Brief Communication

Gonad staging for tropical marine finfishes– Good practices and procedures

U. Ganga, M. Muktha, Swatipriyanka Sen, Shikha Rahangdale, Livi Wilson, K.M. Rajesh, G.B. Purushottama, V. Mahesh, Sujitha Thomas, Shubhadeep Ghosh and Shoba Joe Kizhakudan*
ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala

*E-mail: shoba.joe@icar.gov.in

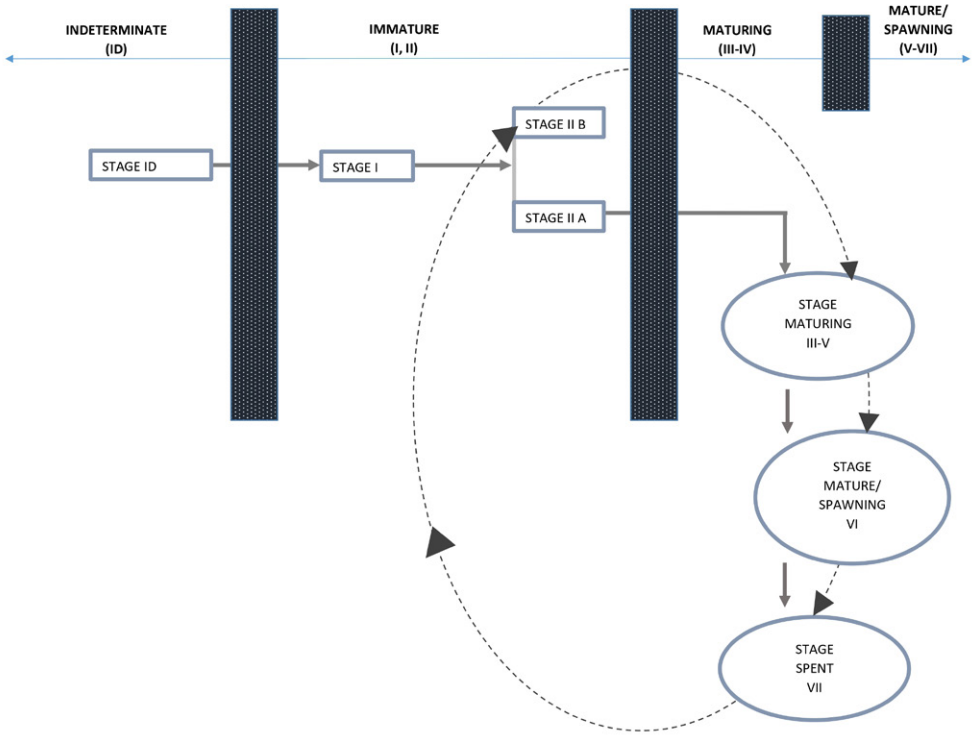
Introduction

Studies on fish reproduction are important as they are related to the regeneration and productivity of the fish populations. The long-term as well as short-term implications of knowledge base on fish reproductive biology for fisheries management, conservation and stock sustainability is increasingly recognized as also the need for collecting such data of various marine fishes with diverse reproductive traits and strategies. In wild capture fisheries information on fish reproduction is crucial in stock assessment exercises and fisheries management decisions that follow. Determining species-specific legal sizes of fish to prevent recruitment (caused by catching too many older fish) or growth (caused by catching too many juvenile fishes) overfishing; seasonal fishing regulations to protect fish spawners, estimating the spawning stock biomass and egg production potential to forecast fisheries production and determine catch limits, if necessary are common approaches to ensure sustainability. Fish breeding programmes in hatcheries also require information on various aspects of reproductive biology of concerned species, as it is related to sourcing of brooders as well as development of a captive broodstock. In all these cases, a simple, consistently used terminology for the various development stages of the fish gonads is essential for comparisons and validation of results across labs and timelines.

Finfish gonad staging

In fishery science, various disciplines typically describe reproductive process at different levels which require an understanding of the context in which they have been used. While whole-gonad development stages take priority in fisheries biology/aquaculture, studies on gamete development related to hormones, stress factors, genetics are more important in fish physiology studies. Fixing the criteria for a fish to be considered as a spawner, based on the cyclical gonad development milestones that apply to all fishes can bring more clarity to fishery biology studies. With specific histological and physiological markers, Brown-Peterson *et al.* (2011) identified and defined critical phases in the reproductive cycle of teleosts, irrespective of their phylogenetic placement, gender or reproductive strategy. Accordingly, fish enter the reproductive cycle (or become sexually mature) when it first becomes gonadotropin-dependant, which enables gonad growth and gamete development. Also, in fishes undergoing multiple spawning, once the fish has attained sexual maturity, it cannot exit the reproductive cycle but re-enters the gonad development cycle through a recrudescence (IIR) stage. Based on this concept, the proposed maturity classification for a sexually differentiated fish will be either of the following states: Immature (stage I & IIA); Maturing (stage III & IV), Mature / Ripe spawner (stages V & VI), Spent (stage VII) and Resting (II-B). The existing species-specific terminologies / stages have to be suitably aligned to this maturity state classification in studies on identification of spawning season, duration and related information.

Studies of fish maturity require large samples that can be easily and rapidly processed which leads to preference for macroscopic staging. While histological validation of the gonad stages is desirable, it is time consuming and expensive. Hence, except for certain species of specific interest, it is seldom employed on a routine basis for all fish species landed. The diagnosis of ovarian development stage at the macroscopic level (gonad appearance, size & colour, development of blood vessels on the gonads, egg size, whole oocyte appearance, gonado-somatic



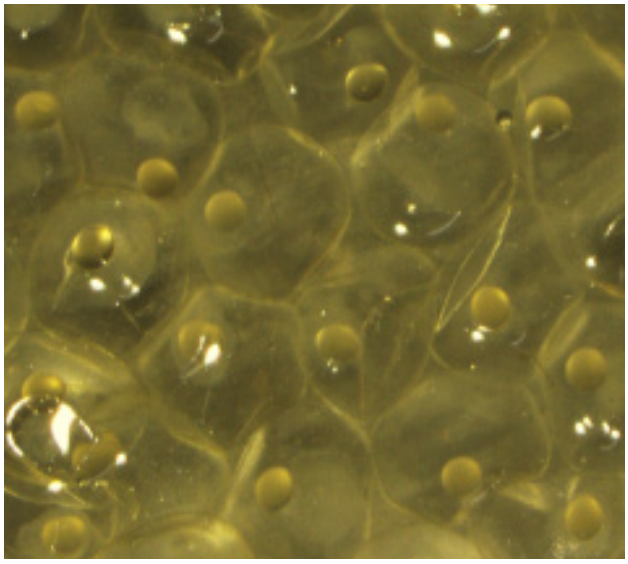
Maturation cycle in fin fishes

index etc.) can be supplemented by microscopic criteria whenever possible (Rhody *et al*., 2013). Most of the bony fishes (teleosts) are gonochoristic, with separate sexes. Initially there is no specific characteristic (Stage 0-Indeterminate/ Undifferentiated with no identifiable gonadal development) associated with the reproductive system. Only at a certain size, through hormonal chemical signaling, the gonadal tissue initiates and completes its differentiation to become physiologically either male or female and the gender of the fish is established. In multiple or continuous spawners, almost all stages of maturity occur in the population throughout the year and hence sampling schemes should ensure that the fishes selected for the analysis are representative of the population on a temporal and spatial scale. Any study that compares species specific spawning trends over time should carefully evaluate the classification scheme followed and ensure that the methodologies reported are uniform to allow such comparisons. Due caution must be exercised in interpreting the observations and reaching conclusions for such comparative studies based on historic databases and secondary information. Also, species specific validated gonad staging through macroscopic and microscopic methods should ideally be prepared for as many species as possible to aid interpretations of seasonal gonad maturation processes.

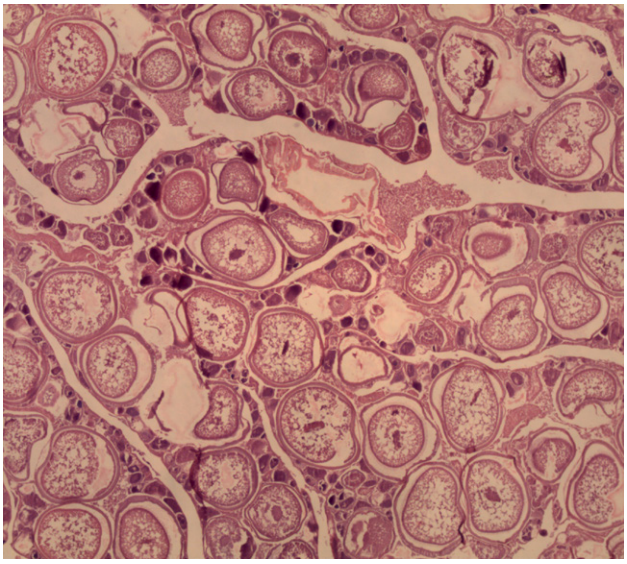
Table 1. Gonad stages of gonochoristic teleost fishes

Stage Number	Stage Descriptor	Macroscopic Stage description	Whole-oocyte appearance	Histology	Gonad identity
Stage 0	Indeterminate or Undifferentiated with no identifiable gonad development	No identifiable gonad	NA	NA	Indeterminate
I Immature	Immature	Small, thin ribbon like transparent ovaries, no blood vessels/ oocytes visible	NA	NA	"Immature"
II A (Immature)	Virgin Developing, never spawned juvenile	Ovaries enlarging, with signs of developing oocytes	oocytes small, transparent and visible under magnification only	Unyolked oocytes in PG phase	

Stage Number	Stage Descriptor	Macroscopic Stage description	Whole-oocyte appearance	Histology	Gonad identity
II R (Regenerating Adult)	Adult but reproductively inactive	Small ovaries with thick ovarian wall, blood vessels and residual eggs inside flabby ovary	Flaccid ovary	Unyolked oocytes in PG phase and more space and interstial tissue around PG oocytes, Post -ovulatory follicles and thicker ovarian wall.	"Resting"
III maturing	Early maturing	Ovaries firm with blood vessels, barely visible eggs present inside.	Oocytes become bigger, numerous small lipid droplets appear.	Oocytes with lipid droplets and cortical alveoli	"Maturing"
IV maturing	Late maturing	Ovaries change colour to deeper tones (red, orange), prominently visible eggs inside. Fully firm and opaque oocytes visible with naked eye, without magnification	oocytes with lipid droplets coalescing progressively	Oocytes with yolk deposition/ vitellogenesis stages (Vtg1 -3)	
V mature	Spawning capable fish (developmentally)	Turgid ovary filling the body cavity and containing ripe translucent oocytes	Oocytes with lipid droplets coalesced and generally, a single oil droplet present	Oocyte maturation process as indicated by germinal vesicle migration and larger oil droplets through coalescence	"Spawning"
VI Ripe/Spawning	Spawning capable fish (developmentally as well as physiologically)	Turgid ovary with transparent oocytes oozing out with slightest pressure	Hydrated oocytes which look transparent	Oocytes with single clear oil globule	
VII Spent (Partially/fully)	Regressing ovaries with cessation of spawning	Flaccid ovaries with prominent blood vessels. Oocytes are few or absent	No hydrated oocytes, post-ovulatory follicles and atretic eggs present	Post-ovulatory follicles and occassionally pre-vitellogenic eggs present.	"Spent"



Hydrated oocyte of a ripe stage cutlass fish *Lepturacanthus savala*



Mature gonad of *Rachycentron canadum* with oocytes in different stages of maturation

Data collection and processing

For following the maturity stages, regular fish samples (monthly, fortnightly or preferably weekly), representative of the population must be sampled. Gonads are to be staged based on macroscopic appearance (colour, shape, size in relation to body cavity, oocyte development stage/diameter etc) with species-specific microscopic (histology sections of gonads) validations, wherever possible. Histology based validation will include oocyte characteristics such as the formation of cortical alveoli, degree of yolk accumulation and nuclear migration in females and the presence/absence and relative proportion of spermatogonia, spermatocytes and spermatozoa in males. For the estimation of spawning season, only the gonads in stage V & VI among the adult, spawning capable females (in Stages III, IV, V, VI and VII) is to be considered (Table 1). Immature fishes that have not entered the reproductive cycle (stage I & II) and spent-recovering adults (IIR stage) with gonads superficially resemble that of a juvenile fish are not included. Based on the monthly

percentage of mature fish /spawners, the spawning period and the peaks can be identified based on the formula below.

$$\text{Mature spawners} = \frac{\text{Stages (V+VI)}}{\text{Stages (III+IV+V+VI+VII)}} \times 100$$

Gonad staging in hermaphroditic fishes

Hermaphroditism, defined as the presence of the male and female function (i.e., sperm and egg production, respectively) in the same individual, occurs either sequentially or simultaneously in 34 teleost fish families comprising 370 species. The transition of the functional gonad from one sex to another involves not only the morphological changes in the gonads, but also social and behavioural changes which is manifested as species specific reproductive strategy. Sex change definitions include protandry (individual initially male changes into female later) or protogyny (from female to male) which is the category of "sequential hermaphroditism" and in some cases, bidirectional (individual switches between male and female) which is in the category of "simultaneous hermaphroditism". In the latter, there may be no clear demarcation between the male and female gonadal tissue or a clear demarcation separating the two regions within the ovary (ovotestes), with either male or female tissue dominating at any particular time in a mature fish (Adolfi *et al*., 2023). Sequential hermaphroditism is explained by a model that predicts sex change occurs when reproductive success of one sex increases more rapidly with size (or age) than for the other eg, reef fishes like serranids, polynemids and sparids. The 'simultaneous hermaphroditism" model suggests that it is associated with low probability of finding a partner and associated reproductive success in certain environments and is common in several deep-sea fish families including lancetfishes (Alepisauridae) and greeneyes (Chlorophthalmidae).

Hermaphroditism is most common in perciform fishes of the families Epinephelidae, Latidae, Lethrinidae, Polynemidae, Pomacentridae and Sparidae. Estimating the spawning season for a sequential protogynous hermaphrodite such as *Epinephelus diacanthus* where female changes sex into male in the early year classes (< 2 years), followed by transitional stage (2-5 years), and males become dominant in higher year classes (> 5 years). Spawning season identification will involve assessment of monthly gonadosomatic index (GSI) of the reproductively capable female (stage III onwards) as monitored throughout the year. The months with higher GSI values and highest percentage of females with ripe gonads (stage V & VI oocytes) among the fishes in stage III and above, can be considered as the spawning season of such species.

Gonad staging in fishes showing parental care

In catfishes, parental care and males incubating eggs in their buccal cavity are indicators of their spawning activity and onset of spawning season. However, they are rarely encountered in commercial catches as possibility of dislodging during capture of the specimens in gears like trawls is high. Peak spawning season of marine catfishes based on macroscopic gonadal staging in female specimens into six identifiable stages based on Vazzoler (1981) is recommended as follows.

Stage 1: Immature

Ovary small, slender & thread-like. The gonads occupancy is less than 1/3rd of the abdominal cavity. The ovary appears whitish or translucent. Oocytes are barely visible to naked eye.

Stage II: Early maturing

Ovary slightly enlarged with occupancy of ½ of the abdominal cavity. Gonads with marginal granulation and oocyte are visible to the naked eye. The ovary appear white to cream colour.

Stage III: Late Maturing

Ovary enlarged especially in anterior portion occupying 2/3rd of the abdominal cavity. Prominent oocyte with whitish yellow colour.

Stage IV: Ripe

Ovary enlarged especially in anterior portion occupying over 2/3rd of the abdominal cavity with prominent presence of blood vessels. Prominently large oocyte with golden yellow colour.

Stage V: Spent

Ovary flaccid and wrinkled with hemorrhagic appearance. The ovary occupying ½ of the abdominal cavity. Heterogeneous in color: some bright colour and some pale cream/whitish.

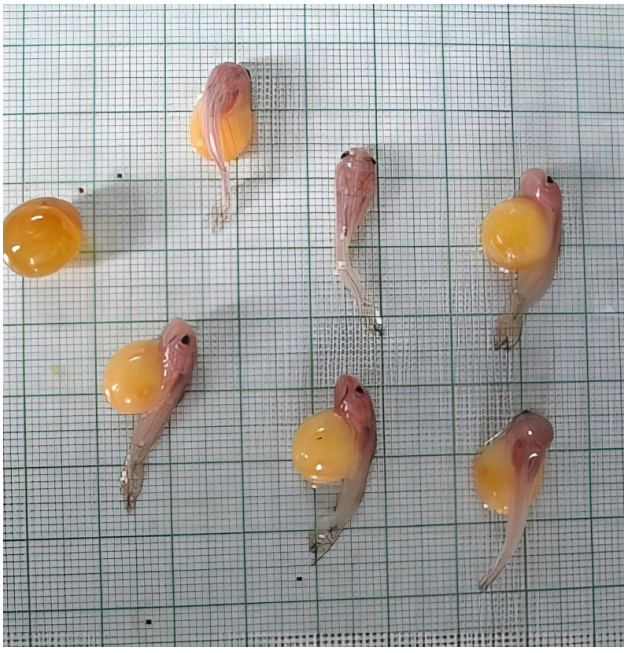
Stage VI: Recovery

Ovary marginally enlarged, ovary occupying > ½ of the abdominal cavity. Oocyte cream to brown colour

$$\text{Monthly mature \%} = \frac{(III + IV)}{(II + III + IV + V)} \times 100$$

and peak spawning season as months with higher presence of fully mature specimens can be used to identify peak spawning season.

Ripe eggs of *Plicofollis layardi*



Larvae collected from incubating males of *P. layardi*

Fertilized eggs collected from incubating males of *P. layardi*

Gonad staging in elasmobranchs

Elasmobranchs are well known for their wide range of reproductive strategies i.e., viviparous, ovoviviparous and oviparous species; viviparous species are further recognized as exhibiting placental and aplacental viviparity. In viviparous species, embryos develop inside the female's body, and they receive nutrients directly from the mother through a specialized structure called a placenta. Ovoviviparity is an intermediate reproductive strategy where eggs develop and hatch within the female's body, but the embryos rely on yolk sacs, not a placenta, for nourishment. Oviparous elasmobranchs lay eggs. The developing embryos rely on the yolk sac for nourishment until they hatch. Variations among elasmobranchs in the reproductive mode, and the period between consecutive birth events and laying of egg clutches make it difficult to determine the exact maturity stages (Walker, 2005). The meaning of the term "maturity" in recent elasmobranch literature ranges from defining the onset of maturation to the period of time when a female elasmobranch undergoes parturition and produces a litter of pups. Since in many elasmobranch species the period between the beginning of the maturation process until pupping can take some years, it is important to define the term "maturity" in an elasmobranch reproductive study

(Conrath, 2005). The sexual maturity stages in females considered for identifying the reproductive seasons are estimated following the criteria of ICES, 2010 and Serra-Pereira *et al.* (2011) prescribed for viviparous and oviparous species. Females are identified as mature or immature by examining the physical and anatomical condition of ovary with the ova, uterus and oviducal glands as described in the Tables 2&3. The females are considered mature if they are ready to reproduce within a short period of time with the evidence of a current or previous pregnancy. In females that are not pregnant, maturity stages are determined by assessing the ova condition in the ovary, uterus condition and the size of oviducal gland (Conrath, 2005).

Viviparous and ovoviviparous elasmobranchs

For viviparous and ovoviviparous species (Table 2), females in stage 3a or above are considered as mature (ICES, 2010). Mature females have well developed yolky eggs in the ovary, developed oviducal gland and expanded uteri. For estimation of breeding season, we have considered the individuals with stage 3c and 3d where we can determine the timing of the reproductive event by directly observing the reproductive tract and tracking the size of the ovarian eggs (ovarian cycle) and the pups within the uterus (gestation cycle) over the months. The resting phase is determined by comparing the timing of the ovulation and gestation cycles following Conrath, 2005. The mean maximum ova diameter (MOD) is estimated for each sampling month and based on the size of ova diameter the peak ovulation season is calculated. Further the timing and length of gestation of viviparous species is determined by following the size of eggs and embryos found within the uterus through time. The gestation time is also determined by observing the size of embryo at birth and the timing of birth.

The parturition time for the elasmobranchs is considered as the spawning period. The parturition time is estimated by observing the time between the observed females with the largest uterine embryos and smallest uterine eggs. The exact size at birth is validated from data on the smallest free-living individuals observed throughout the year in the fishery. The months with an increase in the number of pregnant females carrying near-term embryos and preparing to give birth and followed by a rise in the number of postpartum females are considered as the parturition months. Also, the month with higher mean size of the embryos in the uterus is also considered as major parturition month for the species.

Monthly maturity percentage for viviparous and ovoviviparous species is calculated as:

$$\text{Monthly mature \%} = \frac{\text{Stages (3c+3d)}}{\text{Stages (3a+3b+3c+3d+4a)}} \times 100$$

Table 2. General classification of gonadal stages for viviparous and ovoviviparous elasmobranchs

Females

Stage	Maturation state	Maturity classification	Gonad characteristics	
			Ovary	Uterus and Oviducal gland
Stage 1	Immature	Immature	Ovaries small and whitish in colour, with undistinguishable ovarian follicles.	Uteri very small in size, narrow, thread like and flaccid. Oviducal glands not visible.
Stage 2	Maturing/ Developing	Immature	Ovaries enlarged with different sizes of follicles sometimes present in anterior part of the ovary and small yolked ova filled in ovary,	Uteri begin to enlarge but mostly thin and flaccid and oviducal glands begin to develop.
Stage 3a	Mature, capable to produce, non-pregnant	Mature	Mature ovaries containing fewer visible enlarged yellow coloured follicles/ova of all same sizes, along with smaller maturing white-yellow oocytes.	Uterus developed but not dilated and without yolky matter and embryo. Oviducal gland developed.
Stage 3b	Maternal, early pregnant	Maternal/Pregnant	Ovaries filled with eggs	Uteri with yolked fertilized eggs and general segments cannot be distinguished and small embryos cannot be observed. Oviducal gland size increases.
Stage 3c	Maternal, mid-pregnant	Maternal/Pregnant	Ovaries filled with eggs	Uteri well filled and rounded often with visible segments and embryos are small in size and visible with relatively a large yolk sacs. Oviducal gland further increase in size.

Females

Stage	Maturation state	Maturity classification	Gonad characteristics	
Stage 3d	Maternal, late pregnant	Maternal/Pregnant	Ovaries with less number of eggs	Uteri with fully developed embryos having reduced yolk sacs or absent. Embryos reach a measurable length and sexed.Oviducal gland fully enlarged.
Stage 4a	Regressing	Mature (spent)	Ovaries shrunk with small amount of eggs and degenerating follicles.	The oviducal glands start to reduce in diameter. Very enlarged uteri, reddish in colour, flaccid and empty having recently released young ones.
Stage 4b	Regenerating	Mature (resting)	Ovary with small follicles in various stages of development along with the presence of degenerating follicles.	Uterus enlarged and flaccid. Oviducal glands very small but distinguishable.

Table 3. Maturity stages classification for oviparous elasmobranchs

Females

Stage	Maturation state	Maturity	Gonad characteristics	
			Ovary	Uterus and Oviducal gland
Stage 1	Immature	Immature	Ovaries small and whitish in colour, without distinguishable follicles.	Uterus narrow, thread- like and flaccid. Oviducal glands are absent.
Stage 2	Maturing/Developing	Immature	Ovaries enlarged with small yellow follicles, sometimes present in anterior part of the ovary.	Uterus enlarged, mostly thin and flaccid and oviducal glands developing.
Stage 3	Mature, non-pregnant/ Spawning capable	Mature	Large mature ovaries containing large follicles/ova of all same sizes,	Uterus and oviducal gland well developed.
Stage 4	Mature, early pregnant/ Actively spawning	Mature/Pregnant	Ovary filled with eggs,	Both uteri with yolked fertilized eggs. In some cases, egg capsules present in the uterus and may attach or not attached to oviducal gland. Capsules may be fully developed, dark in colour, hard / begin to develop. Oviducal glands may contain fertilized yolked eggs.
Stage 5	Mature, Spent/ Regressing	Mature (spent)	Ovaries large with few follicles not covering entire surface.	Uterus and oviducal gland enlarged.
Stage 6	Recovering/ Regenerating	Mature (resting)	Ovary with small follicles in various stages of development.	Uterus and oviducal gland enlarged.

Oviparous elasmobranchs

For the oviparous species, the spawning season is determined by considering only actively spawning females using individuals greater than the minimum length at maturity and looking at the ovaries and oviducal conditions (Table 3). The presence of developing or fully developed hard thickened egg capsules attached to the uterus or to the oviducal glands indicates oncoming parturition time. The months with an increase in the number of pregnant females carrying hard thickened egg capsules attached to the uterus or to the oviducal glands and followed by a rise in the number of postpartum females are considered as the parturition months. These parturition months are considered as the spawning months or season for the species (Serra-Pereira *et al.*, 2011)

Monthly maturity percentage for oviparous species is calculated as:

$$\text{Monthly mature \%} = \frac{\text{stage (4)}}{\text{stages}(3+4+5)} \times 100$$

References

Adolfi et al., 2023. *Sexual Development*, 17: 84–98. DOI: 10: 1159/000526008

Brown-Peterson, N.J. *et al.*, 2011. *Marine and Coastal Fisheries*, 3(1): 52-70.

Conrath, Christina L. 2005. *Reproductive biology*.

ICES. 2007. Report of the workshop on sexual maturity sampling (WKMAT).

ICES. 2010. *Report of the Workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL),* pp. 48,32.

Lubzens, E. et al., 2010. *General and Comparative Endocrinology*, 165: 367–389.

Qasim, S.Z. 1973. *Indian J. Fish.*, 20(1): 166 -181.

Rhody, N.R. et al., 2013. *Transactions of the American Fisheries Society*, 142(4): 979-988.

Serra-Pereira, B. et al., 2011. *Mar Coast Fish: Dyn Manag Ecosyst Sci.,* 3:160–175.

Walker, T. 2005. *Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimeras,* p. 81–127.