CHAPTER II

LITERATURE REVIEW

2.1 *Noctiluca scintillans*

Noctiluca scintillans is a large and non-photosynthetic (unarmoured) marine planktonic dinoflagellate species. This large and distinctive bloom forming species has associated with fish and marine invertebrate mortality events (Kofoid and Swezy, 1921).

N. scintillans is a free-living dinoflagellate that exhibits bioluminescence. The bioluminescent characteristic of *N. scintillans* is produced by a luciferin-luciferase system located in thousands of spherically shaped organelles, or "microsources", located throughout the cytoplasm of this single-celled protist. Nonluminescent populations within the genus *Noctiluca* lack these microsources. Originally, *N. scintillans* was classified in Division : Chromophyta, Class : Dinophyceae (Dinoflagellates), Order : Noctilucales (Haeckel, 1894) Family : Noctilucaceae (Kent , 1881)

Life history: *N. scintillans* trophonts usually reproduce by binary fission, but gamonts (cells undergoing gametogenesis) occasionally appear (Calkins 1899; Ishikawa 1893, 1899; Soyer 1969, 1970a, b). Zingmark (1970) described the gametogenesis of *N. scintillans* in more detail, including gamete fusion, but subsequent processes were not elucidated, although the fused gametes (zygotes) were assumed to develop into trophonts. Morphological descriptions of gametes and zygotes have not always agreed. (e.g., Grell 1973; Ishikawa 1893, 1899; Kofoid and Swezy 1921; Sato *et al.* 1998; Schnepf and Drebes 1993; Zingmark 1970) But in 2006, Fukuda and Endoh (2006) described the whole life cycle of *N. scintillans*. Which completed in their clonal cultures and they presented new details of trophont structure, gametogenesis, morphology of the gametes and zygotes (fused gametes) and developmental transformation from zygote to trophont, although they have been unable to determine the location of meiosis. Some characteristics typical of dinoflagellates, such as two grooves and two differentiated flagella, are maintained only in the gametes, while the trophonts are a highly specialized form that lack such traits. They also refer to a possibility that *Noctiluca* may be multinucleate or has a polyploid nucleus. Based on their observation and available

molecular phylogenies, they consider the possible relationships of *Noctiluca* within the dinoflagellates (Fukuda and Endoh, 2006).

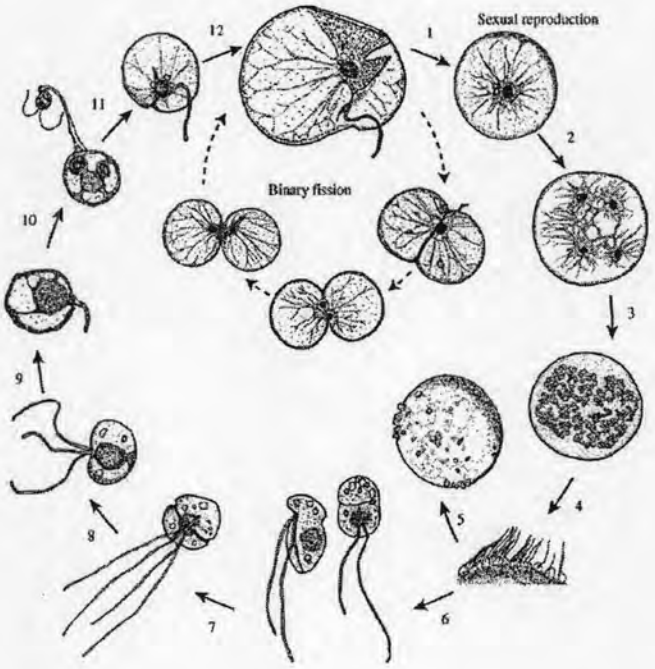

Fig.1. Schematic representation of the complete life cycle of *Noctiluca scintillans*, including both asexual binary fission and sexual reproduction. 1. Initiation of differentiation into gametogenesis cell. 2. Two successive nuclear divisions. This stage may correspond to meiosis. 3. Progamete divisions on the cell surface, resulting in 256–1024 division products. 4. Transformation into gametes (zoospores). 5. Parent cell ghost after releasing zoospores. 6. Isogametes with two flagella. Longitudinal and transverse grooves are discernible. 7. Zygote with four flagella. 8. Transformation into trophont. The number of flagella begins to decrease. 9. Intermediate stage during transformation. Formation of outer crust, tentacle and cytoplasmic network begins. 10. Completion of a tentacle and acquisition of phagotrophic capability. 11. Final stage of transformation, resulting in miniscule trophont. 12. Thickening growth into mature trophont. See the text for detail of Fukuda and Endoh, 2006.

Taxonomic Description: *Noctiluca scintillans* is a distinctively shaped athecate species in which the cell is not divided into epitheca and hypotheca. Cells are very large, inflated (balloon-like) and subspherical (Figs.2 A-D). *N. scintillans* has a ventral groove within which is deep and wide, and located a flagellum, an extension of the cell wall called

a tooth, (Figs.2 A, B and C). Only one flagellum is present in this species and is equivalent to the transverse flagellum in other dinoflagellates (Fig. 2 A). The tooth is a specialized extension of the cell wall (Fig. 2 D) (Zingmark 1970; Dodge 1973; Dodge 1982; Lucas 1982; Fukuyo *et al.* 1990; Hallegraeff 1991; Taylor *et al.* 1995; Steidinger and Tangen 1996) and a striated tentacle involved in ingestion that projects posteriorly. The flagellum does not move the organism and therefore the nonmotile *N. scintillans* depends upon regulation of its buoyancy within the water column perhaps by controlling its cellular concentration of ions and ammonia. At least one study has shown that a string of mucus is produced by *N. scintillans* extending from the tip of the tentacle which then adheres to plankton as it ascends rapidly through concentrations of its prey in the water column (Kiørboe and Titelman, 1998).

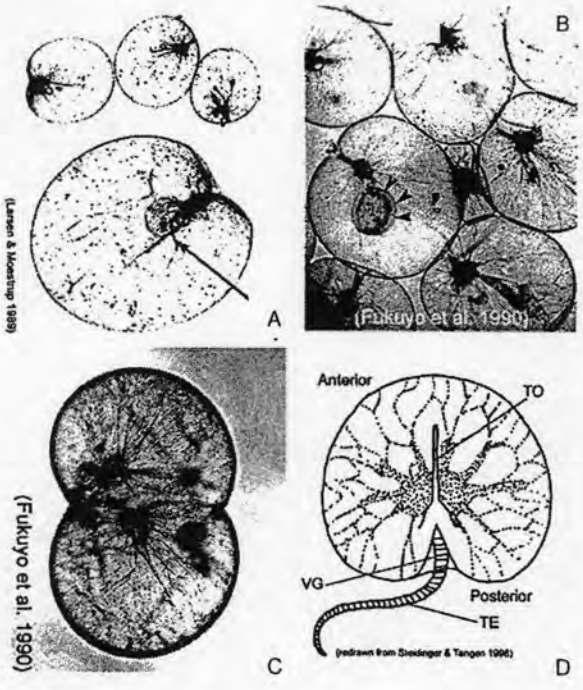

Fig. 2. Cell of *Noctiluca scintillans*) ; (A.) Cells large, balloon-shaped, nearly spherical, and colorless. A single flagellum housed in the ventral groove (arrow). (B.) Cytoplasmic strands extend from nucleus (near the groove) to cell perifery. Engulfed cell (arrowheads). (C.) Asexually dividing cell. (D.) Line drawing. Deep and wide ventral groove (VG) houses the tooth (TO), an extension of the cell wall. Striated tentacle (TE) (Zingmark 1970; Dodge 1982; Fukuyo *et al.* 1990; Hallegraeff 1991; Steidinger and Tangen 1996).

Morphology and Structure: *Noctiluca scintillans* is a nonphotosynthetic heterotrophic and phagotrophic dinoflagellate species; chloroplasts are absent and the cytoplasm is mostly colorless (Figs. 2A and B). The presence of photosynthetic symbionts or intracellular symbiosis (*Pedinomonas noctilucae*) can cause the cytoplasm to appear pink or green in color (Sweeney 1978). A number of food vacuoles are present within the cytoplasm. A large eukaryotic nucleus is located near the ventral groove with cytoplasmic strands extending from it to the edge of the cell (Fig. 2B) (Zingmark 1970; Dodge 1982; Fukuyo *et al.* 1990; Hallegraeff 1991; Steidinger and Tangen 1996).

Size : The size of the single-celled *N. scintillans* ranges from 200 to 2,000 μm in diameter (apparently, they most commonly range from 500-600 μm), assuming the generally spherical shape. *N. scintillans* lacks the armor plates that possessed by other types of dinoflagellates. Unlike many other dinoflagellates, the chromosomes of *N. scintillans* are not clearly-visible and condensed throughout its lifecycle.

Movement : The flagellum does not propel the cell, thus this dinoflagellate can be considered non-motile. However, *Noctiluca* can regulate its vertical position in the water column by controlling its buoyancy. It does this by actively controlling the ionic content of its cell sap (cytoplasm). As a generalization, cells are negatively buoyant and dispersed throughout the water column early in a bloom, and accumulate ammonia and become positively buoyant at the end of a bloom.

Diet : *N. scintillans* is a heterotrophic (non-photosynthetic) organism that engulfs its food (phagotrophic) which primarily consists of plankton, including diatoms and other dinoflagellates, as well as fish eggs and bacteria. Diatoms are often found in the vacuoles (internal membrane-bound storage compartments) within these single celled creatures. It uses its tentacle both for food uptake (it is apparently sticky) and for elimination of feces. Feeding is reported to be greatest at night, and to simply stop just before populations decline.

Reproduction: *Noctiluca scintillans* reproduces asexually by binary fission (Fig. 2 C.). When they do so, several organelles, including the tentacle are resorbed. Cell division tends to be phased, most often occurring at night. The duration of the process has been reported to be dependent on temperature and also sexually via formation of isogametes.

This species has a diplontic life cycle: the vegetative cell is diploid while the gametes are haploid. The gametes are gymnodinioid with dinokaryotic nuclei (Zingmark 1970).

Habitat and Locality: *Noctiluca scintillans* is a cosmopolitan species distributed world wide in cold and warm waters. Populations are commonly found in coastal areas and embayments of tropical and subtropical regions (Dodge 1982; Fukuyo *et al.* 1990; Hallegraeff 1991; Taylor *et al.* 1995; Steidinger and Tangen 1996). *Noctiluca scintillans* is a strongly buoyant planktonic species common in neritic and coastal regions of the world. It is also bioluminescent in some parts of the world. This bloom forming species is associated with fish and marine invertebrate mortality events. *N. scintillans* red tides frequently form in spring to summer in many parts of the world often resulting in a strong pinkish red or orange discoloration of the water (tomato-soup). Blooms have been reported from Australia (Hallegraeff 1991), Japan, Hong Kong and China (Huang and Qi 1997) where the water is discolored red. Recent blooms in New Zealand were reported pink with cell concentrations as high as 1.9 X 10^6 cells/L (Chang 2000). In Indonesia, Malaysia, and Thailand (tropical regions), however, the discolored water is green due to the presence of green prasinophyte endosymbionts; *Pedinomonas noctilucae* (Sweeney 1978; Dodge 1982; Fukuyo *et al.* 1990; Hallegraeff 1991; Taylor *et al.* 1995; Steidinger and Tangen 1996).

Blooms : *N. scintillans* is a well known red-tide organism. Spectacular blooms result from an interaction of biological features and physical concentration mechanisms such as currents, upwelling, fronts (surface slicks can be rapidly mixed away by the wind). The high cells concentrations of their plankton food source that likely result from environmental conditions such as well-mixed nutrient rich waters and seasonal circulation factors are implicated in blooming of *N. scintillans* population (Umani *et al.*, 2004) and runoff from agricultural pollution may contribute to the severity of these blooms, but is not required to cause such events of explosive population growth.

N. scintillans itself does not appear to be toxic, but as they feed voraciously on phytoplankton high levels of ammonia accumulate in these organisms which is then excreted by *N. scintillans* into the surrounding area that may result in the death of other aquatic life in the bloom area. Not all blooms associated with *N. scintillans* are red. The color of *N. scintillans* is in part derived from the pigments of organisms inside

the vacuoles of *N. scintillans*. For instance, green tides result from *N. scintillans* populations that contain green-pigment endosymbiosis (*Pedinomonas noctilucae*) that are living in *N. scintillans* cells . By the way, we could see green discoloration in Southeast Asian water such as Thailand, Malaysia, The Philippines and Indonesia. Extensive toxic blooms have been reported off the east and west coasts of India, where it has been implicated in the decline of fisheries (Aiyar 1936; Bhimachar and George 1950). For example Elbrächter and Qi (1998) could observe *Noctiluca* Red Tide in East South on 20 June 1998. About 21:15 hrs on 19 June, wind speed decreased to < 1 m/sec (southwest), and was recorded as 0 m/sec around 09:00 on 20 June. Massive numbers of positively buoyant cells accumulated at the sea surface, and appeared clotted. About 10:45 on 20 June the wind shifted to the North, and increased to 3-4 m/sec. Wind-forcing created Langmuir cells, and *Noctiluca* concentrated into wind rows. Eventually, the cells mixed into the water column and were no longer visible.

Phylogenetic of *N. scintillans* : The phylogenetic position of noctilucids has been debated. *Noctiluca* was originally classified as a jellyfish (based on detailed observations) of the trophonts until Haeckel (1873) proposed that it should be included in the Cystoflagellata within the dinoflagellates . Later, Kofoid, (1920) placed *N. scintillans* in the newly created order Noctilucales (based on detailed observations of trophont and gamete morphology), which is closely related to the Gymnodiniales and consists of unarmoured dinoflagellates. This classification was widely accepted.

However, in the 1990s, the phylogenetic position of Noctiluca was re-evaluated using molecular information. The phylogenetic analysis at first that included noctilucids was performed on LSU rDNA sequences of domain 1 and domain 8 (Lenaers *et al.* 1991).

Later, Saunders *et al.* 1997 analyzed *N. scintillans* based on SSU rDNA. Which, both phylogenetic analysis, the results suggested that *N. scintillans* should be placed in an ancestral position within the dinoflagellates. Which similar to Cavalier-Smith and Chao (2004) placed *N. scintillans* in a basal position but Oxyrrhis was regarded as a specialized species derived from core dinoflagellates. By the way, the light-emitting enzyme luciferase (LCF) gene was cloned from *N. scintillans* (Liu and Hastings 2007). According to phylogenetic analysis and a comparison of the genomic structure and domain organization of LCF genes from eight species of dinoflagellates, *N. scintillans* possesses the most ancestral type of LCF gene. However Saldarriaga *et al.* (2004) used

a greater number of species to re-analyzed the phylogenetic relationships of *N. scintillans* within the dinoflagellates but the placement of *N. scintillans* was unstable. In this case, it was depending on the method of analysis or depending on the sequences included.

Shauna *et al.*, (2005) studied, based on small subunit (SSU) RNA data was founded that the dinoflagellates were a monophyletic group and that within this group *N. scintillans* was among the earliest to diverge. In the other hand,another study, based on large subunit rRNA, supports the claim that heterotrophic species of dinoflagellates such as *N. scintillans* was preceded the emergence of photosynthetic species. Photosynthetic species would have evolved by acquiring photosynthetic capability through secondary and even tertiary endosymbiosis. *N. scintillans* is also placed within a classification scheme that has a class Diniferea or Dinophyceae, which includes nonparasitic dinoflagellates that lack armor plating.

Fukuda and Endoh (2008) reported phylogenetic analysis of *N. scintillans* based on β-tubulin and heat shock protein 90 (Hsp90) gene sequences. These two genes are widely used for phylogenetic analyses among protozoa (Fast *et al.* 2002; Harper *et al.* 2005; Leander and Keeling 2004; Nishi *et al.* 2005; Saldarriaga *et al.* 2003; Shalchian-Tabrizi *et al.* 2006). Both results of *Noctiluca* and *Oxyrrhis* suggested that should be placed in a basal. Taken together with the previous reports on gamete morphology (Fukuda and Endoh, 2006), they discussed the origin of core dinoflagellates (which produce haploid trophonts), and proposed that they may have evolved from a common haploid ancestor similar to the zoospore of noctilucids (which produce diploid trophonts). This scenario can rationally explain the change in ploidy of trophonts from diploid to haploid during dinoflagellate evolution.

2.2 Common molecular techniques used in population studies

Following the development of PCR methods, molecular techniques have become widely used for detecting genetic variation in natural populations. Most nucleotide changes can be detected by these techniques. Many of these changes probably reflect silent substitutions that are likely to be selectively neutral, making them particularly suitable to population genetic studies (Silva and Russo, 2000). The PCR technology has spawned many procedures for typing strains and species, some of which have become standard methods for species and strain identifications (Xu, 2006).

Molecular tools provide valuable data on diversity through their ability to detect variation at the DNA level. Identification is of fundamental importance in diversity studies in a variety of different ways (Somasundaram and Kalaiselvam, 2006).Recently, most of the techniques are PCR-based. The basics of the PCR technique involves the use of primers (short oligonucleotides), which may be commercially available, to anneal onto both upstream and downstream sides of the DNA sequence of interest at sites which are complementary to primers. The target DNA or gene is then replicated by thermostable DNA polymerase (Tag polymerase). It can then be amplified by a number of cycles. Generally, the PCR condition involves three steps per cycle of reaction, namely denaturation, primer annealing, and primer extension (see Innis *et. al.*, 1990). This technique thus allows researchers to multiply the copy number of the gene of interest. Only a small amount of DNA is required to serve as a DNA template. Although simple in concept, PCR methods have unrivaled, often overlooked complexity. The source of this complexity includes multi-ionic interactions, kinetic constants, and enzymatic activities etc. These factors can repeatedly affect the reactants in a typically small PCR reaction volume over an extended time period. Despite these potential problems, many methods have been developed and are widely used (Xu, 2006). By the way, A major concern in the field of population genetics is to understand the causes of differentiation between populations across ranges of geographic distribution. Thus, for example, one can study evolutionary forces in action (natural selection, genetic drift, etc.) and note phylogenetic and biogeographic patterns among living organisms through geographical space and time (Silva and Russo, 2000). The knowledge produced by the use of theoretical and experimental tools has also proved useful for more applied purposes, such as forensics, fisheries and conservation (reviews by Avise, 1994, 1996; Cipriano and Palumbi, 1999).

2.2.1 DNA markers

In eukaryotic cells, there are two types of DNA: nuclear DNA (nDNA) located in the nucleus and mitochondrial DNA (mtDNA) located in mitochondria (found in the cytoplasm). In the following sections, Molecular markers used in this study will be discusseg.

2.2.1.1 Random markers

Inter simple sequence repeat (ISSR)

ISSRs (Inter-Simple Sequence Repeats) are segments of DNA found between two simple sequence repeats (usually dinucleotide or trinucleotide repeats) e.g. 5'-CTCTCTCTCTCTY-3' or 5'-TGATGATGATGA-3'.

ISSR techniques are nearly identical to RAPD techniques except that ISSR primers are designed from microsatellite regions and are longer (approximately 14 or more bp) than RAPD primers. ISSR analysis involves the polymerase chain reaction (PCR) amplification of regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat (SSR)-containing primer (Zietkiewicz et al., 1994).

The basic premise of ISSRs is that primer annealing sites are distributed evenly throughout the genome such that the primer will anneal to two sites orientated on opposing DNA strands. If these are within an appropriate distance of one another, the region between the two primers will be amplified through PCR. The region would not be amplified if there was divergence at the primer binding sites, if a binding site was lost, or if structural rearrangements of the chromosomes had occurred (http://www. biosci .ohiostate.edu/~awolfe/ISSR /ISSR.html).

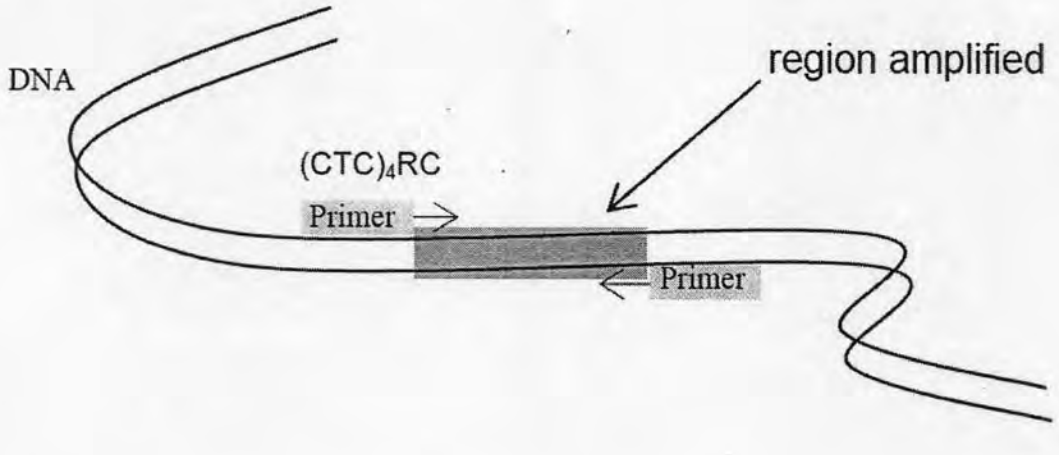

Fig. 3. ISSR technique (http://www.biosci.ohio-state.edu/~awolfe/ISSR/ISSR.html)

ISSR technique was well-known to used with plant. Because of the high polymorphism, ISSRs have been employed successfully in population genetic studies in many cultivated and wild plants (Huang and Sun, 2000; Esselman *et al.*, 1999). Ge and

Sun, (1999, 2001) suggested ISSRs that was useful in evaluating genetic diversity in the mangrove species *Aegiceras corniculatum* and *Ceriops tagal*. Therefore, in the study of Jian *et al.*,(2004), they applied ISSR markers to investigate genetic variation within and among natural populations of *H. littoralis* growing in mangrove and non-mangrove habitat from China and Australia.

Bornet et al., (2004) suggested that ISSR has the potential to fill up the need of more polymorphic tools to distinguish micro algae and ISSR was used as a new marker for genetic characterization and evaluation of relationships among phytoplankton. They also commented the use of ISSR in the future; the use of ISSR should be enlarged, for example, 1) for Harmful Algal Bloom monitoring by species identification of isolated cells, 2) to study genetic relationships among more species and genera to compare results with all the previous data, 3) to the management of species collections especially for strains identification or genetic stability (detection of somaclonal variations), 4) to screen quickly the most abundant SSR motifs in order to develop microsatellite markers.

2.2.2 Animal mitochondrial DNA (mtDNA)

Animal mitochondrial DNA has a close-circular shape, except for a linear form in Hydra (Warrior and Gall, 1985) and Paramecium (Prichard et al., 1990). Its gene contents and arrangement are highly conserved. However, gene rearrangement may occur between phyla (Staton et al., 1997). Normally, an animal mtDNA consists of two rRNA genes, a large (16S rRNA) and small (12 rRNA) rRNA, twenty two tRNA units, thirteen mRNA units coding for proteins (cytochrome b, three subunits of cytochrome oxidase, three subunits of ATP sythetase and six subunits of NADH dehydrogenase), The COX I gene encodes a translation elongation factor, is present in all organisms, like the SSU gene and is well conserved and easy to align (Medlin *et al.*, 2004). Cytochrome c oxidase is the final protein complex in the electron transport chain and is composed of subunits encoded by both nuclear and mitochondrial genes. Thus, cytochrome c oxidase activity reflects, to some extent, the coordinated function of the two genomes (Edmands and Burton 1999). For example, Skelly et al., (1991) studies cytochrome oxidase1 in bacteria species and reported that COX1 intron variation has been recorded also between strains within a species of bacteria. So in this case of *Kluyveromyces lactis* and *S. cerevisiae* that possess strains with one, three or four introns and strains with six or seven introns (Foury *et al.*, 1998), respectively. In this study we sequenced a highly conserved mitochondrial

gene, COX I, which encodes subunit 1 of cytochrome c oxidase, and used these sequences to infer relationships and described genetic variation among 2 groups of *N. scintillans* in the inner gulf of Thailand.

Animal mtDNA has been increasingly used as a molecular tool for population and evolutionary biology because it is a small molecule (about 1.6-1.9 kb) which is easy to access, has a rapid rate of nucleotide divergence, and arises from maternal inheritance (no recombination) (Wilson *et al.*, 1985). However, there are some disadvantages to the use of animal mtDNA such as heteroplasmy(the phenomenon of two or more mtDNA haplotypes occurring in an individual), (Monnerot, 1984, Bermingham *et al.*, 1986), paternal inheritance (Gyllensten *et al.*, 1991, Hoeh *et al.*, 1991, Zouros *et al.*, 1992; Skibinski *et al.*, 1999), and nuclear insertion (Zhang and Hewitt 1996).

The main method employed with animal mitochondrial DNA are the use of restriction enzymes and sequencing of specific genes or regions. The restriction enzymes method is also called restriction fragment length polymorphism. This method involves the use of a restriction endonuclease, which recognize 4-6 bases cutting sites. Polymorphisms can be detected by the difference in length of mtDNA fragments caused by insertions or deletions and the gain or loss of cutting sites brought about base substitution in the recognition site. The sequencing of specific genes can reveal a high resolution of mutation for population and evolutionary studies, but it is expensive, requires laborious work and is time consuming. Recently, automatic sequencers have become available to help to speed up the process, but the expense is still high and experience is also required.

2.2.3 Nuclear marker (Internal transcribed spacer (ITS))

The nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) region has become an important nuclear locus for molecular systematic investigations of angiosperms at the intergenic and interspecific levels. Universal PCR primers are positioned on the conserved rRNA genes (18S, 5.8S, 26S) to amplify the entire ITS spacer region (Jobes and Thien, 1997). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA), defined as the unit containing the ITS1 spacer, 5.8S rRNA gene, and ITS2 spacer (Fig. 4.), has proven to be a useful molecular technique for determining taxonomic relationships in many plant families.

Fig 4. Schematic diagram of the nuclear ribosomal DNA internal transcribed spacer region. There are two *Eco*RI sites (GAATTC; denoted by **E**) located outside the ITS region amplified by the 'universal' primers of White *et al.* (1990).

In Dinoflagellate species, DNA sequencing methods have improved greatly in recent years, both in terms of accuracy and efficiency, making molecular characterization of cells technically easier and less expensive to perform (Haley *et al.* 1999, LaJeunesse and Trench 2000, Godhe *et al.* 2002, Galluzzi *et al.* 2004). The first, in the mid-1980s, ITS region of dinoflagellate was obtained from Prorocentrum micans Ehrenb. (Maroteaux *et al.* 1985). Later the mid-1990s, ITS sequence data were being used to identify a number of harmful algal species (Adachi *et al.* 1996, 1997, Hudson and Adlard 1996). Since that time, species specific molecular assays based on unique ITS sequences have been used to address important taxonomic, phylogenetic, and ecological questions concerning dinoflagellates (e.g., D'Onofrio et al. 1999, Penna and Magnani 1999, LaJeunesse and Trench 2000, Cho *et al.* 2001, Edvardsen *et al.* 2003, Galluzzi *et al.* 2004). Despite the wide use of ITS sequence data, no systematic study has been undertaken to determine if these sequences could be used to recognize putative species across a wide range of dinoflagellate groups (Litaker *et al.*, 2007).

2.3 Population genetics of phytoplankton

Our understanding of phytoplankton population structure is approximately 20 years behind that of other groups of organism (Medlin *et al.*, 2000). Historically, phytoplankton were considered to inhabit a homogeneous environment and as they are primarily asexual it was concluded that there was little scope for genetic diversity. This is a simplistic view of marine environments; for example, coastal seas are a mosaic of water masses with different properties (Mann and Lazier, 1991). Conversely, researchers investigating microalgal physiology have assumed that the high degree of phenotypic

variation, due to physiological diversity within a single species, reflects high levels of genetic variation (Wood and Leatham, 1992) and a multitude of competing genotypes (Doyle, 1975). The molecular markers (e.g. isozymes) used for early studies evolve at such slow rates that diversity within a population cannot be resolved, which contributed to the early assumption of a lack of genetic diversity in microalgal populations (Medlin *et al.*, 2000). Other technical difficulties lie in obtaining the material for analysis. Genetic studies of microalgae rely on the production of clonal cultures from single cell isolates. These isolates will not represent the full genetic diversity of the field population, as the laboratory culture regime will select for some isolates over others. Additionally, the life cycles of many microalgae are unknown and sexual reproduction during culture may further complicate analysis. These issues have been reviewed by Medlin *et al.* (2000). Despite these limitations, isozyme analysis has been used to explore the genetic structure of phytoplankton populations. Gallagher (1980) conducted the first quantitative study of the population genetics of a microalga, isolating 457 clones of the diatom *Skeletonema costatum* from Narragansett Bay over 2 years. She concluded that summer and winter bloom populations were genetically different and that although there were prevalent forms within seasonal blooms, they could not be considered clonal. Soudek and Robinson (1983) examined 101 clones of the diatom *Asterionella formosa* from 20 populations occurring in lakes in Europe and North America. Although there was detectable difference between populations, there was no detectable variation within populations, suggesting that populations were clonal, or at least highly homogeneous. DNA fingerprinting enables greater resolution and has been carried out using random amplified polymorphic DNAs (RAPDs) and microsatellite analysis. Lewis *et al.* (1997) employed RAPD analysis on 126 clones of the diatom *Fragilaria capucina* from seven lakes and concluded that the intrapopulation variation shown by *Fragilariacapucina* but not *Asterionella formosa* (Soudek and Robinson, 1983) was probably due to the greater sensitivity of RAPDs compared to isozyme analysis. Bolch et al. (1999) used both RAPDs and allozyme analysis of populations of the dinoflagellate *Gymnodinium catenatum* and concluded that allozyme analysis was not sensitive enough to use in population comparisons. Bolch *et al.* (1999) found that *G. catenatum* blooms are not clones arising from asexual reproduction and appear to be genetically heterogeneous, outbreeding populations that show distinct population structure on relatively small scales. Rynearson and Armbrust (2000) developed microsatellite markers to investigate the field population of the diatom *Ditylum Brightwellei* in Puget Sound (Washington, USA).

They made the first quantitative assessment of genetic and phenotypic diversity within a micro algal population isolated from a single body of water at a single point in time. The gene diversity index (0.88) indicated that the population was composed of genetically distinct and largely unrelated individuals (for a population of closely related individuals it would be close to 0, approaching 1 for a population of unrelated individuals). Microsatellite markers offer a tool that will enable the investigation of micro algal population genetics with sufficient resolution to look for genetic diversity within a single phytoplankton bloom.

2.4 Why we should study the genetic variation in the inner gulf of Thailand ?

Noctiluca scintillans is a strongly buoyant planktonic species common in neritic and coastal regions of the world. In Thailand, *Noctiluca scintillans* blooms took place throughout the year and could be observed more frequently from July to September in the eastern part and from December to February in the western part of the inner Gulf of Thailand (Lirdwitayaprasit *et al.*, 1993). These seasonal changes in the occurrences of red tides in the inner Gulf were probably due to the water circulation that show in Fig. 5

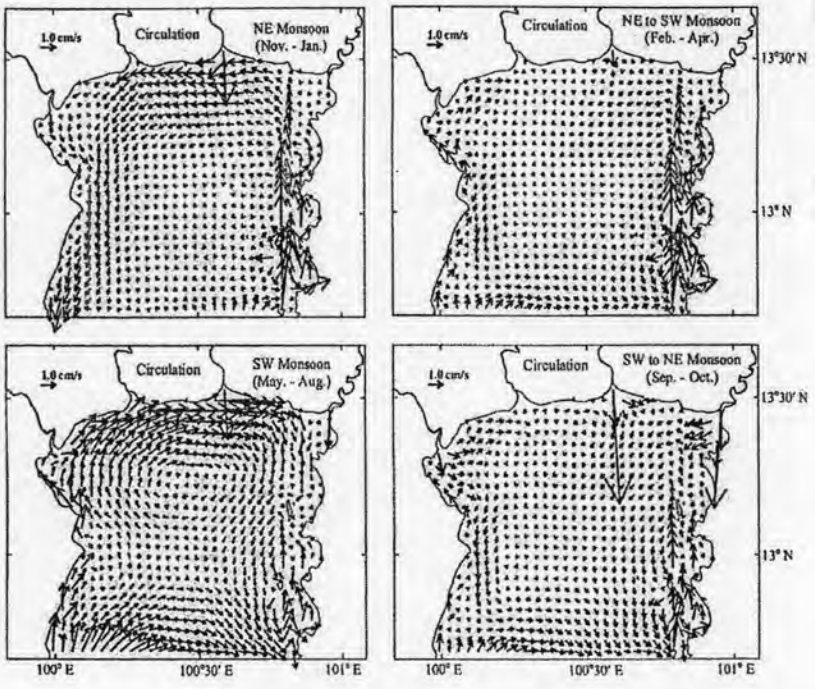

Fig. 5. Seasonal circulations due to seasonal wind fields (Buranapratheprat and Yanagi, 2003).

Counter-clockwise circulation still develops in December which is the northern monsoon period. A clockwise gyre near northern shore is also apparent during the southwest monsoon but the current magnitude is not so large. Current in March is rather complicated and weak. During this time, a clockwise and a counter-clockwise gyres rise up near the northern coast and the middle of the gulf, respectively. Although September is the transition period from the southwest to the northeast monsoons, counter-clockwise flow can develop in almost the same way as it happens during the northeast monsoon. This result is very interesting because wind fields in both seasons are completely unlike but can generate the same circulation pattern. Strong northward flow along the eastern islands appears in all seasons due to imbalance of tidal current around that area. This phenomenon happens due to the characteristic of tidal current in the inner gulf whose major flow is mainly in north-south directions (Neelasri, 1981). The presence of island trend along the eastern coast probably disturbs the magnitude of calculated northward flow during flood tide to become larger than southward flow during ebb tide (Buranapratheprat *et al.*, 2006). Then local wind field on seasonal circulations may be effect with the distribution of cells *N. scintillans* in this area. So this hypothesis into a conversation with influence of local wind field on seasonal circulations in the inner Gulf of Thailand. However, the relationship between groups of *N. scintillans* in the inner Gulf of Thailand has not been known and the knowledge of the cause of blooming or the distribution in this area is rare. Therefore, this study was conducted and aimed at the application of the molecular genetic technique for studying genetic variation of *N. scintillans* in this area. The results obtained from this study may explain the relationship among the different groups of green *N. scintillans* distributed in the inner Gulf of Thailand.