CHAPTER II

LITERATURE REVIEW

General Description of Anchovy

According to Wheeler (1975); Webb, Wallwork, and Elgood (1981); Whitehead, Nelson, and Wongratana (1988), anchovies are pelagic fishes in the family Engraulididae, which distribute around the world in tropical, subtropical, and temperate seas, but are most abundant in the Indo-Pacific. Most are schooling fishes of coastal waters and have relatively small size, usually 10 to 20 cm in standard length. They are slender-bodied and rounded in cross-section, having silvery scales which are easily dislodged. Most anchovies become sexually mature at the end of their first year. Whitehead et al. (1988) stated that the anchovies form very valuable fisheries all around the world, captured chiefly by coastal seine nets and purse seines often operated with attracting lights. The fishes are rarely marketed fresh, most are processed salted, canned, or as pastes. They are also used as animal food and life-baits for tuna fishing. In Southeast Asia stolephorid and encrasicholid anchovies are important food resources and are consumed fresh or as various fermented products (Chullasorn and Martosubroto, 1986).

The Shorthead Anchovy, *Encrasicholina heteroloba*

1. Synonyms of *Encrasicholina heteroloba*

Until 1983, the shorthead anchovy *Encrasicholina heteroloba* was identified to be in the genus *Stolephorus*. According to the most recent revision and clear separation of the genus *Encrasicholina* from *Stolephorus* by Nelson (1983), this particular species is now placed in the genus *Encrasicholina* and the scientific name is *Encrasicholina heteroloba.* Nevertheless, as the name *Stolephorus heterolobus* has been widely used in almost all previous literature (e.g., Munro, 1956 cited in Whitehead et al., 1988; Thosaporn Wongratana, 1985; Whitehead et al., 1988; Dayaratne, 1990; Pirochana Saikliang, 1994), most scientific name of this species in the literature cited in this study is *Stolephorus heterolobus.* There are two other synonyms of this species: *Stolephorus pseudoheterolobus* and *Anchoviella heteroloba* but only a few in the literature (Whitehead et al., 1988). In conclusion, *Encrasicholina heteroloba* is now proved to be the junior synonym of *Stolephorus heterolobus* but it is advisable that the name *Stolephorus heterolobus* should still be given the priority for searching the literature in most database.

2. Diagnostic Features

Described by Thosaporn Wongratana (1985) and Whitehead et al. (1988), the shorthead anchovy (Figure 1) has body rather cylindrical, belly rounded, with 4

to 6 (usually 5) sharp needle-like pre-pelvic scutes. Maxilla tip pointed posteriorly, projecting beyond the second supra-maxilla and reaching to sub-operculum. Lower gill rakers are 22 to 30 (usually 23 to 27). Scales are hexagonal. Unbranched dorsal and anal fin rays are only ii; anal fin is short, with usually ii 14 to 16 fin rays; pectoral fin rays are 12-14. Tip of depressed pelvics just reach below dorsal origin or very slightly behind it. In life, a dull silver or gray band on flank, the back beige. It is distinguishable from the species of *Stolephorus* in its short isthmus, preceded by a small bony plate on urohyal between branchial membrane, while the latter has a long isthmus with no bony plate reaching to the margin of the branchial membrane (Thosaporn Wongratana, 1987).

Figure 1. The shorthead anchovy, *Encrasicholina heteroloba*

(source: Thosaporn Wongratana, 1985)

3. Distribution

Encrasicholina heteroloba is a small species of anchovy which widespreads in the Indian Ocean (Red Sea, East African coast to at least northern Madagascar, eastward to Bay of Bengal) and equally widespreads in western Pacific (Indonesia, Thailand, northward to southern Japan, southward to northern coasts of Australia, eastward to Solomon Islands, New Caledonia, Fiji, Tonga, Samoa, also Palau to Kosrae) (Figure 2). It is one of the two most commercially important species among angraulid fishes in the coastal waters of Southeast region, which are highly abundant along northern Sumatra, Peninsular Malaysia, the east coast of Thailand, Kampuchea, and Viet Nam (Chullasorn and Martosubroto, 1986). Its fishing grounds are generally confined to the inshore waters along these coasts. *E. heteroloba* is the most abundant among total anchovy catch in the Gulf of Thailand (Tongsueb Taweesith, 1979; Pirochana Saikliang and Pismorn Isara, 1984; Piroh Suthakorn, 1985; Pirochana Saikliang, 1990; Department of Fisheries, 1993; Pirochana Saikliang, 1994).

4. Biology

Encrasicholina heteroloba feeds mainly on planktonic crustaceans (Whitehead et al., 1988; Milton, Blaber, and Rawlinson, 1990; Yamashita, Mochizuki, and Piamtipmanus, 1991). Although it appears to breed throughout the year, the spawning intensity of this fish is thought to be regulated by the monsoon (Dalzell,

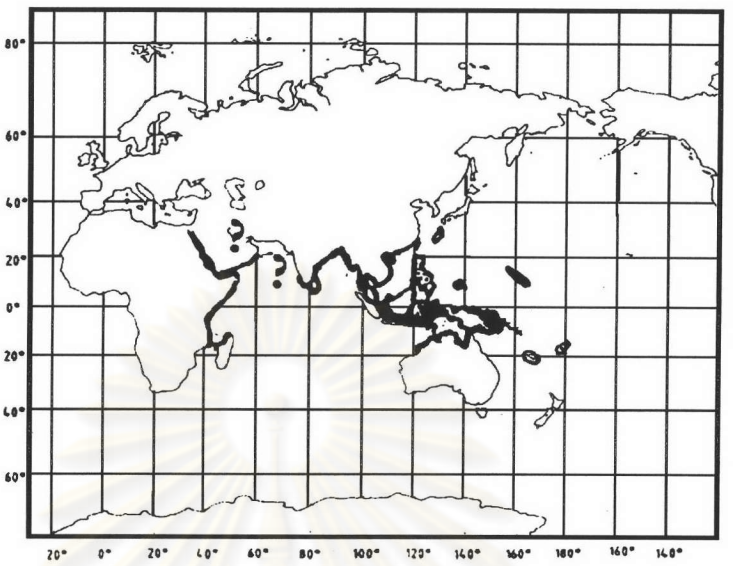

Figure 2. Geographical distribution of shorthead anchovy, *Encrasicholina heteroloba* (source: Whitehead et al., 1988)

1987). It has a peak spawning during the northeast monsoon, October to March, in Manila Bay (Tiews, Ronquillo, and Santos, 1970) or in May to June or July and again in September to November in Papua New Guinea waters, both of which are the transition periods between the monsoons (Dalzell, 1987, 1990). Its eggs are oval, without a knob at one end (Tiews et al., 1970; Whitehead et al., 1988). Published estimates of catabolic growth coefficient (k) based on length-frequency data suggests that its growth rates vary considerably between regions, ranging from 0.198 in the inner Gulf of Thailand to 3.1 in Solomon Islands; and the asymptotic lengths vary from 8.9 cm in Singapore Straits to 12.1 cm in Manila Bay (Tham, 1967; Sakul Supongpan and Pismorn Isara, 1974; Dalzell, 1990; Sommai Yoo-sook-swat, 1990;

Tiroba et al., 1990; Wright, Wiloughby, and Edwards, 1990). Based on means of daily growth rings in its otoliths, growth coefficient and asymptotic length are 3.9 - 4.02 and 8.62 - 8.73 cm, respectively (Dayaratne, 1990). The maximum age of the fish in the genus *Stolephorus*, some of which are now in the genus *Encrasicholina*, is estimated to be 3 years by Tiews, et al. (1970) from their study on the biology of anchovy (*Stolepholus* Lacepede) in the Philippine waters. Dalzell (1990) estimated the maximum age and length of *Stolephorus heterolobus* in Papua New Guinea to be 1.2 years and 8.2 cm, respectively. From the study of Dayaratne (1990) on age and growth of *Stolepholus heterolobus* in Manila Bay, it was concluded that its growth rate is relatively fast, having a larval period of about 20 days, a juvenile stage of 30 days, an adult stage of 50 days, and a life span of about 9 months.

In Thailand, the study on stolephorids were first reported in 1967 by Thongsueb Taweesith on his preliminary survey of stolephorid fisheries in the Gulf of Thailand. From his study, the stolephorids widely distributed in the Gulf of Thailand and *Stolephorus heterolobus* (at present: *Encrasicholina heteroloba*) was the most abundant among stolephorids in the Gulf. No difference is found between the length of male and female fish (Tongsueb Taweesith and Pisamorn Dhebtaranon, 1972). About its growth parameters in the Gulf of Thailand, the catabolic growth coefficients (k) are 0.198 to 1.81, asymptotic lengths ($L\infty$) are 8.89 to 10.57 cm, and hypothetical age at zero length (t_0) are -0.01 to -0.06 years (Sakul Supongpan and Pismorn Isara, 1974; Sommai Yoo-sook-swat, 1990). There are no mature anchovy found in the

period of February to March, whereas the juveniles are obtained in April to May (Sakul Supongpan and Pirochana Saikliang, 1983).

Based on the studies of fish larval distribution in Thailand, anchovy larvae are highly abundant, distributing all year with peaks. In the west coast of the Gulf, their peaks occur in March-April and July-September along Petchburi to Surat Thani coasts, (Somyos Sidtichokpan, 1972, 1976). The highest densities of larvae are found in April and May off Chumporn to Surat Thani coasts(Hayase, 1982; Rangsan Chayakul, 1995), in April, June, and August off Surat Thani to Narathiwat coasts (Chongkolnee Chamchang, 1986). In the Inner Gulf of Thailand, anchovy larvae have their peaks in January, February and November (Somyos Sidtichokpan, 1972; Sa-gna Wattanachai, 1978). The anchovy larvae are reported to be high in September to May in Chang Islands (Sommai Yoo-sook-swat and Anucha Songchitswat, 1990). In Rayong Bay, the high abundance is found in the middle of the Bay during Northeast Monsoon period and the lowest density is found in the inter-period between Northeast and Southwest Monsoon (Wisid Chantarasakul, 1988).

Reproductive Biology of Fish

1. Components of Fish Reproductive Biological Study

1.1 Sex Ratio

Holden and Raitt (1974) stated that the determination of sex ratio is of considerable importance because it forms primary application in providing basic knowledge of the reproductive biology for a stock and it is a part of the basis of stock assessment. The male and female fish of some species have different rates of growth and should be treated as separate stocks in stock assessment. Mortality rates may also differ between sexes. The determination of sexual maturity is always done together with sex ratio for better estimation of stock reproductive potential.

1.2 Maturity Stages

According to Holden and Raitt (1974), maturity stage means the degree of ripeness of ovaries and testes of the fish. Gonad development has always been found with the same meaning for maturity stage. Routine assessment of maturity stages is normally done by assigning individual to stage by characters which can be differentiated with naked eye. There are several keys for maturity staging, but a scale of not more than 8 stages is suitable for most species. A refined distinction

between stages can be made by histological examination. According to the maturity stages in the gonads, a fish can be classified into 2 types of spawner.

1.2.1 Total (Isochronal) Spawner

In this group, after maturation of the gonad , all eggs or sperms which are going to be spawned in a single breeding period develop synchronously. Its release takes place over a short period of a week or so and the breeding season is clearly defined. Maturity staging of total spawner is usually simple because nearly all the developing eggs in the ovary are at the same stage and can fairly easily be allocated to that stage on visual criteria of size, colour, and texture. This is the common type in species of northern latitude.

1.2.2 Partial (Heterochronal) Spawner

Spawning of this fish takes place over a protracted period and the ripening eggs of vary stages in development can be found at any one time in the same ovary both before and during spawning. The construction of a maturity scale for partial spawners is more difficult because there is a range of development stages in an individual gonad at any one time and the differentiation achieved is less precise. This situation is found in a number of species in tropical and sub-tropical waters including anchovies (Hunter and Macewicz,1985; Hunter, Lo, and Leong, 1985; Jiangang and Musick, 1991).

1.3 Gonadosomatic Index

Determination of maturity stages by visual examination using maturity keys always lacks precision because it relies upon subjective judgement (Holden and Raitte, 1974). The alternative practical way of achieving the general trends of reproductive activity, the gonad activity and spawning preparation of fish, with minimum cost and labour is to calculate a gonadosomatic index, which is the gonad weight relative to body weight. Gonadosomatic index (or gonad index) is the expression of the gonad weight as a function of body weight as $GSI = GW/BW \times 10^6$, where GSI is gonadosomatic index, GW is gonad weight, and BW is body weight (Holden and Raitt, 1974).

The expression of GSI in other literatures cited in this study, aparted from Holden and Raitte (1974), is a little difference as it is usually expressed gonad weight as a percentage of body weight.

1.4 Fecundity

Fecundity is usually defined as the number of ripening eggs found in female just prior to spawning (Bagenal, 1978). The knowledge of the fecundity of a species is an important factor in fish stock management as it is used to calculate the reproductive potential of the stock (Holden and Raitte, 1974; Bagenal, 1978). The fecundity together with the survival from egg to recruitment are used to determine

minimum adult stock necessary to maintain recruitment. A knowledge of fecundity and sex ratio of the adult stock is also needed to calculate stock size from estimate of annual egg production. A third use of fecundity data is discrimination between stocks in fisheries exploiting a mixture of two or more stocks with different fecundities.

Holden and Raitte (1974) mentioned the problems of estimating fecundity that it depended upon several factors: absolute number of eggs produced, type of spawners, and degree of differentiation between the size of eggs which will be spawned in that season and any immature eggs present which will be carried over to the next spawning season.

Hunter, Lo, and Leong (1985) illustrated fecundity estimation based on their study of northern anchovy. From their conclusions, the annual fecundity of partial-spawning fishes, which exhibit group-synchronous maturation of oocytes and multiple spawning, cannot be estimated from the standing crop of vitellogenic (yolked) oocytes because oocyte maturation is a continuing process and spawning batches are produced throughout the protracted spawning season. In such fishes, the oocytes usually occur in nearly all maturity stages, ranging in size continuously from unyolked to yolked oocytes. Of these fishes, identification of a predetermined annual spawning batch is impossible, and the only useful fecundity measurement is batch fecundity, the number of eggs produced in a single spawning batch. They stated that in anchovy and other fishes with indeterminate annual fecundity, the oocytes in active ovaries are typically distributed in 2 modes or more, each mode representing a single

spawning batch, and batch fecundity is needed to estimated fecundity in this type of fishes.

Watson et al. (1992), using the method of Hunter et al. (1985) to determine batch fecundity of mackerel, *Scomber scombrus* L., found that the total annual fecundity of this fish during a spawning season was produced by release of discrete batches of eggs and the total fecundity was then the product of the number of batches times the batch fecundity. Estimations of batch fecundity were also done in studies of the reproductive biology in many pelagic fishes of tropical and subtropical zones (Horwood, 1990; Jiangang, and Musick, 1990; Melo and Armstrong, 1991; Dickerson, Macewicz, and Hunter, 1992; Taylor and Murphy, 1992; Watson et al., 1992; Gartner, 1993; Nieland and Wilson, 1993; Taylor and Villoso, 1994; Clarke and Privitera, 1995).

The relationship between fecundity and length or weight is usually expressed in the form of a power function, i.e. fecundity = a . length^b or fecundity = a . weight^b, where a close relationship is usually found between fecundity and length (Bagenal, 1978). To linearize these relationships and to induce homogeneity of variance over the range of observations, Bagenal (1978) suggested performing log-transformation before data fitting using least squares regression, i.e. log fecundity = log a + b log length or log fecundity = log a + b log weight. These log-transformation relationships have been used in nearly all literature of reproductive biology.

Methods of Assessing Ovarian Development in Fishes

Mayer, Shackley, and Ryland (1988) pointed that the macroscopic examination of gonad development changes alone had its limitations. An important component of many studies of fish reproductive biology is the assessment of the stage of gonad development of individual fish (West, 1990). The procedures vary from rough to highly detailed. Visual staging by external appearence of the gonad is possibly the least certain but the most rapid. Histological examination is the most detailed but the most time-consuming. Between the two are measurement of oocyte size and determination of gonadosomatic index. Males have usually been excluded from the study because they are generally more difficult to stage than female, may give a less well defined estimate of the spawning season, and often show little change in gonad weight.

1. Histological Staging

In most studies, ovaries are classified by the most advanced type of oocyte present. Oocyte growth can be confined to the following stages based on West (1990).

1.1 Chromatin Nucleolar Stage

Teleost oocytes initially arise within the ovarian luminal epithelium. Prefollicle cells surround each oocyte resulting in complex buds off the germinal nest

as a primordial follicle. Each oocyte is surrounded by a few squamous follicular cells and has a large nucleus surrounded by a thin layer of cytoplasm. The nucleus contains a single large nucleolus.

1.2 Perinucleolar Stage

The nucleus increases in size and multiple nucleoli appear, generally at its periphery. The cytoplasm stains uniformly, although late perinuceolus oocytes may have vacuoles in the cytoplasm, the presence of which usually characterizes the yolk vesicle stage.

The chromatin nucleolar and perinucleolar stages are sometimes referred to as primary growth phase (Wallace and Selman, 1981; Forberg, 1982).

1.3 Yolk Vesicle Formation

This stage is characterized by the appearance of yolk vesicles in the cytoplasm. The yolk vesicles increase in size and number to form several peripheral rows. Oil droplets or fat vacuole begin to accumulate in the cytoplasm of oocytes in some teleosts, particularly marine species, about the same time as the yolk accumulates. However, no fat vacuoles exist in the oocytes of the eggs of northern anchovy *Engraulis mordax* (Hunter and Macewicz, 1985). The contents of these oil droplets are dissolved during dehydration with alcohols and appear empty with

conventional staining. The zona radiata (chorion) commonly appears at the yolk vesicle stage and the appearance of the zona radiata can vary between species.

1.4 Yolk Stage

This stage is characterized by the appearance of yolk proteins in fluid-filled spheres (yolk granules or yolk globules). The granules are very small when they first appear and are difficult to detect in a light microscope. The yolk globules may maintain their integrity throughout oocyte growth or fuse to form a continuous mass of fluid yolk (yolk mass), which gives these eggs their characteristic transparency. The fusion of yolk granules may begin soon after their initial formation or as late as final maturation.

1.5 Ripe Stage

Following appropriate hormone stimulation, oocytes develop to final stage leading to the release of them into ovarian lumen. The start of this stage is indicated by the peripheral migration of the nucleus and the dissolution of its membrane. The release of first polar body follows before the oocyte is ovulated into the lumen. Photomicrographs of polar bodies are rare in fisheries literature. In some species, coalescence of yolk granules or of lipid droplets or both accompanies the migration and dissolution of the nucleus making oocytes more transparent. In many marine teleosts, there is a further rapid increase in size due to hydration of the oocytes.

This process, which is especially pronounced among marine teleosts that spawn pelagic eggs, renders the eggs buoyant in sea water (Wallace and Selman, 1981).

Ovulation occurs after the fish has reached the ripe or hydrated stage resulting in ruptured empty or postovulatory follicles which are folded in the space left by the egg. New postovulatory follicles are readily identifiable, but they rapidly degenerate: within 2 days in northern anchovy (Hunter and Macewicz, 1985). At higher temperatures as in tropical zone, the resorption of the postovulatory follicles may be accelerated.

Following the final spawning, a small but significant number (2-10%) of yolked oocytes fail to undergo maturation or ovulation and subsequently degenerate and are resorbed, that is become atretic (Hunter and Macewicz, 1985; Mayer et al., 1988). At the onset of atresia, the oocyte breaks up. The follicle granulosa cells proliferate and hypertrophy to form a compact, vascularized structure. These active granulosa cells invade the oocyte through the broken down zonar radiata, digesting and resorbing yolky contents by active phagocytosis. The phagocyte granulosa cells is also degenerate, leaving behind a lightly staining fibrous mass surrounded by connective tissue elements.

2. Classification of Ovaries Based on Oocyte Size-Frequency Distributions

Analysis of oocyte size-frequency distributions has been widely used to assess the pattern of development. On the basis of oocyte size distributions, ovaries have been classified into 3 basic types (Wallace and Selman, 1981).

2.1 Synchronous Ovaries

All oocytes develop and ovulate in unison and there is no replenishment from earlier stages. Such ovaries are found in species that spawn once and then die. The oocyte size-distribution consists of a single mode.

2.2 Group Synchronous Ovaries

At least two distinct size groups of oocytes are present at some time, the larger group or clutch usually being more homogeneous than the smaller.

2.3 Asynchronous Ovaries

Oocytes at all stages of development are present at some time. The oocyte size-frequency distribution is continuous except in ripe ovaries where there may be a clear separation between the ripe and yolked oocytes.

Most species with asynchronous oocyte development protract their spawning seasons with multiple spawnings. However, some species with group synchronous development may also protract their spawning seasons, with the female spawning several times in a breeding season.

According to Holden and Raitt (1974), fishes with synchronous development oocytes are also referred as 'total' or 'isochronal' spawners: the whole clutch of developed oocytes is shed over a short period. Fishes with asynchronous oocyte development are referred as 'partial' or 'heterochronal' spawners: only part of yolked oocytes is spawned and they spawn over a protracted period.

Harris (1986) measured the diameters of fixed whole yolky oocytes of the Australian bass to determine whether the oocytes were developing synchronously or in a cyclic series of batches in each fish. He concluded that cyclic spawning teleost species were usually identifiable by the presence of modes in frequency distributions of yolky oocyte diameters or by the existence of consecutive oocyte developmental stages in various regions of the ovary.

As reviewed by West (1990), the oocyte measurement method is lessened in value for assessment of ovarian maturation if the developmental stage of these oocytes is readily ascertained by other methods. It is appropriate when the stage of development is not readily apparent from the appearence of the oocyte and when all stages of development are not found. However, this method is used in a number of

reproductive biological papers to assesss the type of spawning in the species including the recent work on the spawning of the anchovy *Engraulis capensis* (Melo, 1994a).

3. Gonadosomatic Index

From the study on the use of gonosomatic index (GSI) by DeVlaming, Grossman, and Chapman (1982), GSI is widely used as an index of gonadal activity and as an index for spawning preparation. However, GSI is an inaccurate mean of comparing gonadal activity between specimens of different sizes because the relationship of ovarian to body weights changes with the stage of oocyte development. To overcome this size-dependence for statistical comparisons, West (1990) suggested that the best method was to standardize the size of individuals by selecting a subset of data in which there was no significant difference in body size between samples. Gonad index had traditionally been used as an objective support for field staging, to provide a useful general indication of seasonal trends (West, 1990). He also pointed out the value of gonad index that it reflected the status of all oocytes in the ovary and provided a measure of development additional to that provided by staging methods or oocyte measurements, which were directed only at the upper limit of the oocyte size range.

Coblentz (1995), on his study on reproductive biology of the dwarf herring, stated that GSI's reflected the pattern of gonadal development and the depletion from spawning; the latter was assumed because each sex individual

appeared to have less turgid gonads as the spawning period progressed, the trend of which was confirmed by mean GSI values.

4. Macroscopic Staging

This refers to staging based on the external appearance of the gonad, especially the ovary and the oocytes within it, as viewed with naked eye. Many schemes devised for the study of gonad development have been designed for individual species, though some are intended to be general schemes. Holden and Raitte (1974) proposed an 8-point maturity scale for total spawners and a 5-point maturity scale for partial spawners. The following is a 5-point scale for partial spawners, the characteristic of typical tropical fishes.

4.1 Immature

Ovary and testis are about 1/3 length of body cavity. Ovary is pinkish and translucent while testis is whitish. Ova are not visible to naked eye.

4.2 Maturing Virgin and Recovering Spent

Ovary and testis are about 1/2 length of body cavity. Ovary is pinkish and translucent while testis is whitish, more or less symmetrical. Ova are still not visible to naked eye.

4.3 Ripening

Ovary and testis are about 2/3 length of body cavity. Ovary is pinkish-yellow with granular appearance while testis is whitish to creamy. No transparent or translucent ova are visible.

4.4 Ripe

Ovary and testis are from 2/3 to full length of body cavity. Ovary is orange-pink with conspicuous superficial blood vessels. Large transparent, ripe ova are visible. Testis is whitish-creamy and soft.

4.5 Spent

Ovary and testis shrink to about 1/2 length of body cavity. Walls are loose. Ovary may contain remnants of disintegrating opaque and ripe ova, darkened or translucent. Testis is bloodshot and flabby.

To define spawning seasons in tropical fish, Ntiba and Jaccarini (1990), categorized gonads into 6 stages: virgin, developing virgin or resting and recovering (matured), early developing, late developing, ripe, and spent. These categories have more details in staging between virgin (immature) stage and ripe stage in the way of examining the gradually increasing size and the blood vessels appear from internal to

external wall of the ovary. These criteria were used by N'Da and Deniel (1993) for their study of red mullet in the southern coast of Brittany.

From the conclusion of West (1990), the 'spent' ovary, in which egg production has ceased for the current season, presents particular problems in some fish, and distinguishing between degenerating and normal ripe eggs requires microscopical examination. The characters typically used to define a spent ovary are flaccid, empty, often blood shot in appearance, and containing a small number of remnant ripe eggs. In the species with asynchronous development, in which batches of eggs are produced throughout a prolonged spawning season like in the northern anchovy which reported by Hunter, Lo, and Leong (1985), the spent condition often difficult to detect, and in continuous spawners it may not occur at all. In other words, in asynchronous development, there are usually no external indications that spawning has occurred. The flaccid nature of the spent gonads may only be apparent in the field as this character is lost after they are preserved. In additions, detecting remnant ripe eggs usually be difficult since normally they are present in only small numbers (Hunter and Goldgerg, 1980).

Generally, the presence of fish with ripe gonad is used as an indicator of spawning season. In application, the presence of ripe oocytes, whether ovulated or not, is normally taken as an indication that spawning is imminent, especially if the late yolk stage oocytes can be distinguished from ripe oocytes and if degeneration (atresia) of ripe oocytes can be recognized (Hunter and Macewicz, 1985).

3. Determination of Spawning Season

3.1 Postovulatory Follicle Method

The postovulatory follicle method assesses the frequency of spawning based on the occurrence of follicles remained in ovary following ovulation of hydrated oocytes. For the studies on some anchovies, the criteria used for spawning frequency estimation are the incidences of postovulatory follicles.

Postovulatory follicles are used as the evidence for recent spawning of the fish in many studies. Alheit (1985) determined the spawning frequency of Peruvian anchovy *Engraulis ringens* by recording the incidence of postovulatory follicles. Hunter and Macewicz (1985), and Hunter et al. (1985) described the measurement of spawning frequency in multiple spawning fishes in natural using postovulatory follicle method, and stated that the structure of postovulatory follicle of teleosts was relatively similar to each other. From their studies, the new postovulatory follicle was very convoluted in shape with many folds or loops, a lumen containing some granular or particulate material, a definite granulosa epithelial cell layer lining the lumen, linearly arranged granulosa cells of cuboidal or columnar shape which contained a prominent nucleus, a definite thecal connective tissue layer with blood capilaries, and, most importantly, no degeneration of the follicle. The characteristics of degenerating postovulatory follicles are irregular shape that is smaller and much less convoluted than a new postovulatory follicle, with the presence

of a lumen the size of which is greatly reduced, the degeneration of granulosa cells (pycnotic nuclei, few cell walls, vacuoles usually present, and lack of alignment of nuclei), and an identifiable thecal layer. Hunter and Macewicz (1985) stated that the anatomical stages that seem to be equivalent to postovulatory ovaries were too ambiguous to be used to estimate spawning frequency. It might be possible using a laboratory calibration to identify some macroscopic characteristics that could be used to identify postovulatory ovaries for a short time after spawning.

This technique was also applied successfully to the African anchovy *Engraulis capensis* by Melo (1994b). He determined the spawning frequency of this anchovy using the incidence of female with postovulatory follicles described by Hunter and Macewicz (1985). Melo (1994b) pointed out that this method had advantages in that it could be carried out on fish taken at any time of day after spawning and obviated the need to correct for bias caused by actively spawning fish being more available to the trawl gear.

West (1990) stated that in asynchronous development ovary, which was the typical characteristic of tropical and sub-tropical fishes, there usually were no external indications that spawning had occurred. It can be observed internally or histologically by the presence of degenerating (atretic) ripe eggs in the lumen of ovary or the empty follicles as the indication of recent spawning.

3.2 Hydrated Oocytes Method

Hydration is the final stage of maturation characterized by a rapid secretion of fluid of low specific gravity into the advanced eggs by the granulosa cells of follicle making the volume or wet weight of the eggs increases 3 to 4 folds (Hunter et al., 1985; West, 1990). This fluid causes more or less complete fusion or solution of yolk granules producing the translucent appearence of hydrated eggs. Hunter et al. (1985), found that hydration begined about 12 hours before spawning in northern anchovy. They also concluded that ovulation and spawning soon followed completion of hydration in most clupeoids (anchovy, pilchard, sardines, and others).

Although the incidence of females with hydrated ovaries can provide a quantitative estimate of spawning activity in natural populations, Hunter and Macewicz (1985), by their study on northern anchovy, mentioned the three disadvantages of this method. First, sampling for the incidence of females with hydrated ovaries can be done only during a very limited time, whereas females with postovulatory follicles can be sampled at any time of day. The second possible disadvantage is that females with hydrated ovaries may be more vulnerable to trawls and perhaps other fishing gears than females in other reproductive stages causing bias on an spawning estimate based on hydrated ovaries. The third disadvantage of this method is that the incidence of females with hydrated ovaries is irregularly distributed among fish samples whereas females with postovulatory are not.

3.3 Gonadosomatic Index Method

Gonadosomatic index (GSI), the ratio of ovary weight divided by fish weight, can be used to detect hydrated ovaries since the wet weight of hydrated ovaries is 2 to 4 or more times that of other maturity stages. However, the GSI had the inherent problem of dividing by an expression of body size which did not compensate completely for the effects of fish size (Hunter and Macewicz, 1985). For the same reproductive state, small fish usually has a lower GSI than do larger fish, and this effect increases with maturation of the ovary. They concluded that this methodology alone cannot be used to accurately identify in multiple spawners either postovulatory female or post-spawning female, but it was possible that the GSI could be calibrated using histological detection of spawning frequency. This method, however, as stated by West (1990), provides a useful general indication of seasonal trends and has traditionally been used as an objective support for field staging.