An Improved and Simplified Terminology for Reproductive Classification in Fishes[*]

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As the number of fish reproductive studies has proliferated, so has the number of gonadal classification schemes and terms. This has made it difficult for managers and scientists to communicate and for comparisons to be made between studies. We propose the adoption of a simple, universal terminology for the phases in the reproductive cycle that can be used with all male and female elasmobranch and teleost fishes. These phases were chosen because they define key milestones in the reproductive cycle representing critical parameters such as size at maturity, duration of spawning season, location and diel periodicity of spawning, and fecundity. The phases we propose include: Immature, Developing, Spawning Capable, Actively Spawning, Regressing and Regenerating. Although the histological criteria identifying each phase may vary for different species and phases may not always occur sequentially, each phase is conceptually universal. The Immature phase can only occur once. The Developing phase signals entry into the gonadotropin-dependent stage of oogenesis and spermatogenesis and gonadal growth. The Spawning Capable phase indicates fish that will spawn this season because development within ovaries (fully grown vitellogenic oocytes) or testes (spermatozoa in lumens/ducts) is sufficiently advanced. Actively Spawning females are those that show recent evidence of spawning (i.e., hydrated or ovulated oocytes). Females of many species cycle between the Spawning Capable and Actively Spawning phases during the reproductive season and these phases are necessary to determine fecundity, spawning frequency, location and diel periodicity. Spawning Capable and Actively Spawning phases are difficult to differentiate histologically in males. The Regressing phase indicates fish that are completing the spawning season. Fish in the Regenerating phase are sexually mature but reproductively inactive. We show how researchers can incorporate species-specific histological criteria or classes within each of the universal phases, allowing more specific divisions yet preserving the overall reproductive terminology for comparative purposes.

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Phase	Previous Terminology	Female	Male
I**mmature** Never spawned	Immature, Virgin	Only oogonia and primary growth oocytes present, including chromatin nucleolar and perinucleolar oocytes. Usually no atresia.	Small testes, only primary spermatogonia, no lumen in lobules.
Developing Gonads beginning to develop, will not spawn soon	Maturing, early developing, early maturation, ripening, previtellogenesis	Oocytes in the following stages may be present: primary growth, cortical alveolar, and/or early vitellogenic. No evidence of POFs. Some atresia can be present. *Fish with determinate fecundity:* mid-vitellogenic oocytes present.	Initiation of spermatogenesis and formation of spermatocysts. Secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa can be present in spermatocysts. Spermatozoa not present in lumen of lobules or sperm ducts. Germinal epithelium continuous throughout.
Spawning Capable Fish will spawn in this cycle (or season).	Late developing, late maturation, late ripening, gravid, vitellogenesis	Vitellogenic oocytes present. Some atresia and old POF may be present. *Determinate fecundity:* Late vitellogenic oocytes, fecundity estimates made in sub-phase with no POF. *Indeterminate fecundity:* Mid and late vitellogenic oocytes prevelant (with or without evidence of previous spawning, POFs); or early vitellogenic oocytes with evidence of previous spawning (POFs). Less-developed oocytes often present.	Spermatozoa in lumen of lobules and/or sperm ducts. All stages of spermatogenesis (spermatogonia, spermatocytes, spermatids) can be present. Spermatocysts throughout testis. Germinal epithelium continuous or discontinuous. Macroscopically, testis large but milt not released with gentle pressure. Histologically undistinguishable from Actively Spawning phase.
Actively Spawning Imminent, active or recent spawning.	Ripe, running ripe, FOM, spawning	Ovulating (spawning) or approximately 12 hr prior to or after spawning as indicated by either GVM, GVBD/hydrated oocytes, or POFs < ~12 hr old. Atresia of late vitellogenic/hydrated oocytes may be present. *Indeterminate fecundity:* Less-developed oocytes often present. Fecundity and spawning frequency estimates made in 12 hr pre- and post-spawn sub-phases.	Spermatozoa in lumen of lobules and/or sperm ducts. All stages of spermatogenesis (spermatogonia, spermatocytes, spermatids) can be present. Spermatocysts throughout testis. Germinal epithelium continuous or discontinuous. Macroscopically, milt freely flowing with gentle pressure. Histologically undistinguishable from Spawning Capable phase.
Regressing Cessation of spawning	Spent, regression, post spawning, recovering	Atresia present (any stage). Vitellogenic oocytes undergoing alpha or beta atresia common. Less-developed oocytes often present. POFs may be present.	Residual spermatozoa present in lumen of lobules and sperm ducts. Widely scattered spermatocysts containing spermatids. Spermatogonial proliferation and regeneration of germinal epithelium common in periphery of testis. Macroscopically, testes small and milt not released with pressure.
Regenerating Sexually mature, reproductivel y inactive	Resting, regressed, recovering	Only primary growth oocytes present, including chromatin nucleolar and perinucleolar oocytes. Muscle bundles, enlarged blood vessels, thick ovarian wall and/or gamma, delta atresia, may be present.	Testes small. No spermatocysts. Lumen of lobule small or nonexistent. Proliferation of primary, occasionally secondary, spermatogonia throughout testis. Residual spermatozoa occasionally present in lumen of lobules and sperm duct.

Table legend: General description of the phases in fish reproductive cycles. Timing within each phase is species-dependent. Previously developed histological classification schemes for individual species may be added as sub-phases within each of the defined phases. Some criteria listed for phases may vary depending on species, reproductive strategy or water temperature.

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Abstract

Accurate determination of the reproductive state of fishes is crucial for understanding reproductive potential and spawning seasonality. Histological inspection of gonadal tissue is the definitive method for accurately identifying both the reproductive class and if the fish has recently spawned. Unfortunately, there is a plethora of terminology in use to describe various reproductive classes in fishes, such that comparison among studies and communication among researchers and managers is often difficult and confusing. We propose the adoption of a simplified, universal reproductive classification system based on phases in the reproductive cycle that can be used with all male and female elasmobranch and teleost fishes: Immature, Developing, Spawning Capable, Actively Spawning, Regressing and Regenerating. The Developing phase signals the entry into the gonadotropin-dependent stage of oogenesis and spermatogenesis. Spawning Capable females are those with fully developed ovaries (late vitellogenic oocytes), but no histological evidence of recent spawning. Spawning Capable and Actively Spawning males have spermatozoa in the lumen of the lobules and/or sperm ducts and are not distinguished histologically. Actively Spawning females are those that show histological evidence of spawning within a 12 h period (i.e., females undergoing final oocyte maturation, hydrated and ovulated oocytes, or POF < 12 h). Batch spawning females cycle between the Spawning Capable and Actively Spawning phases during the reproductive season. Regressing fish are found at the end of the reproductive season and are not spawning capable (females with predominance of vitellogenic oocytes undergoing alpha or beta stage atresia, males with testes undergoing minimal spermatogenesis and shrinking in size). Fish in the Regenerating phase have only primary growth oocytes or spermatogonia and are sexually mature but reproductively inactive. Researchers can incorporate their own classes within each of the new terms, allowing more specific divisions (sub-phases) based on histological observations yet preserving the overall classification terminology for comparative purposes.

Introduction

There are a number of terms and classifications used in the literature to describe the reproductive biology of fishes, resulting in confusion and a hindrance in communication among researchers in fish-related disciplines. This proliferation of terminology is often due to the needs of various disciplines to describe reproductive processes on either the macroscopic or microscopic level. For instance, classification of ovarian condition has been based on methods such as 1) the macroscopic (external) appearance of the gonad, 2) whole oocyte size, 3) the gonadosomatic index, and 4) histology (West 1990). Furthermore, gonad class names apparently are chosen based on either the frequency with which they are used in the literature or how descriptive they are of the process being identified (i.e., developing, spawning, regressing), and thus are somewhat subjective and inconsistent among studies. Unfortunately, attempts to introduce standardization and consistency into reproductive classification (i.e., Pollard 1972, Hilge 1977, Bromley 2001) have met with limited to no success in the past, no doubt due to researcher's reluctance to adopt an unfamiliar nomenclature that may not be the best "fit" for the species they are working with. Therefore, we suggest that rather than erecting a new classification system, communication among scientists studying fish reproductive biology can be improved by describing and naming the major phases within the fish reproductive cycle. These phases would act as a framework within which individual classification schemes can then be integrated.

Purpose

1. To describe and name the major phases within the annual reproductive cycle of fishes,
2. To demonstrate the applicability of this system to fishes with varying reproductive strategies, and
3. To show how existing classification systems can fit under the umbrella of this new terminology.

Methods

This terminology was developed during the Third Workshop on Gonadal Histology of Fishes, held in New Orleans, Louisiana, in July 2006. The conceptual Reproductive Phases Cycle was developed through discussions among 35 fish reproductive biologists at the workshop. Specific histological criteria presented for each phase, as well as descriptions of varying reproductive strategies, is a collaboration among the six person Terminology Committee formed at the workshop. Each phase in the reproductive cycle is illustrated by five species with differing spawning strategies.

Reproductive terms used in this poster (from Murua and Saborido-Rey 2003)

1) **Number of breeding opportunities in lifetime**
   a. Iteroparous (multiple spawning events)
   b. Semelparous (single spawning event)
2) **Sexuality**
   a. Gonochoristic
   b. Hermaphroditic (sex change)
3) **Egg Production (prior to spawning season)**
   a. Determinate (all eggs produced prior to season)
   b. Indeterminate (eggs continually recruited)
4) **Oogenesis**
   a. Synchronous (all oocytes undergo gametogenesis simultaneously
   b. Asynchronous (multiple stages of gametogenesis in same ovary)
5) **Egg release**
   a. Total (all eggs released simultaneously)
   b. Batch (eggs released in batches throughout spawning season).

Females

Immature (fish has never spawned): Only oogonia and primary growth oocytes present, including chromatin nucleolar and perinucleolar oocytes; usually no atresia. **Previous terminology:** virgin

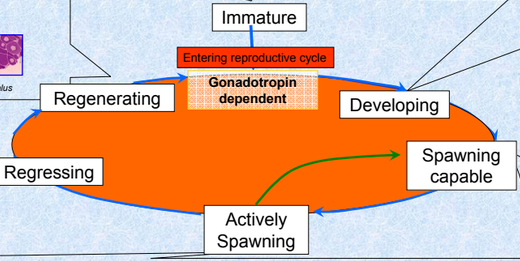
Batch spawner/indeterminate: *Cynoscion nebulosus* (Sciaenidae), *Sardinops sagax* (Clupeidae)
Batch spawner/determinate: *Gadus morhua* (Gadidae)
Total spawner: *Clupea herrengus* (Clupeidae), *Mugil cephalus* (Mugilidae)

Regenerating (sexually mature, reproductively inactive): Only primary growth oocytes present, including chromatin nucleolar and perinucleolar oocytes. Muscle bundles, enlarged blood vessels, thick ovarian wall and/or gamma, delta atresia may be present. **Previous terminology:** resting, regressed, recovering

Batch Spawner/indeterminate: *C. nebulosus*, *S. sagax*
Batch Spawner/determinate: *G. morhua*
Total Spawner: *C. herrengus*, *M. cephalus*

Regressing (cessation of spawning): Atresia present (any stage). Vitellogenic oocytes undergoing alpha or beta atresia common. Less-developed oocytes often present. POFs may be present. **Previous terminology:** spent, regression, post spawning, recovering

Batch Spawner/indeterminate: *C. nebulosus*, *S. sagax*
Batch Spawner/determinate: *G. morhua*
Total Spawner: *C. herrengus*, *M. cephalus*



Developing (gonads beginning to develop, will not spawn soon): Oocytes in the following stages may be present: primary growth, cortical alveolar and/or early vitellogenic. No evidence of POFs. Some atresia may be present. *Fish with determinate fecundity:* mid vitellogenic oocytes also present. **Previous terminology:** maturing, early developing, early maturation, mid maturation, ripening, previtellogenesis, recrudescence

Batch spawning/indeterminate: *C. nebulosus*, *S. sagax*
Batch spawner/determinate: *G. morhua*
Total spawner: *C. herrengus*, *M. cephalus*

Spawning capable (fish will spawn this cycle): Vitellogenic oocytes, POF and some atresia present. *Fish with determinate fecundity:* Late vitellogenic oocytes prevalent. *Fish with indeterminate fecundity:* mid-and late-vitellogenic oocytes prevalent (with or without evidence of previous spawning) or early vitellogenic oocytes with evidence of previous spawning (POFs). Less-developed oocytes usually present. **Previous terminology:** late developing, late maturation, late ripening, gravid, vitellogenesis.

Additional sub-phase useful for determinate spawners: Late vitellogenic oocytes only with no POF, upon first entry into Spawning Capable phase, to be used for potential fecundity determinations.

Batch Spawner/indeterminate: *C. nebulosus*, *S. sagax*
Batch Spawner/determinate: *G. morhua*
Total Spawner: *C. herrengus*, *M. cephalus*

Actively Spawning: Ovulating (spawning) or approximately 12 hrs prior to, or after spawning, as indicated by either germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), hydrated oocytes, or POFs < ~ 12 hr old. **Previous terminology:** ripe, running ripe, FOM, spawning

Spawning: Rupture of follicles and release of fully mature oocytes. Can include fish with hydrated oocytes (pelagic spawners), oocytes with complete yolk coalescence (demersal spawners) as well as fish with ovulated oocytes in the lumen.

Batch Spawner/indeterminate: *C. nebulosus*, *S. sagax*
Batch Spawner/determinate: *G. morhua*
Total Spawner: *C. herrengus*, *M. cephalus*

Additional sub-phases useful for Indeterminate Batch Spawners: Fecundity estimates are made ~12 h prespawning, when fish have oocytes undergoing lipid coalescence, GVM, GVBD, yolk coalescence or hydration. The oocyte developmental stage 12 h pre-spawning will differ depending on water temperature. The appearance and degradation of 12 – 24 h old POF immediately following spawning will differ depending on water temperature. Spawning frequency estimates are made during the ~12 h postspawning sub-phase.

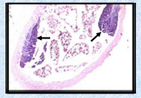
~ 12 h prespawn: *C. nebulosus*, *S. sagax*
~ 12 h postspawn: *C. nebulosus*, *S. sagax*

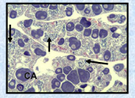
Batch Spawners

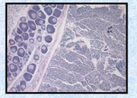
Many fish species release more than one batch of eggs during the reproductive season, and are referred to as batch spawners, multiple spawners, or fractional spawners. Batch spawning fishes can have an extended reproductive season (5-7 months) or a relatively brief spawning period (1-3 months) and oocyte development in the ovary can be asynchronous or group synchronous. Regardless of the spawning or oocyte development pattern, all fishes that release more than one batch of eggs during a single reproductive season will cycle between the Actively Spawning phase and the Spawning Capable phase (green arrow in diagram). At the end of the reproductive season, the fish enters the Regressing phase, indicating that no more batches of eggs will be matured and released. Fish that have spawned during the current reproductive season cannot return to the Developing phase, as this phase represents initial ovarian development prior to commencement of spawning. *Modification of the Reproductive Phase Cycle for multiple spawning fishes:* Once a multiple spawning fish has released her first batch of eggs, oocyte development and maturation for future spawning events takes place in the Spawning Capable phase. Fish in this condition have often been classified as partially spent, recently spawned, or redeveloping.

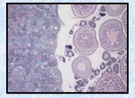
Hermaphroditism

Hermaphroditic fishes present a variation to the Reproductive Phases Cycle in that sexual transition (female to male or male to female) must be accommodated within progression among phases. Prior to sex transition in the protogynous *Centropristis striata* (Serranidae), the alamellar regions in female gonads contain bands of testicular precursor cells with a connection to the ovarian wall. The active proliferation of spermatogenic tissue (i.e., sex transition) occurs after the spawning season in *C. striata*, when females are reproductively inactive and in the Regenerating Phase. Increased degeneration of ovarian tissue coincides with the proliferation of testicular tissue. For *C. striata*, sex transition represents a link between the Regenerating phase in females and the Developing phase in males within the Reproductive Phase Cycle, a pattern that occurs in many other protogynous hermaphrodites. In contrast, some protogynous hermaphrodites such as *Epinephelus flavolimbatus* (Serranidae) undergo transition from female to male after females enter the Developing Phase, such that an individual will enter the Developing Phase as a female and exit that phase as a male. Many protandrous hermaphrodites, such as *Diplodus annularis* (Sparidae), begin sexual transition while males are still in the Spawning Capable/Actively Spawning phase, and complete transition to the female gender occurs in the Regressing phase. Simulataneous hermaphrodites, such as *Serranus scriba* and *Diplectrum formosum* (Serranidae), have gonads with both male and female tissues in the Spawning Capable phase, although typically the ovaries and testes are not both in the Actively Spawning phase at the same time. Simultaneous hermaphrodites do not undergo a sex transition stage.


Proliferation of spermatogenic tissue (arrows) in alamellar regions of Regenerating female *C. striata* during sexual transition.

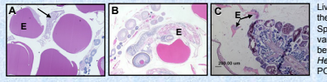

Proliferation of spermatogenic tissue (arrows) in Developing female *E. flavolimbatus* during sexual transition. CA—atretic cortical alveolar oocyte.


Spawning capable male *D. annularis* with ovarian tissue during sexual transition.


Simultaneous hermaphrodite *S. scriba* with both ovaries and testis in the Spawning Capable phase.

Fishes with Internal Fertilization

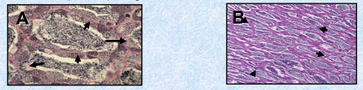
There are varying levels of embryonic development in fishes with internal fertilization, ranging from viviparity (release of free-living young) to zygoparity (release of fertilized eggs). Most sharks accumulate yolk in the oocyte but produce few oocytes per season, as full embryonic development occurs within the maternal reproductive tract. However, some species, especially those in the family Scorpaenidae, produce thousands of oocytes in each breeding season, following the same pattern as in the Reproductive Phases Cycle presented here. These oocytes are fertilized internally and undergo varying levels of embryonic development within the ovary until parturition. Thus, the length of the Actively Spawning phase in fishes with internal fertilization differs from the definition presented in the Reproductive Phases Cycle. This phase is longer than in species releasing unfertilized eggs because it includes the additional sub-phases of embryonic development and parturition.



Live bearing fishes from the family Scorpaenidae in the embryonic development sub-phase of the Actively Spawning phase. Embryos in the ovarian lumen are in various stages of development (species-specific) before parturition. A, B. *Sebastes mentella*. C. *Helicolenus dactylopterus*. E—embryo; arrow—POF.

Males

Terminology describing the reproductive phases of males is the same as that for females, with one important difference. It is difficult to histologically differentiate between Spawning Capable and Actively Spawning males, as both can have spermatozoa in the lumen of lobules and/or sperm ducts. The definitive method for differentiating these phases in males is to apply light pressure to the abdomen and look for any release of milt. In the absence of macroscopic observations, males should be considered Spawning Capable and able to actively participate in breeding. Most males will remain in the Spawning Capable phase for the duration of the spawning season. Males undergo dramatic changes in the abundance of spermatocysts and thus the amount of spermatogenesis occurring during the spawning season, as outlined by Grier and Taylor (1998) and further discussed by Brown-Peterson et al. (2002). The previously defined classes of early maturation, mid maturation, late maturation and regression, based on the appearance of the germinal epithelium rather than the presence/absence of spermatozoa (Grier and Taylor 1998), can all be placed as sub-phases within the Spawning Capable and/or Actively Spawning phases, thus providing additional information on the seasonal progression of spermatogenesis in a male that is capable of spawning for several months.



Histological variations in the male Spawning Capable/Actively Spawning Phases. **A**. *Rachycentron canadum* (Rachycentridae) undergoing active spermatogenesis with many spermatocysts (arrows) present. Classified as mid-maturation sub-phase following Grier and Taylor (1998). **B**. *Archosargus probatocephalus* (Sparidae) with reduced spermatogenesis and few spermatocysts (arrows) present. Classified as regression sub-phase following Grier and Taylor (1998).

Summary and Conclusions

- We propose standardized terminology describing the major phases of the reproductive cycle of all fishes to improve communication among scientists and thereby their ability to work collaboratively.
- Reproductive Phases terminology is applicable to males and females of all fish species, including marine teleosts, freshwater teleosts and elasmobranchs, and is based on histological criteria supplemented partly with macroscopic observations. This universal conceptual model will allow scientists to better highlight both the similarities and the differences in fish reproduction.
- Sub-phases can be added within each of the defined phases to accommodate species-specific differences in histology and reproductive strategies. Existing classification systems can be incorporated into these researcher-defined sub- phases.
- Key female differences addressed in this poster include batch versus total spawners, determinate vs indeterminate fecundity, sequential hermaphrodites, and fishes with internal fertilization.
- The amount of time a species spends in each phase is dependent on water temperature, physiology and reproductive strategy.
- Researchers in fish-related disciplines, including those working with fresh water species, are encouraged to apply and evaluate the Reproductive Phases terminology relative to the species they are investigating.

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