CHAPTER I

INTRODUCTION

Oysters are important cultured shellfish species in Thailand. Five important species; *Crassostrea belcheri* (Sowerby, 1871), *C. iredalei* (Faustino, 1932), *Saccostrea cucullata* (Born, 1778), *S. forskali* (Gmelin, 1791) and *Striostrea* (*Parastriostrea*) *mytiloides* (Lamarck, 1819) belonging to the subfamily *Crassostreinae* are found in Thai waters. Differentiation of these oysters at the genus level can be carried out using the chomata (ridges or tubercles along the margin of the shell). Oysters having this morphological character is regarded as members of the genus *Saccostrea* whereas those do not exhibit this character are from the genus *Crassostrea*. The Thai common name of oysters varies locally but generally, is hoi-nangrom. The small oysters (*Saccostrea*) is called hoy-joe, hoy-pakjip or hoy-tieb while the big oyster (*Crassostrea*) is called hoy-takrom. The latter can be further divided into takrom kram dum (*C. iredalei*) and takrom kram kho (*C. belcheri*) due to the color of adductor musscle scars. If it is black, it will be regarded as the *iredalei* species. The white scar oyster is *C. belcheri*.

The cultured production of oysters in Thailand ranged from 580 tons to 23,420 tons during 1986 to 1996 (Table 1.1). The annual production tremendously increased from 3,774 tons in 1992 to 17,810 tons in 1993 and consistently increased annually to 23,420 tons in 1996. The costs of cultured oysters was increased from approximately 54 Mbaht in 1992 to 655 Mbaht in 1996 (Department of Fisheries, 1999a).

The total cultivated areas were 8,127 rai in 1996 (Table 1.2). Of these, Surat thani occupied the largest cultured area at about 4,040 rai (49.7% of the total cultured areas) followed by Chonburi (1,697 rai accounting for 20.9%) and Chanthaburi (1,230 rai accounting for 15.1%) (Department of Fisheries,1999b).

Table 1.1 Production and value of oysters in Thailand between 1986-1996

Year	Production (tons)	Value (Mbaht)
1986	580	10.997
1987	1,483	17.486
1988	1,858	25.434
1989	1,399	23.466
1990	1,370	24.755
1991	3,311	49.291
1992	3,774	54.053
1993	17,810	576.383
1994	19,273	522.623
1995	23,037	591.983
1996	23,420	655.512

Source : Fisheries Statistics and Information Technology Sub-Division, Department of Fisheries, 1999a

Table 1.2 Cultured area, production and values of oysters from aquaculture section in 1996

Province	Area (Rai)	Production (Tons)	Value (MBaht)
Total	8,127	23,420	655.512
Coastal Zone 1 (Eastern part of the Gulf of Thailand)	2,006	2,886	32.156
Trat	776	670	18.111
Chanthaburi	1,230	2,216	14.045
Rayong	-	-	-
Coastal Zone 2 (Inner part of the Gulf of Thailand)	1,697	7,744	182.665
Chonburi	1,697	7,744	182.665
Chachoengsao	-	-	-
Samutprakan	-	-	-
Bangkok	-	-	-
Samutsakon	-	-	-
Samutsongkram	-	-	-
Phetchaburi	-	-	-
Coastal Zone 3 (Central part of the Gulf of Thailand)	4,357	10,888	390.653
Prachuapkhirikhan	18	82	2.375
Chumphon	299	24	0.122
Surat Thani	4,040	10,782	388.156
Coastal Zone 4 (Southern part of the Gulf of Thailand)	3	13	0.081
Nakhon Si Thammarat	-	-	-
Songkhla	0	4	0.031
Phatthalung	-	-	-
Pattani	-	-	-
Narathiwat	3	9	0.050
Coastal Zone 5 (Andaman Sea)	64	1,889	49.957
Ranong	16	10	0.469
Phang-nga	48	1,879	49.488
Phuket	-	-	-
Krabi	-	-	-
Trang	-	-	-
Satun	-	-	-

Source : Fisheries Statistics and Information Technology Sub-Division, Department of Fisheries, 1999b

Cultivation of oysters in Thailand has been carried out for approximately 5 decades but it had been limited due mainly to a lack of appropriate growing-out techniques and limitation of seed supply. Basically, oyster seed are entirely collected from nature resulting in overexploitation of natural populations and seasonal inconsistency of the seed supply (Jarayabhand et al., 1995). Laboratory scale production of *S. cucullata* and large scale hatcheries of *C. belcheri* were successfully developed in 1985 and 1988, respectively (Jarayabhand et al., 1985; Sahavacharin et al., 1988). These provided the possibility to develop a sustainable breeding programme for increasing culture and management efficiency of oysters in Thailand.

The basic data on genetic diversity of oysters in Thailand are essential for construction of appropriate breeding programes of these taxa. This knowledge is also important for broodstock selection and management scheme in these species. At present, taxonomic identification of oysters in Thai waters is still unclear limiting culture efficiency and development of closed life-cycle culture of these species due to the possible use of a part of species complexes for aquaculture.

Classification of these taxa has been carried out base principally on morphological characters. However, oysters could display ecomorphological variation (xenology). The external characteristics (e.g. shell morphology) are influenced by a variety of habitats and environmental conditions (Tack et al., 1992). Differentiation of oysters based on this method may not be suitable because two sympatric species may be morphologically similar and misidentified to be a single species. On the other hand, allopatric populations inhabiting different habitats may show variation in shell morphology but the species status is still questionable. Accordingly, species-specific marker of a particular species and phylogenetic relationships of oysters in Thailand are needed at present.

1.1 Taxonomy of oysters

The taxonomic definition of Thai oysters is as follows (Vaught, 1989) :

Phylum Mollusca
Class Bivalvia
Subclass Pteriomorphia
Order Ostreoida
Suborder Ostreina
Superfamily Ostreoidea
Family Ostreidae (oysters)
Subfamily Crassostreinae
Crassostrea belcheri (Sowerby, 1871)
Crassostrea iredalei (Faustino, 1932)
Saccostrea cucullata (Born, 1778)
Saccostrea forskali (Gmelin, 1791)
Striostrea (*Parastriostrea*) *mytiloides* (Lamarck, 1819), formerly called *Saccostrea echinata* (Torigoe,1981)

1.2 Shell morphology

Identification of oysters is based on morphology of the shell (Figure 1.1). The conchological characters employed in the descriptions are general sizes, shapes, radial ribs, commissural plication, hyote spines, commissural shelf, shape and color of adductor muscle scar, umbonal cavity and attachment area of the left valve, and chomata.

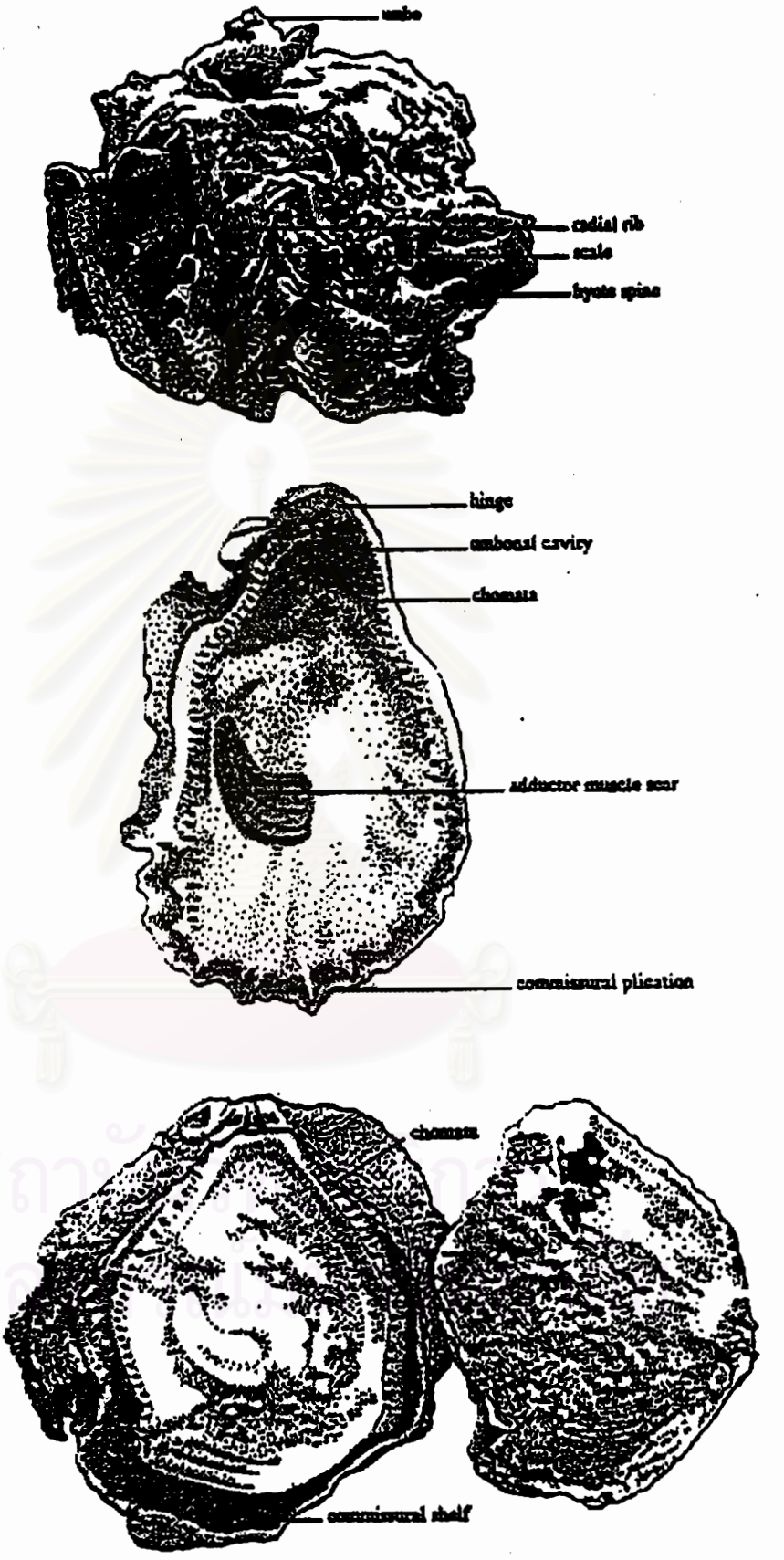

Figure 1.1 General shell morphology of oysters (W. Yoosukh, unpublished data)

1.2.1 *C. belcheri* (Figure 1.2A)

synonym : *Ostrea belcheri* (Sowerby, 1871)

English common name : Belcher's oyster, white-scar oyster

Thai common name : Takrom kram khao

Characteristics : Large sizes (H 13.0 cm x L 9.0 cm). Outline is elongated orbicular to subtriagonal. The surface is lamellated, chalky white inside. Chomata is absent. Adductor muscle scar is white and cresent in shape. Commissural shelf is narrow. Attachment area is medium in size.

1.2.2 *C. iredalei* (Figure 1.2B)

synonym : *Ostrea iredalei* (Faustino, 1932)

Ostrea lugubris (Sowerby, 1871)

Crassostrea lugubris (Bernard et al., 1993)

English common name : Slipper-shaped oyster, Black-scar oyster

Thai common name : Takrom kram dum

Characteristics : Medium sizes (H 7.0 cm x L 5.5 cm). Outline is obliguely subtriagonal grayish purple and whitish within. Umbonal cavity is shallow. Chomata is absent. Adductor muscle scar is reniformed and nearly black. Commissural shelf is narrow. Attachment area is variable from small to medium.

1.2.3 *S. cucullata* (Figure 1.3A and B)

synonym : *Ostrea cucullata* (Born, 1780); (Hanley, 1856); (KÜster & Kobelt, 1868)

Ostrea mordax (Saville-Kent, 1891)

Saxostrea amasa (Iredale, 1939)

Crassostrea amasa (Thomson, 1954); (Carreon, 1969)

Saxostrea mordax (Kira, 1965)

Saxostrea parasition (Habe & Kosuge,1979)

Saxostrea mordax (Torigoe, 1981)

English common name : Rock oyster, Hooded oyster

Thai common name : Hoy nang rom pak jip, hoy tieb, hoy joh

Characteristics : Small to medium sizes (H 6.5 cm x L 4 cm). Shell is oval to subtriangular. The external color is light purple, interior opaque white with dark violet

around the marginal area. Commissural plications are clearly seen. Right valve flat, but left valve deeply concave forming a cup shape. Chomata is rod in shape, encircle entirely vary deep and commissural shelf is wide. Adductor muscle scar is elliptical, white in left valve but dark violet in right valve. Attachment area is large.

1.2.4 *S. forskali* (Figure 1.3E)

synonym : *Ostrea forskali* (Chemnitz, 1785); (KÜster & Kobelt, 1868);
(Sacco, 1897)
Ostrea forskali (Gmelin,1791)
Ostrea cucullata var. Forskali (Lynge, 1909)
Ostrea cucullata (Awati & Rai, 1931)
Saccostrea forskali (Arakawa, 1990)

English common name : Indian rock oyster, Bombay rock oyster

Thai common name : Hoy nang rom pak jip, hoy tieb, hoy joh

Characteristics : Small to medium sizes (H 6.5 cm x L 4 cm). Outline is variable, iregularly elongated, oval to subtriagonal depending upon substrates and spaces. The left valve is deeply cupped with a distinct umbonal cavity. The right valve is flattened or slightly concave. The color of the exterior is grayish white. Commissural plications are weak. The interior is white with purple staining at margin. Commissural shelf is narrow. Strong chomata, appear as tubercles but often obscure at ventral margin. Adductor muscle scar is reniformed, light brown or stained with dark bands on both valves. Attachment area is large and completely cemented.

1.2.5 *S. mytiloides* (Figure 1.3 C and D)

synonym : *Ostrea mytiloides* (Lamarck, 1819); (Hanley, 1856); (Martens, 1897)
Ostrea echinata (Hanley, 1856)
Ostrea spinosa (Iredale, 1939)
Crassostrea echinata (Thomson, 1954)
Saccostrea echinata (Torigoe,1981)

English common name : Black bordered oyster, Black edge oyster

Thai common name : Hoy nang rom

Characteristics : Medium sizes (H 7.0 cm x L 6 cm). Shell is orbicular or elongated oval. Commissural plications are unclearly seen. Shell exterior is dull white, interior coloration is lustrous white with pale purple blotches. Shell margin raised verically particularly at ventral area. Both valves flat or slightly concave with no umbonal cavity. Commissural shelf is wide. Chomata is small and often obscure at the ventral margin. Adductor muscle scar is reniformed and white. Attachment area is large.

1.3 Biology of oysters

1.3.1 Orientation

The hinge is considered to be dorsal and that of the opposite is ventral (Figure 1.4). When one has the outer surface of the right, or unattached, valve facing them. With the hinge uppermost, the anterior is to the right, the posterior is on the left. The height of a specimen is measured along a line perpendicular to the axis of the ligament, and the length is that along a line perpendicular to the height and paralleled to the ligamental axis. The width or thickness of valves when right dovetail into left valve. (Dilokrattanatrakul, 1998).

1.3.2 Anatomy of Oysters

Anatomy of oysters is shown by figure 1.5. The adductor muscle is the muscle that function in closing the shells. It is usually whitish and is a little chewier than the rest of the oyster. The mantle is the meaty body part. The entire soft part of the oyster, including the stomach and the gills, is eatable. The hinge is the ligament that joins two shells and acts to open the shell (in oppostion to the adductor muscle). The oyster has two valves and is a member of bivalves. (Dore, 1991).

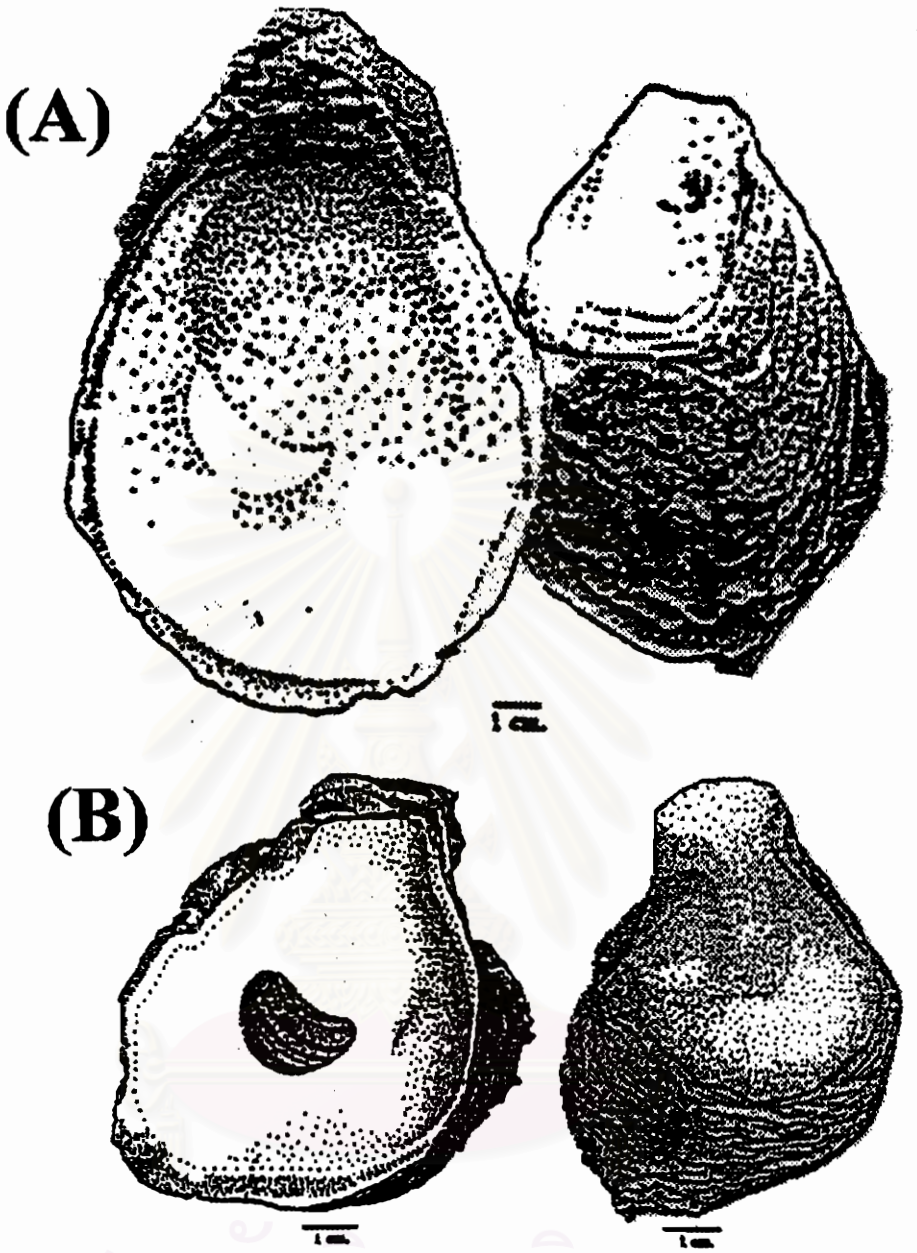

Figure 1.2 External shell morphology of *Crassostrea* oysters (W. Yoosukh, unpublished data)

A: White scar oyster *C. belcheri*

B: Black scar oyster *C. iredalei*

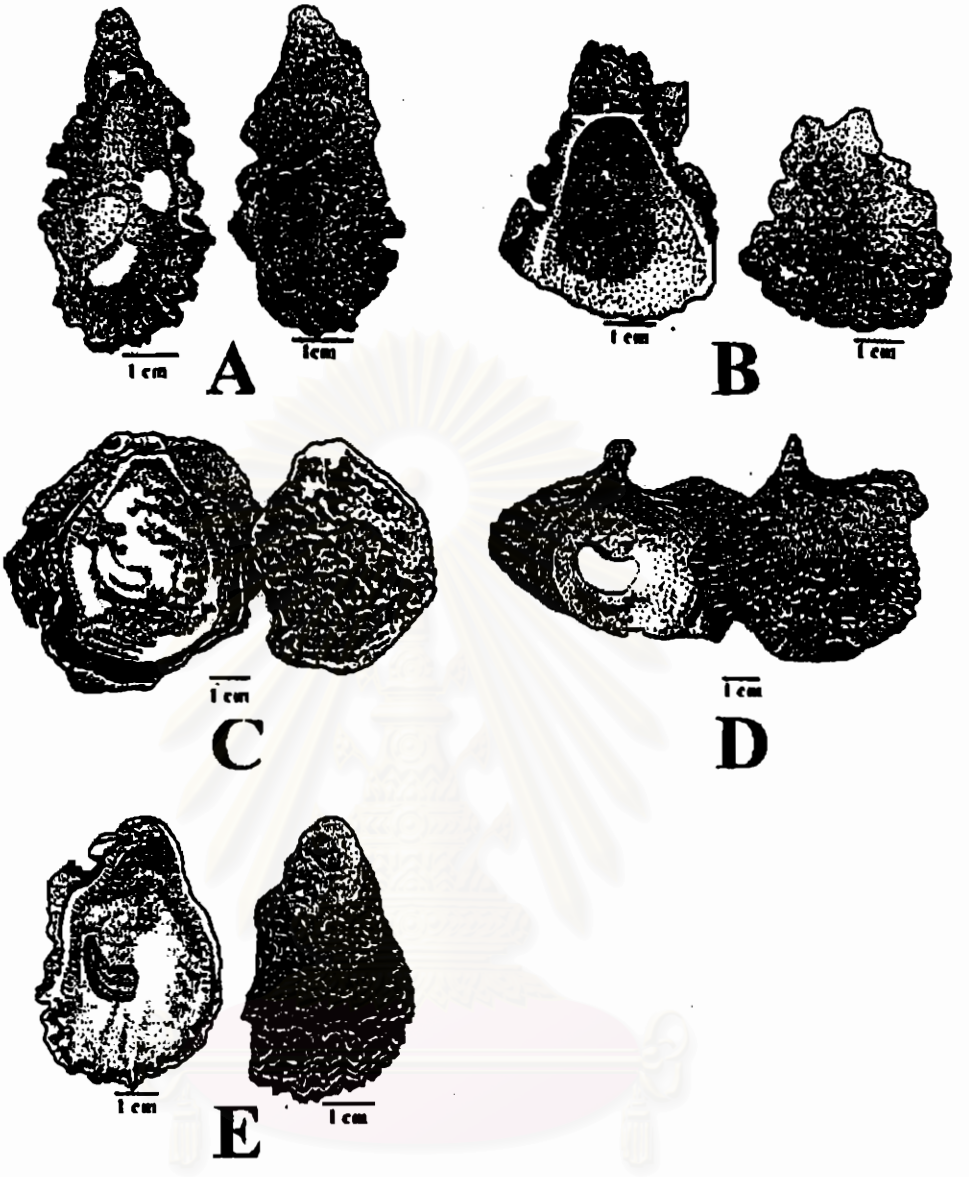

Figure 1.3 External shell morphology of *Saccostrea* oysters (W. Yoosukh, unpublished data)

A and B: *S. cucullata*

C and D: *S. mytiloides*

E : *S. forskali*

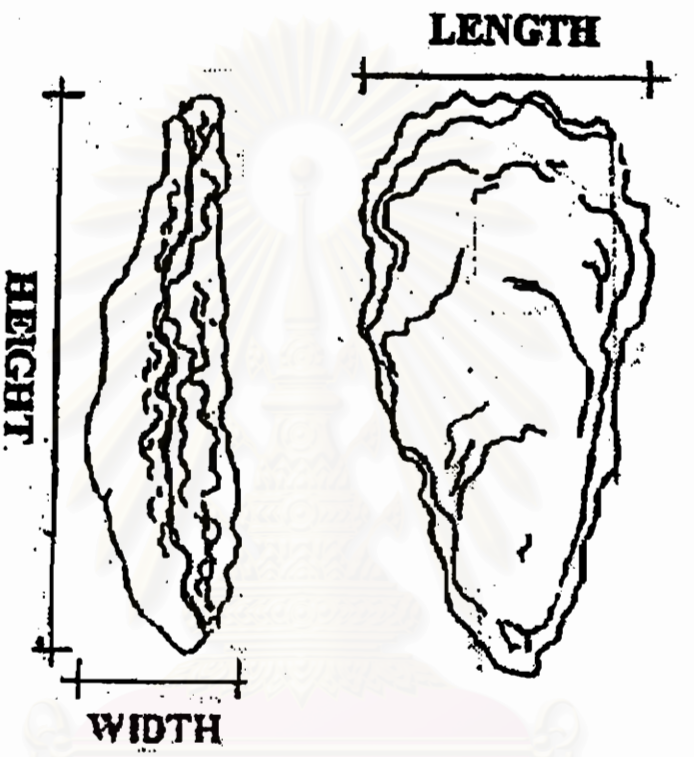

Figure 1.4 Dimension terms applied to oysters (Dilokrattanatrakul, 1998)

1.4 Life cycle of oysters

Two main types of oysters, *Ostrea* and *Crassostrea*, have different breeding habits. In *Ostrea*, the eggs released from gonad are retained in the mantle cavity within the shell while the sperms are discharged externally. Eggs are fertilized by sperms from outside and about half of the larval development period takes place inside the shell before released into the open water. In *Crassostrea*, both eggs and sperms are discharged directly outside into the open water during spawning where fertilization and all subsequent development takes place externally (Quayle, 1980). After fertilization, the embryo undergoes cleavage and passes through blastula and gastrula stages within 24 hours (Fig 1.6). At this stage two tiny shells were developed. The trochophore larvae can swim by beating of minute hair (called cilia). In both genera, the fully formed larva is about 0.2 mm long. It still remains as a zooplankton for 1-2.5 weeks. During this time, the primarily feed is algal cells which are smaller than 0.01 mm in diameter. The larva reaches a maximum size at about 0.3 mm in length during this period (Dilokrattanatrakul, 1998)

At the end of free-swimming period, a suitable spot (often a small crevice) are found for settlement. This is checked by releasing of the cement deposited in a gland of the foot into which it crawls with the left of cupped valve down. The cement quickly hardens, the oyster is then permanently attached to the substratum for life. This process is called spatting or setting, the young oysters at this stage is spat or seed (Quayle, 1980). The oysters at this stage is transferable and could can be replanted in different geographic areas to commercial sizes.

In *Ostrea*, an alternation of sexuality, usually within the some spawning season is occurred. The oyster may firstly spawn as a male after which the gonad are changed to the female phase, and eggs are released at the next spawning. In the *Crassostrea* type, the oyster spawn either as a male or a female in one season but the sex may change before the breeding season of the following year (Quayle, 1980).

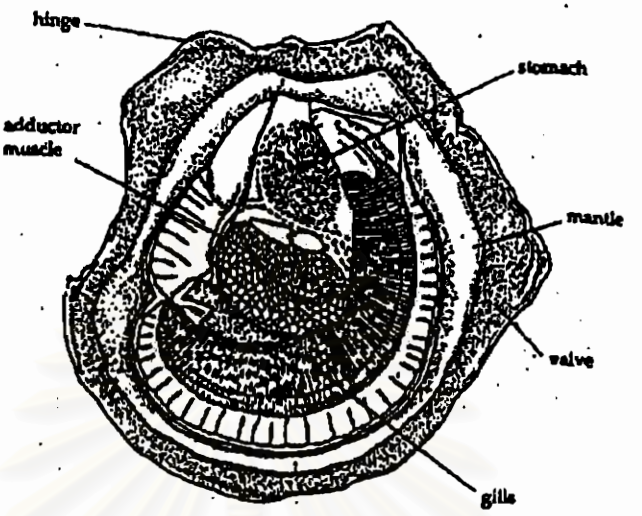

Figure 1.5 Anatomy of oysters (Dore, 1991)

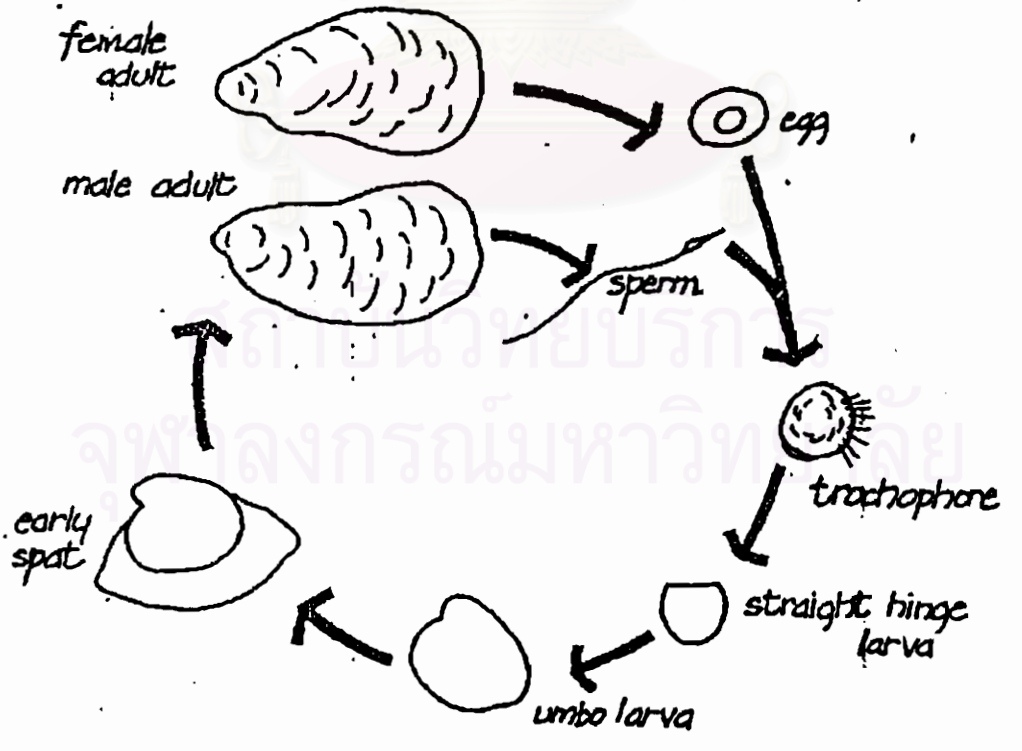

Figure 1.6 Life cycle of oysters (Quayle, 1980)

1.5 Distribution of oysters

Oysters are widely distributed throughout tropical and subtropical areas. Different species of oysters can grow in waters from full sea strength to intertidal streams (Matthiessen, 1991). Oysters are found abundantly in shallow water along rocky coastlines and in intertidal mangrove areas. They attach to rocks and other substrata in intertidal streams. In the mangrove areas, oysters attach roots or other vegetative substrata. They also attached to coral or rocks around islands. In Thailand oysters are found along shorelines of both the Gulf of Thailand and the Andaman Sea coasts (Brohmanonda et al., 1988)

1.6 Molecular genetic markers

Molecular markers are useful for various genetic studies. These, generally, include protein and DNA markers. The former refers to markers from allozyme and protein analyses. The latter is composed of those obtained from several approaches including restriction analysis (restriction fragment length polymorphism, RFLP; DNA fingerprinting and polymorphism of single copy nuclear DNA), PCR-based techniques (e.g. randomly amplified polymorphic DNA, RAPD and microsatellites) and DNA sequencing.

1.6.1 Protein markers

Protein polymorphism is regarded as one of the genetic markers detected by electrophoresis of proteins (usually enzymes). Proteins with different net charges migrate at the different rate through a gel matrix when exposed to an electric field (Avise, 1994). The protein bands can be visualized by a specific histochemical stain of a particular enzyme.

Analysis of protein polymorphism is a technique of choice to starting with when any molecular data of a species under investigation has not been reported. The majority of protein markers are represented by allozymes which are different

molecular forms of an enzyme coded by different alleles at one gene locus. The allozyme markers are transmitted in a co-dominant manner, therefore, heterozygosity of individuals within populations or species can be determined. The advantages of allozyme approch are its cost-effective, less tedious and time consuming compared to other molecular genetic techniques. Therefore, large numbers of samples can be processed within the limiting period of time.

However, allozyme analysis has some limitations. For instance, synonymous mutations can not be detected. Likewise, nucleotide substitutions changing one non-polar amino acid to another do not alter the electrophoretic mobility of proteins. Scoring of gels can be complicated and need experienced scientist to interprete the precise results from electrophoresed gels (Kocher and Stepien, 1997).

1.6.2 DNA markers

DNA can be compared in several different ways. Genetic variations at the DNA level can be generalized roughly into two categories : base substitutions and insertions/deletions. The polymorphism is usually represented by differences of nuclear and mitochondrial DNA.

1.6.2.1 Animal mitochondrial DNA

Mitochondrion is one of the cell organelles found in cytoplasm of eukaryotic cells. The size of animal mitochondrial genome is approximately 16,000-20,000 base pairs in length (Carvolho and Pitcher, 1995) and is available for several thousand copies of the mitochondrial genome per cell.

The animal mitochondrial genome is composed of 13 protein coding genes : NADH dehydrogenase, (ND, subunits 1, 2, 3, 4, 4L, 5 and 6); cytochrome b (cyt b), three subunits of cytochrome oxidase (COI, COII and COIII), two subunits of ATP synthetase (ATPase 6 and 8), 2 genes coding for ribosomal RNAs (16S and 12S rRNAs) and 22 transfer RNA coding genes.

Animal mitochondrial DNA is widely employed in evolutionary and population genetic studies because it is small in size reflecting its easiness to purify from the target sources. The maternally inheritant nature and high mutation rate in most organisms make the animal mtDNA suitable for various genetic applications. Typically, the number of gene contents and organization of gene order of organisms within the same phylum are conserved. Nevertheless, in terms of polymorphism based on point mutations, mtDNA, particularly in vertebrates, evolves five to ten times faster than does single copy nuclear DNA (scnDNA) (Vawter and Brown, 1986).

1.6.2.2 Nuclear DNA

Nuclear DNA contains both unique single copy and repetitive regions. The single copy region generally codes for a particular gene product. The structure of protein coding genes in the nuclear genome consists of coding regions (exons) and non-coding regions (introns or intervening sequences). Typically exons are highly conserved. In contrast, much higher polymorphism can be observed in introns (Krawczak and Schmidtke, 1994).

Repetitive DNA consists of core sequences that are repeated in varying degrees. They may be made up of coding segments such as the ribosomal RNA (rRNA) genes, or non-coding tandem repeated units (Parker et al., 1998).

Variable number of tandem repeats (VNTRs) are composed of satellite, minisatellite and microsatellite DNA. Large repetitive units of satellite DNA are often associated with heterochromatin located near the centromere of chromosomes. Smaller regions (made up of repeat units < 65 base pairs) are known as minisatellite DNA and can occur throughout the genome (Jeffreys et al., 1985) whereas microsatellite DNA has consecutive repeat units of only 2-6 base pairs (Tautz, 1989).

1.6.3 DNA techniques commonlyused in genetic variation studies

1.6.3.1 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analysis is one of the initial techniques widely used to indirectly detect genetic variation at the DNA level. It examines size variation of specific DNA fragments following digestion with restriction enzymes.

Conventional RFLP analysis is carried out by digestion of genomic DNA with a specific endonuclease. The products are then size-fractionated using gel electrophoresis and transferred onto a membrane. The investigated fragment (s) is identified by hybridized with the specific radiolabeled probe. In more comprehensive studies, restriction sites are mapped and the presence or absence of restriction sites (rather than sharing of fragment lengths) is scored (Kocher and Stepien, 1997).

1.6.3.2 PCR-RFLPs

The polymerase chain reaction (PCR) is an effective approach applied for population genetic and systematic studies. The method is based on an *in vitro* enzymatic amplification of DNA. Million copies of a particular target DNA fragment are produced from a very low amount of starting template DNA (mostly in the nanogram range). PCR is a simple but powerful technique. The PCR reaction contains the target double-stranded DNA, two primers that hybridize to flanking sequences on opposing strands of the target sequence, deoxyribonucleotide triphosphates and a *Taq* DNA polymerase. The amplification reaction consists of three steps; denaturation, primer annealing and elongation step. The cycle is repeated for 25-50 times. In each cycle newly synthesised strands act as the templates for subsequent replication resulting in exponential increasing of the specific product within only a few hours. The product obtained are usually electrophoretically analyzed using agarose gels.

The PCR-amplified fragment can be further analyzed for restriction site and / or fragment length polymorphism. After specific DNA is amplified through the PCR, the product is then digested with restriction endonuclease before electrophoretically analyzed. The most important advantage of this technique is that hybridization of labeled DNA probes to the target restricted DNA is not necessary. This technique is much simpler than the conventional RFLP approach.

1.6.3.3 DNA sequencing analysis

Polymorphism at the DNA level can be studied by several methods but the most direct strategy is determination of nuclotide sequences of a defined region.The sequences obtained can be aligned by comparing with an orthologous region in the genome of related organisms (or populations).

PCR-amplified fragments can be directly sequenced using the typical chain termination reaction (Sanger, 1977) or alternatively by cycle-sequencing. Nevertheless, more accurate sequences of DNA fragments are obtained through the cloning approach.

However, DNA sequencing is tedious, time consuming and expensive. The number of bases which can be determined by sequencing usually cover a few hundred bases. Increasing the length of investigated DNA with a large number of samples are prohibited by several factors described above. With an introduction of automatic DNA sequencers, the experiments can be carried out much faster than that based on manual sequencing allowing its wider applications for genetic studies on long DNA sequences (i.e. genome projects) at present.

1.6.3.4 Randomly amplified polymorphic DNA (RAPD) analysis

Randomly amplified polymorphic DNA (RAPD) analysis was concurrently developed by Williams et al. (1990) and Welsh and McClelland (1990). Technically, it is a simple method for determination of genetic variations using arbitrarily primed PCR-based technique. The amplification conditions of RAPD differs from that of the standard PCR in that only single random primer (e.g. 10 mer with GC content usually at least 50%) is employed. RAPD amplified target DNA on the basis that the nuclear genome contains several priming sites closed to one another that and are located in an inverted orientation. Accordingly, the primer is utilized to scan genome for the small inverted sequences resulting in amplification of DNA segments of variable length. The amplification products are separated on agarose gels and detected by staining with ethidium bromide.

The advantages of using RAPD markers are as follows; first, RAPD analysis is a simple, rapid and inexpensive method for detecting DNA polymorphism, second, RAPD does not require knowledge of the genome under investigation, third, RAPD is a PCR-based method. It requires small quantity of DNA template per reaction. Forth, RAPD-PCR does not require the use of radiolabelled probes for hybridization. Finally, unlimited numbers of RAPD primers can be screened for suitable molecular markers of various applications within a short period of time.

There are some disadvantages of the RAPD approach for population genetics, genetic mapping, and taxonomic studies. Fragments (especially those arising from mispairing of a primer with the genomic DNA) may not be reproducible among different laboratories because amplifications are sensitive to slightly changes in amplification conditions.

The allele distribution of RAPD amplified fragments is treated in a dominant fashion. Accordingly, the presence of amplified fragment may reflect either a homozygous (AA) or heterozygous (Aa) situation. Only the absence of the fragment reveals the aa genotype. This disadvantage of RAPD results in an inability to estimate

heterozygosity and the actual status of interested alleles because homozygotic can not be dissociated from heterozygotic states.

1.7 Genetic studies in oysters

The allozyme studies is one of the most popular approaches for examination of population genetic studies in oysters. Buroker et al. (1979) investigated levels of genetic variation of six oyster species; *C. belcheri* (N=36), *C. iredalei* (N=154), *C. gigas* (N=96), *C. rivularis* (N=108), *C. rhizophoreae* (N=160) and *C. virginica* (N=160) using 38 allozyme loci. The levels of genetic polymorphism in these oysters were high. Coincidentally, four (*Ald, Idh-1, Mdh-1* and *To-2*) and six species-specific (fixed) alleles (*Acp-3, Ald, Aat-1, Mp-1, To-1* and *Xdh*) were found in *C.belcheri* and *C. iredalei*, respectively.

MtDNA-RFLPs has been widely used for population differentiation analysis of oysters. Genetic discontinuity of the American oyster, *C. virginica*, collected from continuously distributed populations from the Gulf of St. Lawrence (Canada) to Brown ville (Texas) were examined using polymorphism of mtDNA digested with 13 restriction endonucleases (*Ava*I, *Ava*II, *Bgl*I, *Bgl*II, *Cla*I, *Eco*RI, *Hin*cII, *Hin*dIII, *Msp*I, *Nde*I, *Pvu*II, *Spe*I and *Stu*I). Eighty-two haplotypes were found from analysis, of 212 *C. virginica* individuals. Using the UPGMA clustering analysis, mtDNA haplotypes were allocated into two distinguishable clusters having approximately 2.6% nucleotide sequence divergence. A large genetic differences (genetic break) between *C. virginica* collected from the north and south regions of the Atlantic and the mid-coast of Florida were observed (Reeb and Avise, 1990).

The Pacific oyster, *C. gigas* from Miyaki Prefecture (Japan) had been transplanted to the British Columbia coastals between 1927 to 1977. Four populations of introduced oysters (Nootka Sound in Hisnitt Inlet, Barclay Sound in Pipistem Inlet, Pendrell Sound and Hotham Sound) were collected. MtDNA polymorphism was analyzed using nine restriction enzymes (*Ava*I, *Ava*II, *Hpa*I, *Hpa*II, *Hae*II, *Hae*III, *Hin*cII, *Hin*dIII and *Hin*fI). Forty-four composite haplotypes were identified from 141

individuals. Two haplotypes, possessed by 60% of individuals, were shared by all four populations at approximately the same frequencies. A total of forty haplotypes (9-11 haplotypes per population) were uniquely found in investigated populations. Thirty-four of these were singletons (observed in single individuals) (Boom et al., 1994).

Banks et al., (1993) succesfully developed species-specific PCR primers, restriction analysis of PCR-amplified 16S rDNA and dot-blot hybridization to discriminate two closely related oyster species, *C. gigas* and *C. sikamea*. The molecular discriminatory techniques are directly applicable to eliminate *C. gigas*-like oysters from commercial production of *C. sikamea*.

Differentiation of the Portuguese oyster (*C. angulata*) and the Pacific oyster (*C. gigas*) has often been questionable. Using restriction analysis of cytochrome oxidase c subunit I (COI) of 253 individuals with *Hha*I, *Mse*I, *Sau*3AI and *Taq*I, two common hapoltypes; C (dcad) and A (ccab) were found in 76% and 88% of *C. gigas* and *C. angulata*, respectively. These mtDNA markers offered partial differentiation between *C. angulata* and *C. gigas* (Boudry et al., 1998).

Karl and Avise (1992 and 1993) developed oligonucleotide primers for determining polymorphism of "anonymous" scnDNA loci in the American oyster (*C. virginica*) and found significant differences of allele frequencies of populations originating from the Atlantic and the Gulf of Mexico at four polymorphic loci (cv07, cv19, cv32 and cv195).

PCR-RFLP of five anonymous single-copy nuclear DNA (cv07, cv19, cv32, cv195, and cv233) were also used to examine genetic polymorphism in juveniles of *C. virginica*. Two types of RFLP were observed : (I) simple presence or absence of a restriction site, such as that in cv-32 (*Nsi*I) and cv-195 (*Ava*II), resulting in a diallelic system; and (II) multiple polymorphic restriction sites resulting in three or more polymorphic alleles, for instance, four alleles in cv-07 (*Hin*fI) and three alleles in cv-233 (*Eco*RI). For the locus cv-19 digested with *Bst*NI, *Stu*I and *Xba*I, the

fragment profiles were rather complicated. Frequency of non-Mendelian genotypes of offspring across overall loci was approximately 7%. Strict Mendelian inheritances of alleles were observed for loci cv-07 and cv-32 (Hu and Foltz, 1996).

Molecular phylogenetics of cupped oysters were studied using partial sequencing of the large subunit of ribosomal RNA (28S rDNA) gene amplified from eight species of oysters (*C. belcheri, C. gigas, C. rhizophorae, C. rivularis, C. virginica, S. commercialis, S. cucullata and O. edulis*) and the green mussel (*Mytilus colulis*). A total of 315 polymorphic sites was found and 90 of these were phylogenetically informative. Maximum parsimony and maximum-likelihood analyses showed idential phylogenetic lineages and could separate investigated taxo to 3 groupings; *C. belcheri; C. gigas;* and *C. rivularis* (group A), *C. virginica*; *C. rhizophorae; S. commercialis* and *S. cucullata* (B) and *O. edulis* (C). Molecular data support a later divergence of the tropical Pacific *Saccostrea* from a common ancestor of the Atlantic *Crassostrea* species (Littlewood, 1994).

Anderson and Adlard (1994) sequenced an internal transcribed spacer 1 (ITS I) and its immediate flanking portions (18S and 5.8S rDNAs) of the Sydney rock oyster (*S. commercialis*) and the New Zealand rock oyster (*S. glomerata*). Nucleotide sequences of the ITS 1 (438 bp) and portions of its flanking 18S (216 bp) and 5.8S (92 bp) regions were precisely identical for both oyster species indicating that *S. commercialis* and *S. glomerata* should be regarded as a conspecific taxon.

There have been no publication concerning molecular population genetic studies of oysters genera *Crassostrea, Saccostrea* and *Striostrea* in Thailand. The basic knowledge on this discipline is essential for development of effective breeding and management programs in these economically important taxa. Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis has been successfully used for various applications of population genetic studies (Hadrys et al., 1992). These included determination of genetic diversity and identification of useful genetic markers at different taxonomic levels of various mollusc species (Heipel et al., 1998; Jacobsen et al., 1996; Stothard and Rollinson, 1996 and Chambers et al., 1998).

The objective of this thesis is to determine levels of genetic diversity of local oysters in Thailand and to identify species-specific markers of commercially cultured oyster species (*C. belcheri*, *C. iredalei*, *S. cucullata*) by the RAPD analysis. The knowledge obtained can be applied for construction of the effective fisheries management scheme and for identification of seed and broodstock of commercial cultured oysters to ensure sustainable culture of these oysters in Thailand.