was supposed to 80 to 100 days. Laboratory analysis and field results indicate that increase in water temperature triggers early hatching of saffron cod and occurs simultaneously with melting of ice.

A-557 YOKO GOTO, YOSHIAKI KAI, TAKANORI HORIMOTO, KENJI SAKAGUCHI and TADASHI MISAKA

Multiplex PCR - based molecular identification of species of *Ammodytes* (Perciformes: Ammodytidae) caught off the northern part of Sea of Japan, Hokkaido, Japan

Japanese sand lances (*Ammodytes* spp.) are among the most important fish resources around northern Hokkaido. In this region, three sand lance species (western sand lance *A. japonicus*, Arctic sand lance *A. hexapterus*, and peaceful sand lance *A. heian*) are known to be sympatrically distributed. Therefore, to accurately evaluate the status of resource distribution of this genus, it is necessary to thoroughly understand their distributional characteristics. In this study, using multiplex PCR, which can easily identify species, as indicated in previous studies, an easier and more rapid method of identifying species belonging to this genus was developed. Using this method, the western and Arctic sand lances could be easily differentiated. However, the precise identification of peaceful sand lance species was slightly difficult with this technique. This technique was shown to be useful for initially screening the species from samples. This study suggested that for understanding the distributional characteristics of these species in Hokkaido, detailed sampling of each area in each season is necessary.

A-558 MAKOTO HATAKEYAMA, MAKOTO FUJIWARA and SHINYA MIZUNO

The concentration of *Flavobacterium psychrophilum* in the ovarian fluid of returning chum salmon (Short paper)

We determined the concentration of *Flavobacterium psychrophilum* in the ovarian fluid of returning chum salmon that were captured from 6 rivers in Hokkaido, Japan. The detection rate of the bacterium ranged from 28%-90% of the samples from each river. The concentration found in each specimen

was below 7 log CFU/mL. The incidence of specimens carrying a high concentration of this bacterium tended to be low. We also examined the effects that different sample storage methods had on the concentration of viable bacteria. Incubating samples at 4 ° C for 24 h resulted in a reduction in bacterial concentration. Freezing and thawing produced an approximately 90% reduction. These results indicate that bacterial counts based on CFU may lead to underestimation depending on the method of specimen storage.

A-559 TATSUYA KOYAMA, AKIYOSHI SHINADA, HIROSHI KURODA AND YASUYUKI MIYAKOSHI

Consideration on northward transport of juvenile chum salmon in the Sea of Japan off Hokkaido based on particle-tracking experiments (Short paper)

Using particle-tracking experiments, based on a high-resolution ocean model, we examined the northward transport of juvenile chum salmon (*Oncorhynchus keta*), released into the Sea of Japan, by assuming that the particles represented juvenile chum salmon, which were transported passively by ocean currents. The experiments were undertaken for the two brood year classes of juvenile chum salmon; the 2009 brood year class released in the spring of 2010, which showed the lowest return rate among the 2007 to 2010 brood year classes, and the 2007 brood year class released in the spring of 2008, which showed the highest return rate. A remarkable difference in the distribution of the particles was observed between the two brood year classes; i.e., for the 2009 class, particles were transported across a wider range in offshore waters, and the timing at which particles were transported into the Okhotsk Sea through the Soya Strait was delayed. Moreover, the ambient sea water temperature experienced by the particles was lower for the 2009 year class. These results indicated that the post-release transport of juvenile chum salmon by ocean currents was remarkably different between the two years and it may contribute to the low return rate of the 2009 class on the Sea of Japan side of Hokkaido.

A-560 HIDETSUGU YOSHIDA AND MAKOTO KANAMORI

Link between abundance of toxic dinoflagellate, *Alexandrium tamarense*, and certain environmental factors, particularly river runoff, in eastern part of Funka Bay, Hokkaido (Note)