Amnesic shellfish poisoning (ASP) potential in Japan

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Objective and outline

Objective: Estimation of the ASP potential in Japan

1994-

1. Monitoring of domoic acid (DA) contamination in shellfish in Ofunato Bay
2. Screening of DA-producing *Pseudo-nitzschia* in Ofunato Bay
3. Isolation of *P. multiseries* from Ofunato Bay
4. Culture experiments of *P. multiseries* for DA production
5. Screening of other *Pseudo-nitzschia* producing DA in Ofunato Bay

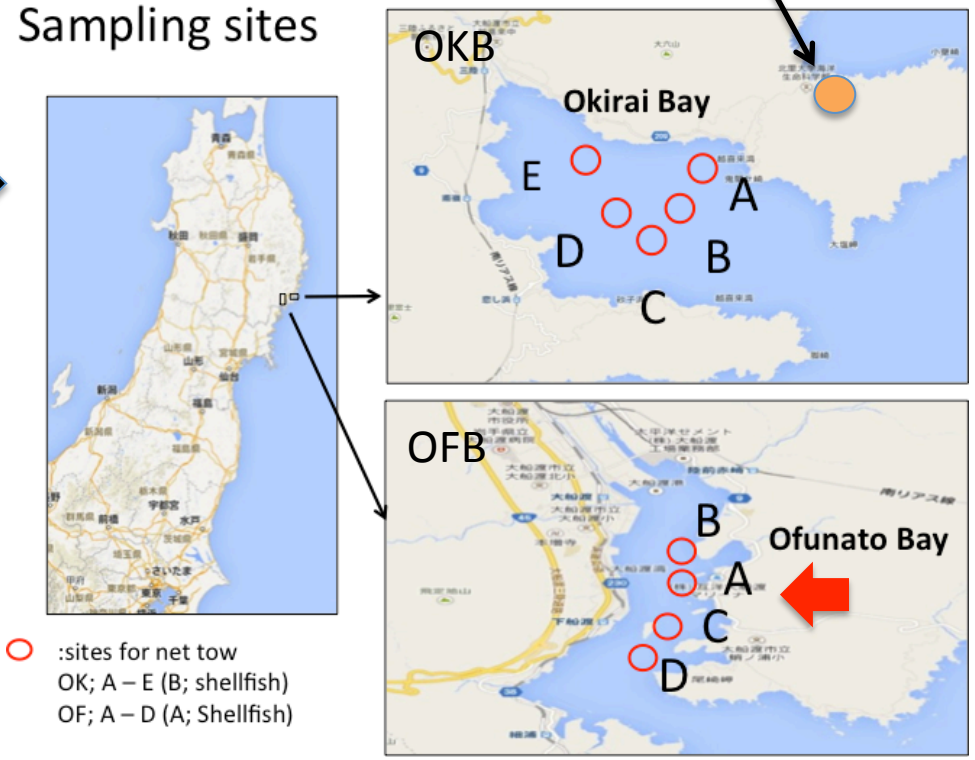
2012-

6. Monitoring of DA contamination in shellfish in Ofunato and Okirai Bay
7. Monitoring of DA in net tow samples
8. Screening of *P. multiseries* in the bays
9. Isolation of *P. multiseries* from both bays
10. Culture experiment of *P. multiseries* for DA production

Monitoring was first started at one station (Ofunato Bay A)

Map of East Asia

Sampling sites



Ofunato Bay point A (Shizu Station):
Famous station for highly contaminated shellfish with PSP toxins （Ogata et al. 1982)

Monitoring result

Domoic acid concentration of shellfish at station A in Ofunato Bay

Date of collection*1	Mussel (μg g-1)*2	Scallop (μg g-1)*3
Nov. 16, 1994	2.8	0.3
Dec. 14, 1994	0.5	ND
Jan. 11, 1995	0.5	0.8
Feb. 15, 1995	1.2	ND

*1; Shellfish was collected from April, 1994 to February, 1995.

*2; Edible part was used for the analysis.

*3; Digestive gland was used for the analysis.

Domoic acid level was enough lower than the regulatory limit (20 ppm)

(Kotaki et al. 1996)

DA monitoring in shellfish was continued for several years in Ofunato Bay

Monitoring in other areas done by others

The results were all negative (ND or trace)

Japanese government decided no regular DA monitoring

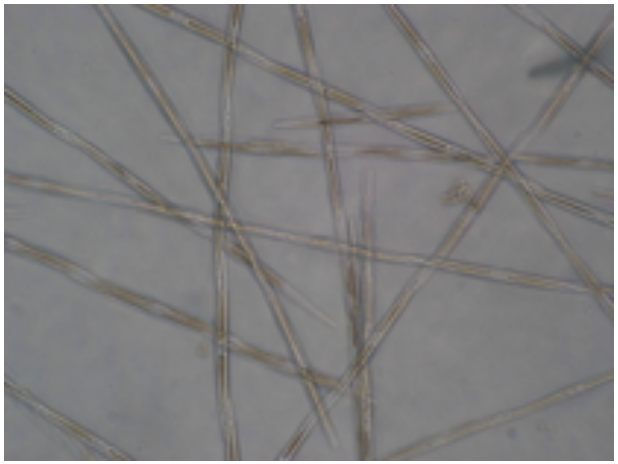
During the first stage of the preliminary monitoring , water parameters of temperature, salinity and pH was measured.
But these were lost during the transfer to the main campus after 2011 disaster.
Data obtained at the same point A by prefectural team will be added.

P. multiseries was isolated from Ofunato Bay

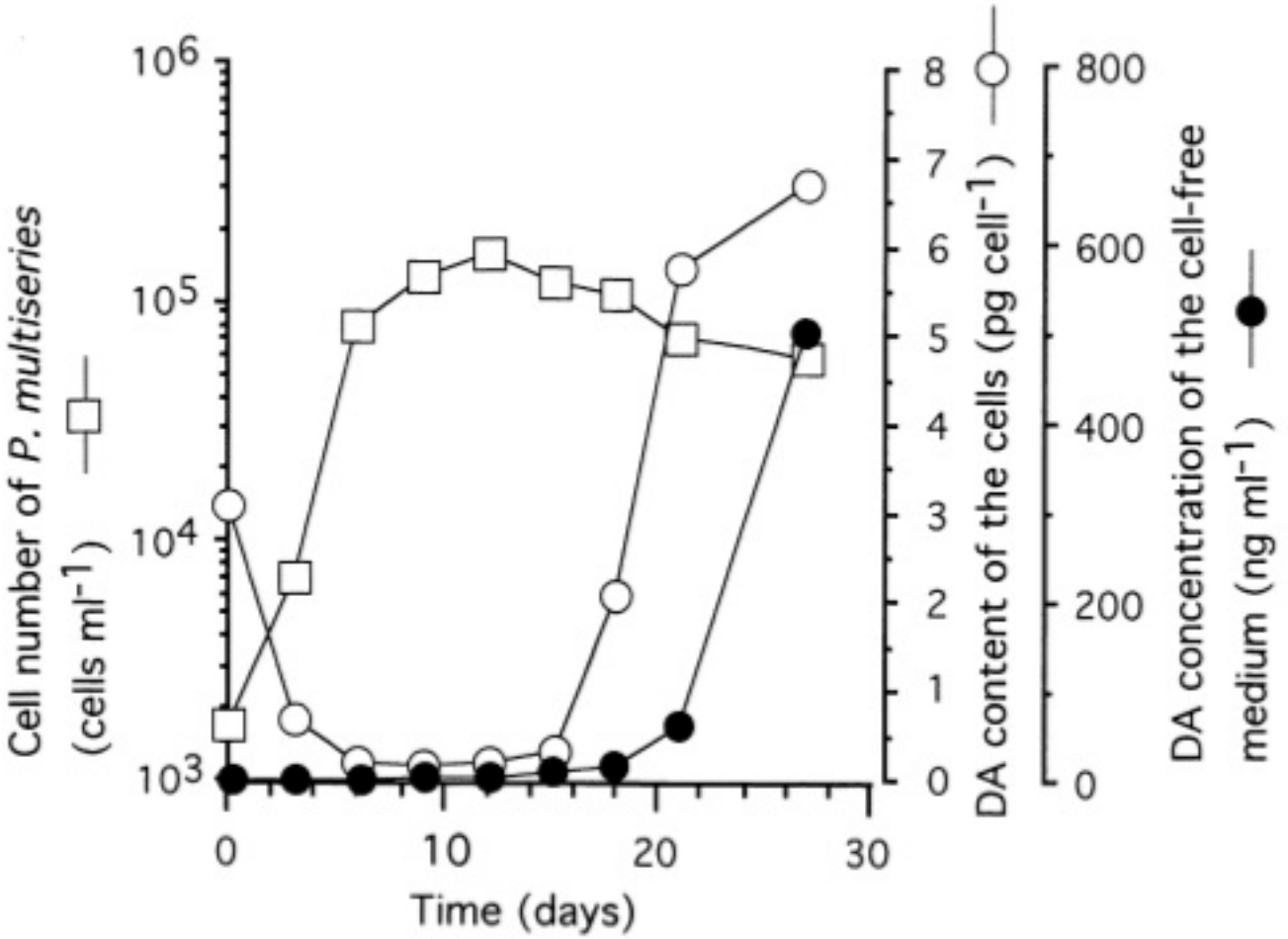
- Basic toxin production characteristic was investigated by culture experiment.
- Strong DA production was confirmed, showing that the origin of the DA in shellfish was *P. multiseries*.
- *P. multiseries* was isolated almost every year after this not only from Ofunato Bay but also from Okirai Bay, showing that *P. multiseries* is the common species in this area.

Sampling

Pseudo-nitzschia multiseries



Growth and domoic acid production of *P. multiseries* by batch culture experiment



P. multiseries

Significant increase of DA at the late stationary growth phase.

Significant DA increase needs the severe bloom and its maintenance for a long time in the bay.

Fig. 1. Cell growth and DA production of *Pseudo-nitzschia multiseries*. (Kotaki et al. Toxicon, 1999)
P. multiseries strain acclimatized to the conditions was inoculated into 2 l of f/2 medium (Guillard and Ryther, 1962) with the initial cell concentration of 1,700 cells ml^{-1} and cultured for 27 days at 15°C under light intensity of 100 µmol photon m^2 s^{-1} with 16:8 LD cycle. DA levels of the cell and culture medium were monitored separately by the HPLC-Fluorescent analysis according to Pocklington et al. (1990). The growth was monitored by counting cell number under light microscope.

(Kotaki et al. 1999)

Conditions necessary for the ASP occurrence

- Presence of highly toxic *Pseudo-nitzschia* such as *P. multiseries*
- Bloom occurrence of the *Pseudo-nitzschia* in the bay
- Sustaining of the bloom for more than 2 weeks without nutrient supply
- Shellfish fed with the bloomed *Pseudo-nitzschia*
- Consumption of the shellfish by human

Bays replete with above conditions are few in Japan

ASP potential seems low in Japan

Screening of other *Pseudo-nitzschia* spp. producing DA in Ofunato Bay

Result

Toxin (DA) production level: low (< 1 pg/cell)

P. pseudodelicatissima

P. delicatissima

P. pungens

P. turgidula

P. fraudulenta

P. cuspidata

P. subpacifica

P. subfraudulenta

P. heimii

Two unidentified *Pseudo-nitzschia*-like pennate diatoms

These diatoms seemed not to contribute the increase of ASP potential in Japan. (Kotaki. 2008)

Factors affecting the domoic acid production in *P. multiseries* culture

1. Si, P, Fe depletion *
2. Irradiance *
3. Bacteria*
4. pH increase
5. Stop of cell division

* Investigated and confirmed by our culture experiment

Our Results

- Change of iron both in axenic and non-axenic *P. multiseries* culture (dissolved iron in the culture medium and increase of iron in the cells) co-related well with the increase of DA level, showing the possibility that DA was produced as a result of iron uptake to *P. multiseries* cells.
- High level of DA production was observed in non-axenic culture, and axenic culture reintroduced with bacteria.
- Even axenic culture showed the very low level of DA production, indicating the existence of bacteria inside cells.
  (Kobayashi et al. Fish. Sci. 2003).
- Decrease of N, P, Si was seen during/before the significant DA increase.
- These phenomena was not enough to explain the DA production mechanism.
- Irradiance was confirmed to be necessary for the DA production but photosynthesis activity did not increase in stationary growth phase.

Results of the culture experiments showed that iron is the most reasonable factor influencing the DA production.
Bacteria were also one of the most reasonable factor influencing the DA production.

Is it possible to combine the two factors for understanding the DA production mechanism?

Is the attachment between bacteria and *P. multiseries* always needed for the DA production?

Culture experiment using dialysis membrane; Will bacterial effect in DA production occurred when axenic culture is divided with non-axenic culture by dialysis membrane?
In case of m.w. 12,000 ; Negative
In case of m.w. 50,000 ; Positive

Results show the possibility that attachment between bacteria and *P. multiseries* is not always necessary for the DA production.
Some compounds of m.w. 12,000 – 50,000 might have some role in DA production followed by DA occurrence.

Re-monitoring started after 2011 East Japan Disaster

East Japan Tsunami March 11, 2011

Ofunato, Iwate

Miyako, Iwate

2011 Tsunami attacked east Japan including Ofunato and Okirai Bay

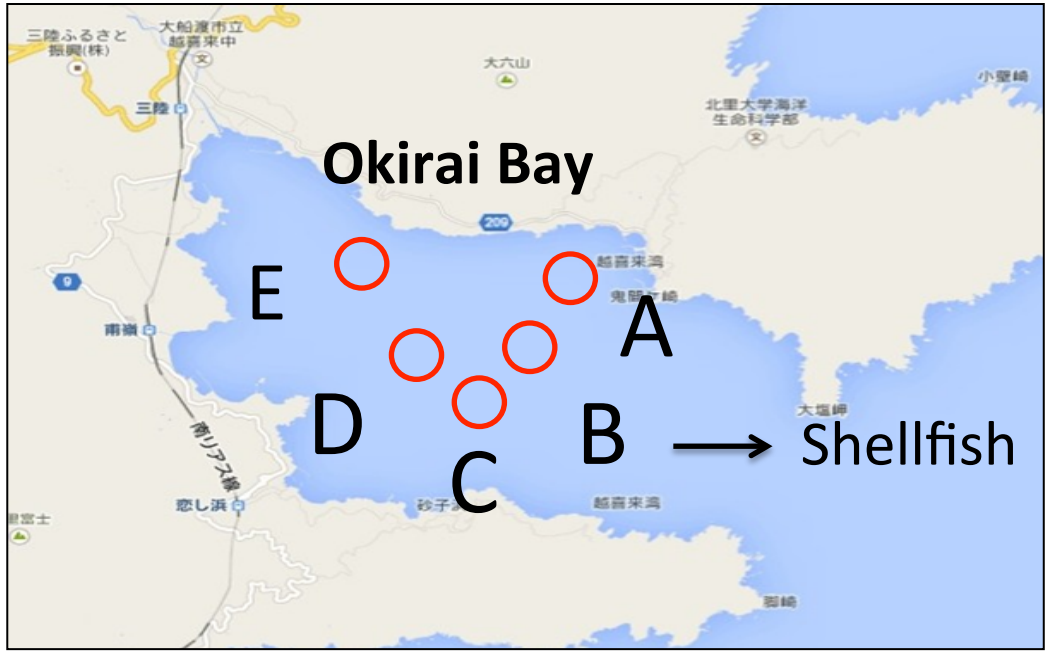
Planktons flew out and newly came?
Bacteria flew out and newly came?

From offshore
or old sediment

Possibility of change with DA-producing plankton
or DA production ability

Monitoring seemed to be needed

Sampling sites



○ :sites for net tow
OK; A – E (B; shellfish)
OF; A – D (A; Shellfish)

Monitoring Method

DA contamination in shellfish was monitored at the point of Ofunato A and Okirai B

Plankton net sample
(20 µm, vertical tow from 20m, twice)
Cells were obtained on the 3 µm filter, extracted with water by sonication and analyzed by HPLC with fluoresence detection after reaction with FMOC fluorescence reagent. Representative of positive result samples were confirmed for DA by LC-MS/MS.

Isoation of *P. multiseries* and culture experiment for DA production was done after this

Result (2013 – 2015)

DA in net tow sample
Ofunato Bay:
Negative (ND <1.7 pg/L net tow seawater, n=21)
Positive (7–720 pg/L net tow seawater, n=11)
Okirai Bay:
Negative (ND <1.7 pg/L net tow seawater, n=29)
Positive (17-205 pg/L net tow seawater, n=18)

DA in shellfish (Blue mussel and scallop)
Ofunato Bay:
Blue mussel; Negative (ND <0.5 μg/g edible part, n=10)
Scallop; Negative (ND < μg/g digestive gland, n=10)
Okirai Bay:
Blue mussel; Negative (ND <0.5 μg/g edible part, n=14)
Scallop; Negative (ND < μg/g digestive gland, n=14)

P. multiseries isolates

2013 from Ofunato Bay
- Three strains
- Culture experiment; 0.64±0.38 pg/cell
  because of weakness (died within 2 weeks)

2014 from Ofunato Bay
- One strain
- Culture experiment; 317 ng/mL (9 pg/cell) at 36 day

Discussion

P. multiseries exists in Ofunato Bay (might exist in Okirai Bay), and still has the ability to produce high level of DA, but it might not bloom after the East Japan Tsunami

Difference of ASP potential between west and east Pacific Ocean might depend on the difference in iron concentration of seawater.

Conclusion

The ASP potential in Japan seems to be low as well as before the 2011 East Japan disaster, due to the DA production characteristics of *P. multiseries*, because bays replete the necessary conditions for ASP occurrence are few in Japan as shown above.

Factors affecting the DA production of *Pseudo-nitzschia* should be studied more.

References

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Thank you for your attention!!