Occurrence of *Pseudo-nitzschia* species in San Pedro Bay, Leyte, Philippines

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1. Introduction

The genus *Pseudo-nitzschia* has 31 member species. At present 11 species of this genus are known to produce domoic acid (DA). DA is an algal toxin that causes amnesic shellfish poisoning (ASP). Critical identification of each species in this genus requires transmission electron microscope. This technical criteria is the reason why ecological studies of *Pseudo-nitzschia* at the species level remain wanting. Counting the species under TEM is logistically impossible while viewing the cells under LM cannot provide for proper identification. This pilot study aims therefore to use LM for initial identification and quantification of *Pseudo-nitzschia* species. Not only will this aid species ecological studies of the genus, this will also be useful for early detection of probable toxic *Pseudo-nitzschia* species so that decisions for issuance of warning for proper food security can be made ahead of time.

Studies on this genus are numerous particularly in Europe and North America. In contrast studies of *Pseudo-nitzschia* in Southeast Asia remain scant. The second purpose of this study thus addresses this gap of knowledge.

San Pedro Bay is found in the middle and eastern part of the Philippine Islands in between the so-called "twin islands" of Leyte and Samar. It is composed of a smaller Cancabatoc Bay, and the very narrow San Juanico Straits. San Pedro Bay eventually leads to the Pacific Ocean.

2. Materials and Method

Field surveys in San Pedro Bay were conducted from December 2006 to May 2008 (Fig. 1). Samples were taken twice a month using Van Dorn sampler. Based on *Pseudo-nitzschia* cells' shape, lengths and widths, six identification -groups were established as follows:①AUS-group (composed of 15 species), ②GAL-group (6 species), ③MICRO-group (2 species), ④CAC -group (9 species), ⑤AME -group (3 species) and ⑥SUBC -group (1 species). Species of *Pseudo-nitzschia* were grouped and counted using these six identification-groups. All counted cells were picked up and placed in centrifuge tubes corresponding to the groups made. The cells in the tubes were then washed with distilled water, and cleaned using $KMNO_4$, H_2SO_4 and Oxalic Acid. The cells were washed several times with distilled water again before these were mounted and observed under the transmission electron microscope. Thus it was insured that the all cells counted, grouped and observed under TEM were the same cells.

3. Results and Discussion

Results of the sampling from December 2006 to May 2008 showed that *Pseudo-nitzschia* was present in San Pedro Bay the whole year round. The highest cell density (50,000 cells/L) of *Pseudo-nitzschia* species was spotted in February 2007. More diverse species of the genus tended to appear during the wet season while higher cell densities are likely to occur during the dry season (Fig.2).

In the six identification-groups made, five groups were found in San Pedro Bay. These were :①AUS-group (about 8,700 cells picked up) ② MICRO-group (22,000 cells) ③ CAC-group (87,000 cells), ④ AM-group (40,000cells) ⑤ SUBC-group (5,900 cells). Result of TEM observations showed that for the MICRO-group, the identification-group was exact. All cells in the tube for this group were identified under the TEM as *P. micropora*. However for the CAC-group, three different species were found under the TEM. These were *P. pseudodelicatissima, P. caciantha* and *P. pungens*. *P. pungens* should have been picked up and placed in the AUS-group and not in the CAC-group. This discrepancy was because *P. pungens* in San Pedro Bay appeared very thin than its temperate counterparts and was mistaken to be straight shaped (CAC-group) instead of spindle shaped (AUS-group). The identification -groups will then be fine-tuned to answer this discrepancy. TEM observations for AUS-group, AME-group and SUBC-group are still undergoing.

Thus far there are five species of *Pseudo-nitzschia* in the bay. These are *P. pungens, P. pseudodelicatissima, P. brasiliana* (identified from culture strains), *P. caciantha* and *P. micropora*. The presence of probable DA producing species, *P. pseudodelicatissima* is a signal that ASP could occur in the area and thus monitoring should be done especially since aquaculture cages are present here.

Based from the preliminary results made, the identification-groupings are acceptable. Aside from *P. pungens* that was found in the CAC-group, the other species identified using the groupings under the light microscope matched with the species that belonged in the groups made when these were viewed and confirmed under the TEM. The identification-groupings will be tried in other biomes such as in the temperate and Antarctic areas so that these can be tested on other *Pseudo-nitzschia* species as well.

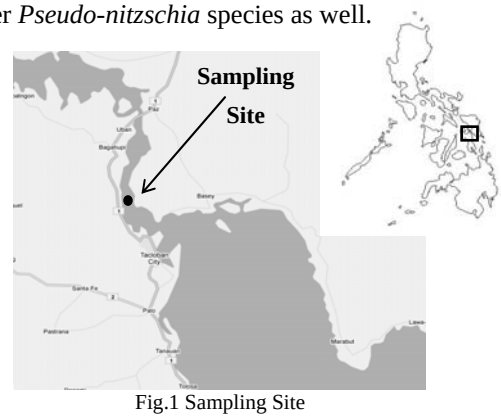

Fig.1 Sampling Site

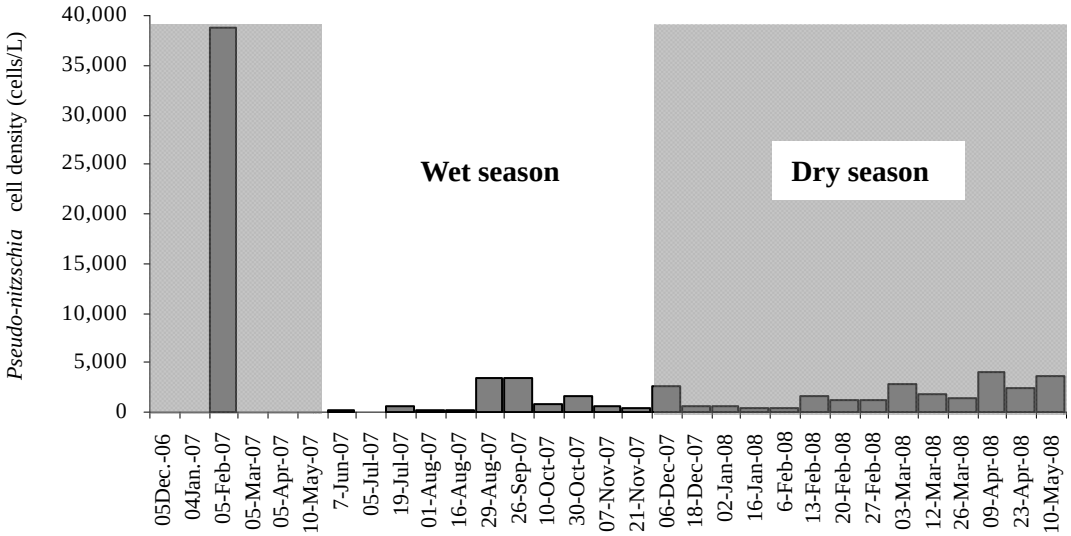

Fig. 2 Cell densities of *Pseudo-nitzschia* during December 2006 to May 2008 in San Pedro Bay, Philippines.