

Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes

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Abstract The literature presents a confusing number of macroscopic maturation scales for fish gonads, varying from over-simplified scales comprising three to four stages to highly specific and relatively complicated nine-stage scales. The estimation of some important life history traits are dependent on a correct assessment and use of the gonadal maturation scales, and frequent mistakes have been made in many studies. The goal of this report is to provide a synthetic, relatively simple, yet precise maturation scale that works for most oviparous teleost fishes. The synthetic scale proposed here is based on the correspondence between key physiological and cytological processes of gamete development and corresponding modifications observed at the macroscopic level. It is based on previous and ongoing studies of several fish species pertaining to some of the most important African and Neotropical taxa, including Characiformes, Siluriformes, Osteoglossiformes and Perciformes. This scale should allow for standardized protocols of field studies and improve

intra- and inter-specific comparisons of life history traits. Guidelines on the correct use of this scale to estimate these life history traits are provided.

Keywords Amazon fishes · Maturation scale · Oocyte · Ovary · Spermatogenesis · Testes · Vitellogenesis

Introduction

The estimation of fish basic life history characteristics, such as breeding season, age and size at maturity and fecundity, is fundamental to being able to make predictive generalizations on the responses of different species to environmental modification, understanding the adaptive responses of species to exploitation, guiding fisheries management, developing appropriate culture conditions, fueling general ecological studies at the community or ecosystem level and/or designing broad reproductive strategies in fishes (e.g. Winemiller 1989; Winemiller and Rose 1992). However, the precise determination of these life history characteristics relies on a correct estimation of the gonadal condition and maturity stages of the studied species, and this estimation relies on results from a combination of precise histological studies and extensive field observations. Although the gonado-somatic index (GSI) is often used instead of gonadal maturation stages to estimate the breeding

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season, this method lacks precision for multiple-spawners, where crucial stages, such as just spawned or recovering, cannot be distinguished from resting stages (Duponchelle et al. 1999). In addition, estimation of the size and age at first maturity requires a precise determination of gonadal maturation stages, whatever the method used for the estimation of the breeding period.

The physiological processes involved in the gonadal development of fishes vary according to their taxonomic status and reproductive guild (Balon 1975). For this reason, the study reported here did not focus on viviparous or ovo-viviparous teleosts or chondrichthyan fishes, but on oviparous teleost fishes, with most examples belonging to Neotropical Amazonian species. Oviparous teleost fishes can be separated into two groups according to their spawning strategy: the semelparous species, which have a single spawning event during their life time, such as some species of salmon (Crespi and Teo 2002), and the iteroparous species, which have several breeding events during their life time. Iteroparous species can be divided into two subcategories: (1) the annual single-spawners (i.e. species that reproduce only once during the breeding season each year), such as the large Amazonian characids *Colossoma macropomum*, *Piaractus brachipomus* (Loubens and Panfili 1997, 2001; Muñoz and Van Damme 1998), *Brycon* spp. (Gonçalves et al. 2006) or *Prochilodus* spp. (Loubens and Panfili 1995) and most large commercial catfishes, such as *Pseudoplatystoma* spp. (Muñoz and Van Damme 1998; Loubens and Panfili 2000; Brito and Bazzoli 2003); (2) the annual multiple-spawners (i.e. species that reproduce several times during the breeding season each year), such as cichlids *Cichla* spp. (Magalhães et al. 1996; Muñoz et al. 2006), some small characids, such as *Serrasalmus* spp. (Lamas and Godinho 1996; Teles and Godinho 1997), *Pygocentrus* spp. (Ferreira et al. 1996; Lamas and Godinho 1996; Teles and Godinho 1997; Duponchelle et al. 2007), *Tetragonopterus* spp. (Ricardo et al. 1998), *Hemigrammus* spp., *Moenkhausia* spp., *Roeboides* spp. (Bazzoli et al. 1997), *Apareiodon* spp. (Fonseca Ratton et al. 2003), *Schizodon knerii* (Ferreira and Godinho 1990), some small catfishes, such as *Loricariichthys* spp. (Suzuki et al. 2000), freshwater Engraulids (*Anchoviella vailanti*; Bazzoli et al. 1997) and Osteoglossiformes, such as *Osteoglossum* spp. and *Arapaima gigas* (unpublished data). Only a few examples have been provided

for each taxa, as a complete review of spawning strategies of Neotropical fishes would be outside the scope of this article. However, complementary information can be found in the literature (Lowe-McConnell 1964, 1969, 1987; Loubens et al. 1984; Loubens and Aquim 1986; Winemiller 1989).

This study does not aim at reviewing the gametogenesis in fishes nor the cytological processes of vitellogenesis, and excellent reviews already exist on these topics (see, for example, Selman and Wallace 1989; Tyler and Sumpter 1996; Sumpter 1997). Although this study did not aim at reviewing all of the existing maturation scales for teleost fishes, it must be pointed out that a confusing number have been published: from simple three-stage (Spadella et al. 2005), four-stage (Brito and Bazzoli 2003; Ravaglia and Maggese 2003) and five-stage (Perez-Vega et al. 2006; Yamaguchi et al. 2006) scales to more complicated ones with seven (Coward and Bromage 1998; Robillard et al. 2008), eight (Legendre and Ecoutin 1989; Poortenaar et al. 2001) or nine stages (da Silva et al. 2003). Rather, the aim of this study was to provide a synthetic, yet precise scale that could be used in most teleost fish species and to clarify the terminology found in the literature relative to oocyte or ovarian stages and their use for determining some life history traits. Our scale based on the key physiological processes occurring in developing gonads (estimated from histological analyses) and the associated crucial macroscopic changes that can easily be observed on the field.

Materials and methods

The scale presented here is based on the synthesis of data from previous and ongoing studies on several fish species pertaining to some of the most important African (Nunez Rodriguez et al. 1995; Duponchelle and Panfili 1998; Otemé et al. 1996; Duponchelle et al. 1999; Duponchelle and Legendre 2001; Duponchelle and Ribbink 2000), European (Nuñez Rodriguez et al. 1996; Mañanos et al. 1997; Le Menn et al. 2000; Ndiaye et al. 2006) and Neotropical taxa (Duponchelle et al. 2006; Muñoz et al. 2006; Nuñez et al. 2006a, b; Duponchelle et al. 2007), including the Characiformes, Siluriformes, Osteoglossiformes and Perciformes. Nevertheless, the illustrations presented in this study will concern only

Amazonian species for which histological or macroscopical illustrations have never been published.

The development of spermatogenesis and oogenesis was observed on histological sections of immature and mature males and females during a reproductive cycle and macroscopically on entire fresh gonads. *Colossoma macropomum* (923 specimens), *Piaractus brachipomus* (377), *Pygocentrus nattereri* (1464), *Pseudoplatystoma fasciatum* (763), *Pseudoplatystoma tigrinum* (273) and *Cichla pleiozona* (3237) were sampled in the Bolivian Amazon (Mamoré, Itenez, Béni and Madre de Dios basins) between 2001 and 2005. Arahuanas specimens (*Osteoglossum bicirrhosum*, 432 specimens) were captured in the Peruvian Amazon near Iquitos in 2007, and *Arapaima gigas* (20 specimens) were sampled at the IIAP's (Instituto de Investigaciones de la Amazonía Peruana) Quistococha facilities (Iquitos).

Macroscopic maturation stages were determined in the field on freshly captured specimens, and samples of gonads were also sampled and placed in Bouin's fluid for subsequent histological analysis. Length, total weight and gonad weight were also recorded.

Gonad samples were maintained in Bouin's fluid for 1 week and then dehydrated in a series of graded ethanol (70, 90, 96 and 100%) for 2 h. The dehydrated sample was then transferred successively through three series of xylene (xylene I, II and III) for 2 h and embedded in cytoparaffin. Gonads were sectioned (7- μ m thick slices), stained with hematoxylin–eosin and finally mounted with Canada's balm.

In the text and figures, the term “mature” will refer to fish that have reached sexual maturity, as opposed to “ripe” fish, which are ready to reproduce (as indicated by the developmental stage of their gonads and gametes).

Results

Ovogenesis–vitellogenesis

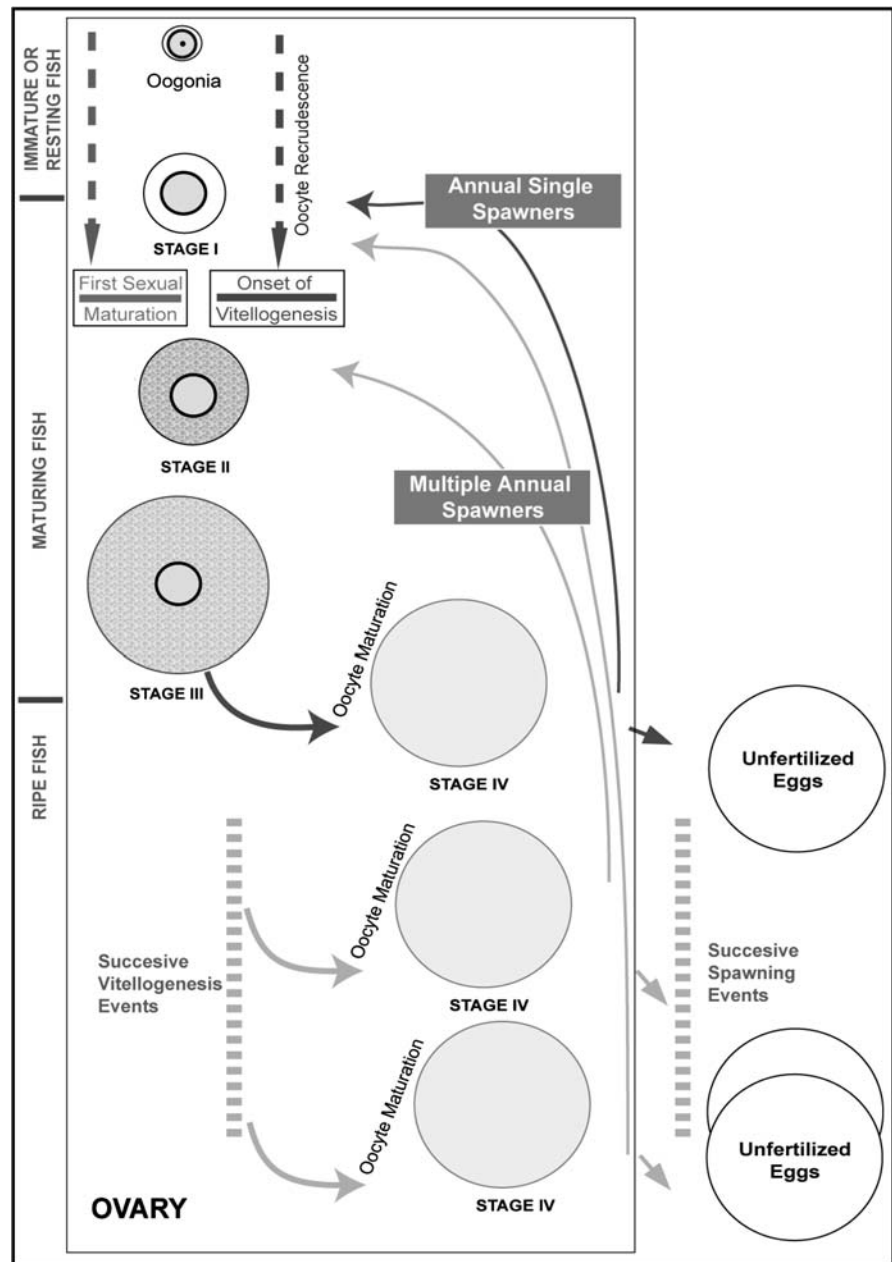
Oocyte characteristics during oogenesis and specific physiological stages have been divided into four stages (Fig. 1), and ovarian development has been divided into five stages according to macroscopic appearance (Fig. 2). Arabic numbering (1–5) in these figures refers to macroscopical ovarian stages, whereas Roman numbering (I–IV) refers to oocyte stages observed by

photonic microscopy. The species described in this study can be categorized into two groups depending on their spawning strategies: (1) the annual single-spawners (*Pseudoplatystoma*, *Colossoma*, *Piaractus*) and (2) the annual multiple-spawners (*Pygocentrus*, *Cichla*, *Arapaima*, *Osteoglossum*). Oocyte (Fig. 1) and ovarian dynamics (Fig. 2; Table 1) are similar in both single- and multiple-spawners until ovarian stage 3.

Stage I oocytes correspond to previtellogenic oocytes and are characterized by a small size (<150–200 μ m), a basophilic homogenous ooplasm, central or sub-central nucleoli and a high nucleoplasmic ratio (Figs. 3a, 4a, e). From a physiological point of view, fishes presenting such oocytes are either immature (ovarian stage 1; Figs. 2, 5a, 6a) or mature in the “resting” or recrudescence period (stage 5-1; Figs. 2, 6d). Nevertheless, on transversal sections, the diameter of the ovary and thickness of the ovarian wall are larger, the vascularization is more developed and the color is a darker pink or red in mature resting females (stage 5-1) than in immature ones (see below). Stage I oocytes are also visible in all other ovarian stages (Figs. 3d, f, 4b, e), but they represent 100% of the oocytes stages in a stage 1 ovary.

Stage II oocytes (Figs. 3d, 4a, b) indicate the onset of vitellogenesis (endogenous and exogenous) and are easily distinguished from stage I oocytes by the presence, in the peripheral ooplasm, of lipid droplets (endogenous vitellogenesis), cortical alveoli and small yolk granules that are sometimes difficult to observe at this stage (exogenous vitellogenesis). The average diameter increases, the nucleus envelope becomes crenellated, nucleoli are distributed at the periphery of the nucleus envelope and nucleo-plasmic ratio is reduced. These oocytes are typical of the macroscopic gonadal stage 2 (Table 1; Figs. 2, 3b, d, 4a, b, 6b). This ovarian stage is generally identified at its beginning by the appearance of small white or yellow spots visible to the naked eye that correspond to stage II developing oocytes (Fig. 6b). This ovarian stage clearly indicates that the vitellogenesis has started. At the end of stage 2, the ovaries are completely filled up with clearly visible oocytes of different sizes, whitish, yellowish or orange depending on the species. Owing to the different appearances it can take, ovarian stage 2 is sometimes divided into two or more distinct stages in the literature on a specific species (Casadevall et al. 1993; Giulianini et al. 1994; Jackson and Sullivan 1995; Grau et al. 1996; Coward and Bromage 1998; Arocha 2002).

Fig. 1 Diagram of a simplified fish maturation scale for females considering four oocyte development stages. *Stage I* previtellogenic, *stage II* early vitellogenic, *stage III* late vitellogenic, *stage IV* oocyte maturation. *Immature Fish* denotes animals that never reproduced, *Resting* indicates sexually mature fishes between two reproductive cycles, *Maturing* indicates fishes undergoing vitellogenesis, *Ripe* indicates females ready to spawn

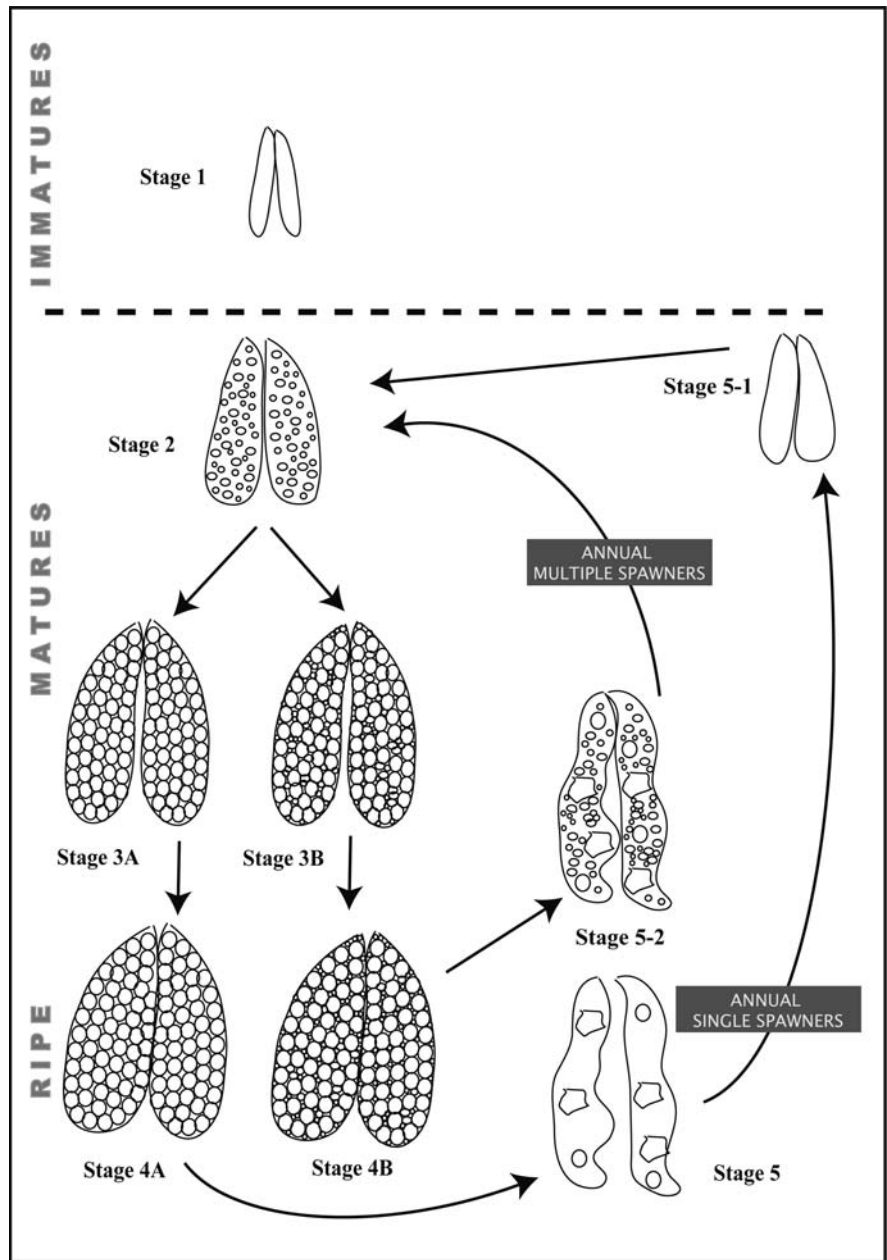


However, as it represents a single continuous physiological state (initiation and development of the vitellogenesis) and as its aspect may vary a great deal depending on species, it appears simpler and more logical to keep it as a single stage in this synthetic scale.

In stage III oocytes (Figs. 3b, 4c), the chorion (or zona radiata) is clearly visible, and the theca (follicular epithelium + thecal and meso-epithelial cells + blood vessels) is generally well developed. The entire

ooplasm is progressively filled with yolk granules, globules or platelets and lipid globules, and the cortical alveoli are generally located only in the peripheral ooplasm although they are less observable since the entire ooplasm is almost filled with yolk globules at the end of vitellogenesis. The nucleus or germinal vesicle is still visible and located in a central position (Figs. 3b, 4c). Stage 3 ovaries occupy a significant part of the abdominal cavity and present visible

Fig. 2 Female ovarian cycle of single- and multiple-spawners. See Table 1 for the definition of gonadal stages



yellowish, orange or greenish oocytes, depending on the species (Table 1; Figs. 2, 5b) and a well-developed vascularization. However, the oocytes are still included in the ovarian lamellae, and the ovary lumen is empty. These ovaries characterize fully vitellogenic females, close to the spawning period. This ovarian stage is the more appropriate one to estimate the absolute fecundity (fecundity per spawning event), since the number of stage III oocytes is very close to the number of released eggs. The distinction between

single- and multiple-spawners starts at this ovarian stage 3. In single-spawners, a single oocyte size mode (stage III) is observed (ovarian stage 3A), whereas in multiple-spawners, vitellogenic oocytes (stage II) of different sizes can be observed in between the larger stage III oocytes (ovarian stage 3B; Fig. 2; Table 1). These larger stage III oocytes will evolve into stage IV oocytes, which are released at the next spawn, whereas the smaller stage II oocytes in between will continue their maturation process and will be eventually

Table 1 Macroscopic gonadal maturation stages of females and males

Females ^a	
Annual spawners	Multiple-spawners
1 <i>Immature</i> : ovaries of circular section, small, thin, partly translucent and opaque or sometimes pinkish. Oocytes invisible to the naked eye.	
2 <i>Maturing</i> : ovaries much larger, occupying a significant part of the abdominal cavity. They are filled with white or yellowish oocytes of different sizes.	
3A <i>Advanced maturation</i> : ovaries are larger and fuller. The oocytes, yellow, orange or greenish depending on the species, are now larger and more homogeneous in size.	3B Similar to stage 3A, but vitellogenic oocytes of different sizes can be seen in between the larger oocytes to be released at the next spawn.
4A <i>Ripe</i> : aspect almost identical to stage 3A, but the oocytes are partially ovulated (free in the ovarian cavity = ovules) and can be expelled with a gentle pressure on the fish flanks. The ovary reaches its maximal development. This is an ephemeral stage just before the actual spawning event.	4B Similar to stage 4A, but vitellogenic oocytes of different sizes can be seen in between the oocytes undergoing maturation and ovules are partially filling the ovarian cavity.
5 <i>Spent</i> : the ovaries are still large but almost empty, flaccid, often bloody. Some remaining ripe oocytes can still be visible along with atretic follicles.	5-2 <i>Spawned and Recovering</i> : aspect similar to stages 5 and 2. The ovaries are still relatively large and flaccid with remaining empty spaces, and sometimes atretic follicles. However, it differs from a stage 5 by the presence of developing yellow (vitellogenic) oocytes of different sizes. When time since spawning increases, it may be difficult to distinguish from stage 2. This stage characterizes the beginning of one or more new spawning cycles until the end of the breeding season.
5-1 <i>Resting</i> : the ovaries are similar to stage 1, but are usually larger, wider and pink to dark red in color. The ovarian wall is also thicker. It distinguishes between a mature resting female and an immature one.	5-1 <i>Resting</i>
Males ^a	
1 <i>Immature</i> : testes are like two silvery or translucent threads, thinner and longer than stage 1 ovaries.	
2 <i>Maturing</i> : testes are longer, wider, often of triangular or circular section and whitish to pinkish color. In many Siluriformes, testes are reticulated: they show fringes or vesicles along their entire length. Resting adults are usually at this stage.	
3 <i>Ripe</i> : testes are larger, fuller and completely white. Sperm emitted with a gentle pressure on the abdomen.	
4 <i>Spent</i> : testes still large as a stage 3, but flaccid, empty-like.	

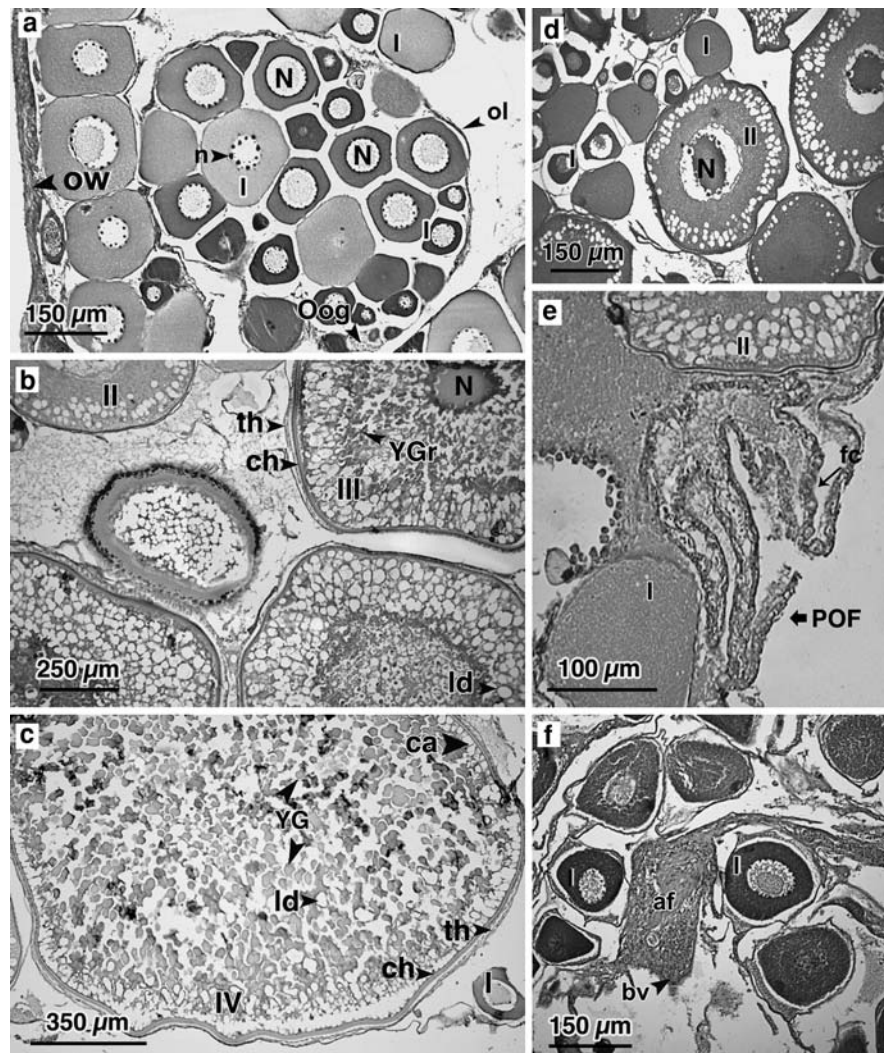
^a In Osteoglossiformes, only one ovarian or testicular lobe is functional, generally the left one

released at a subsequent spawning event. In stage IV oocytes (Figs. 3c, 4d), the ooplasm is completely filled with large yolk globules and lipid droplets, and cortical alveoli are still visible in an almost continuous layer at the periphery of the oocyte. The ooplasm becomes progressively completely transparent and occasionally contains visible oil droplets. At the end of this stage, the germinal vesicle migrates to the periphery of the ooplasm (Fig. 4d), determining the animal pole, where the micropyle is generally visible (Fig. 4d). At this stage, females are considered to be ripe, and spawning generally occurs within a very short time span. Ovulated eggs (or unfertilized eggs) are sometimes

present in the ovary lumen. This oocyte stage is typical of the macroscopic gonadal stage 4 (Table 1; Figs. 2, 6c) and is an ephemeral stage that usually lasts only a few hours; it may be easily overlooked in field studies. In the ovarian stage 4, as for stage 3, multiple-spawners (ovarian stage 4B) are distinguished from single-spawners (ovarian stage 4A) by the presence of vitellogenic oocytes of different sizes in between the full grown oocytes (Figs. 2, 6c; Table 1).

After spawning, the ovaries of single-spawner females (ovarian stage 5; Fig. 2) are large, flaccid, empty-like bloody sacs. They contain the remains of some stage IV oocytes, and post-ovulatory follicles

Fig. 3 Female oocyte maturation stages in Piranha, *Pygocentrus nattereri*. (a) Immature stage 1 ovary from a juvenile female, (b) detailed view of a vitellogenic stage 2 ovary, (c) detailed view of a stage IV oocyte from an advanced vitellogenic ovary, (d) general view of an early vitellogenic stage 2 ovary of a mature female during the breeding season, (e) post-ovulatory follicle in a stage 5-2 ovary during the breeding season, (f) stage 5-1 ovary during the resting period after the breeding season. *ol* Ovarian lamella, *ld* lipid droplet, *Oog* oogonia, *OW* ovarian wall, *n* nucleoli, *N* nucleus, *th* theca, *ch* chorion, *YG* yolk globule, *YGr* yolk granule, *POF* post-ovulatory follicle, *fc* follicular cells, *af* atretic follicle, *bv* blood vessel



are easily visible within the ovarian lamellae among the few unovulated or atretic oocytes (Figs. 3e, 4f) that were not spawned and a few stage III oocytes that did not undergo oocyte maturation. All this remnant material will undergo atresia during the next few days or weeks. The rest of the ovary contains post-ovulatory follicles and previtellogenic stage I oocytes. Such ovaries will evolve into a resting stage (stage 5-1), which is relatively similar to an immature stage (stage 1), but larger, with a thicker ovarian wall, a flattened aspect, a darker pink to dark red color and the existence, at least at the beginning of the resting period, of large empty spaces within the ovarian lamellae. The ovaries will remain at that stage until the next breeding season, when they will evolve into stage 2 again. Close to the re-initiation of

vitellogenesis, they are larger and will become turgescient due to the important increase in recruited oocytes (recrudescence period). Analysis of histological sections reveals the ovarian wall to be much thicker than in immature females. It is important to correctly identify an ovarian stage 5-1 from a stage 1, since the biological significance is completely different: the first indicates a mature resting female, a few weeks after spawning, and the second is characteristic of an immature female.

On the other hand, the ovaries of spawned multiple-spawner females (stage 5-2) have a similar general appearance as stage 5 ovaries (large, flaccid, bloody with post-ovulatory follicles), but they contain new batches of developing vitellogenic oocytes (stage II and III), which makes the ovary partially

Fig. 4 Oocyte stages (I, II, III, IV) of *Cichla pleiozona* (a, b) and *Colossoma macropomum* (c, d, e, f). *ch* Chorion, *YG* yolk globule, *N* nucleus, *n* nucleus, *oopl* ooplasm, *GV* germinal vesicle, *pof* post-ovulatory follicle, *atr. ooc* atretic oocyte. (a, b) Stage 2 ovaries, (c, e) stage 3 ovaries, (d) stage 4 ovary, (f) stage 5-1 ovary of a post-spawned female

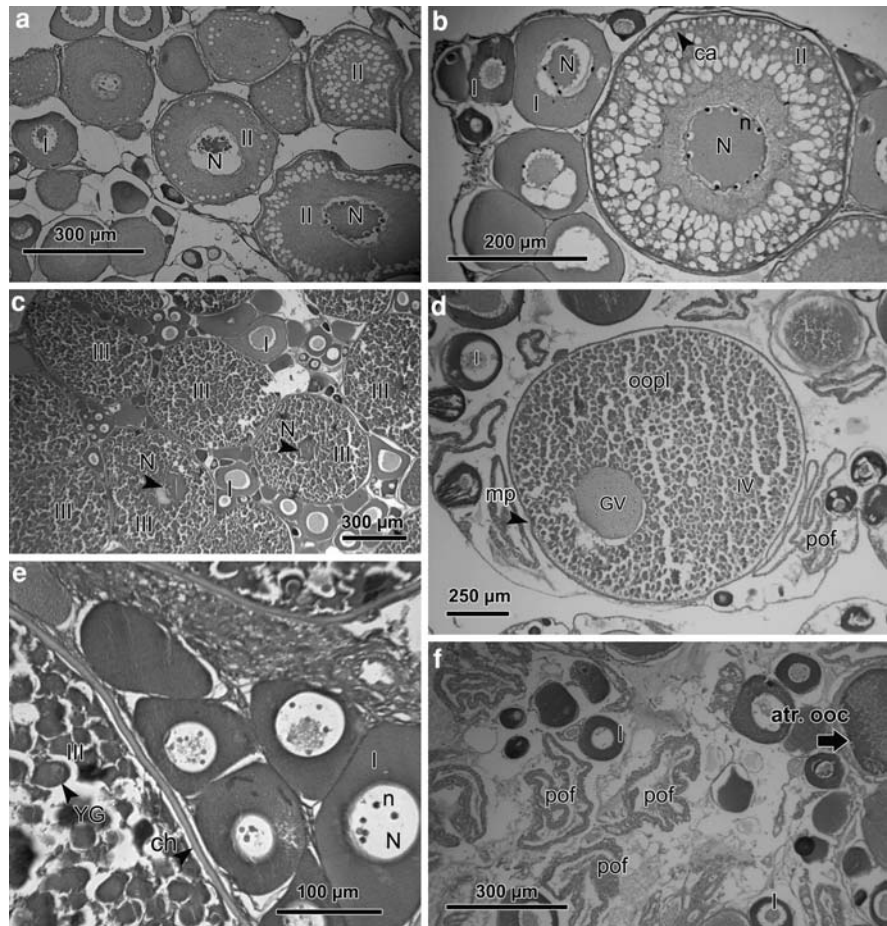
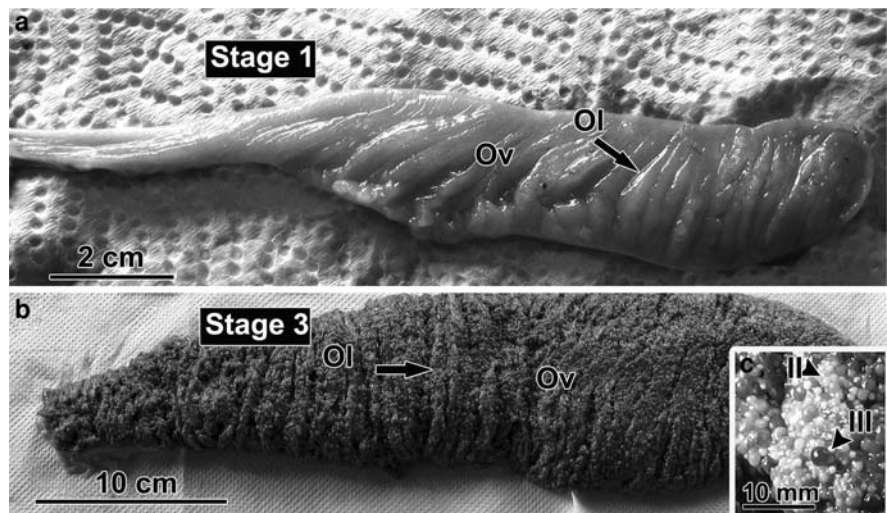


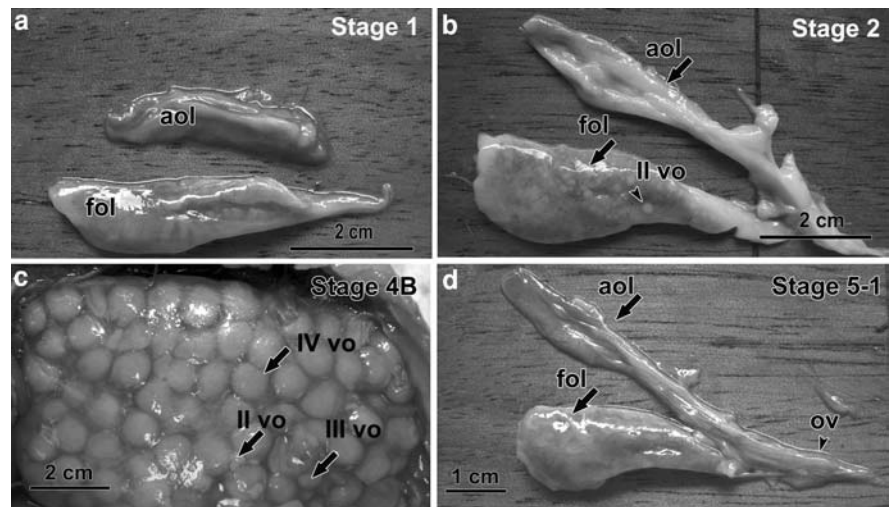
Fig. 5 Ovarian stages 1 and 3 in *Arapaima gigas*, a multiple-spawner. *Ov* Ovary, *Ol* ovarian lamella, *II* Stage II oocyte, *III* stage III oocyte



filled (Figs. 2, 3e). These will evolve directly into the maturing stage (stage 2) to initiate a new spawning cycle. In some species (fractional-spawners), the

gonad will evolve directly to stage 3. Depending on the species, females may complete two or more spawning events. At the end of the breeding season,

Fig. 6 Ovarian stages 1, 2, 4B and 5-1 of the Arahuaana (*Osteoglossum bicirrhosum*). *fol* Functional ovarian lobe, *aol* atrophied ovarian lobe, *vo* vitellogenic oocyte, *ov* oviduct



females will eventually be spawned out, and their ovaries will evolve into stage 5-1 (resting) until the next breeding season (Figs. 2, 3f, 6d).

Spermatogenesis

Four testicular stages were defined according to the maturation stage of the males (Table 1). As for females, the males' testicular stages are referred to by Arabic numbers. However histological stages were not defined as in females by roman numbering. Instead, the terminology of spermatogenetic stages common to all vertebrates was used as it also allows for the correct description of spermatogenesis in fishes.

Stage 1 testes are characterized by the presence of spermatogonia nests embedded in abundant connective tissue (Fig. 7a). This structure is characteristic of immature males (stage 1) but is also observed in resting adults. Stage 1 testes are generally small silvery or translucent filaments (Fig. 8a), thinner and longer than stage 1 ovaries.

Stage 2 testes are characterized by numerous well-organized cysts with different spermatogenetic stages (Fig. 7b), varying from spermatogonia to spermatids (Fig 7c). Stage 2 males are characterized by whitish to pinkish, relatively large and turgescient testes of triangular or circular section, depending on the species. However, semen is never present within the spermiduct and even when squeezed vigorously, nothing comes out.

Stage 3 testes are characterized by lobules filled up with spermatozoa in the central part of the testes,

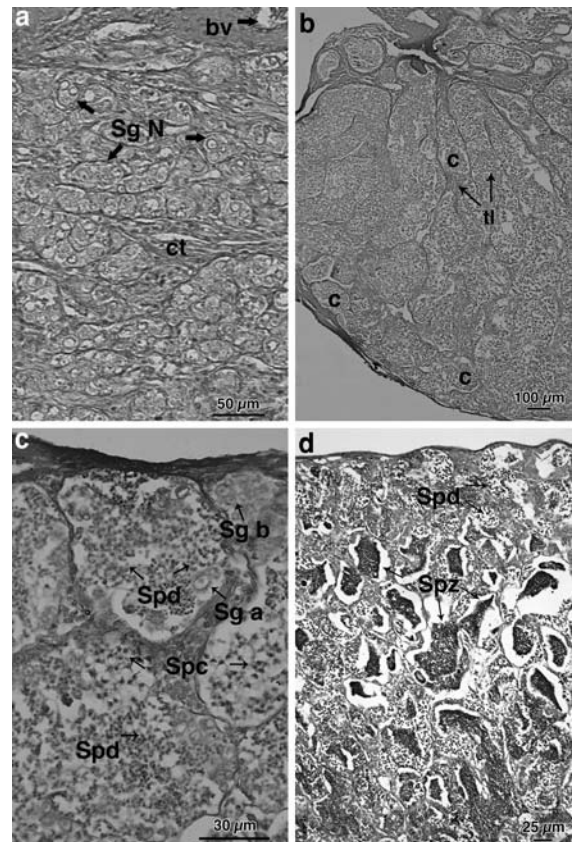
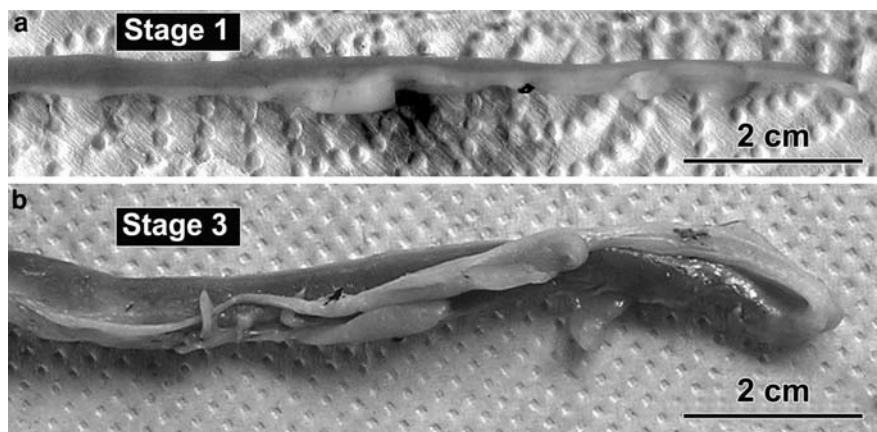


Fig. 7 Testicular maturation stages in Piranha, *Pygocentrus nattereri*. (a) Transversal section of an stage 1 immature testis, (b) partial view of a stage 2 testis, (c) detailed view of an advanced stage 2 testis, (d) partial view of an advanced stage 3 testis. *bv* Blood vessel, *ct* connective tissue, *Sg N* spermatogonia nests, *c* cyst, *tl* tubule lumen, *Sg a* spermatogonia a, *Sg b* spermatogonia b, *Spc* spermatocytes, *Spd* spermatids, *Spz* spermatozoa

Fig. 8 Testicular stages 1 and 3 in *Arapaima gigas*. (a) Partial view of stage 1 immature male testes, (b) partial view of a stage 3 adult male



which present a darker hematoxylin staining (Fig. 7d). In the cortical area of the testes, some spermatids remain in the lobule's lumen; however, all other stages of spermatogenesis are absent. Stage 3 males (Fig. 8b) are characterized by a larger, fuller, well-developed testes when compared to previous stages. The entire gonad or only some areas, depending on where semen has accumulated before being stored in the inner part of the testis, has a whitish color. A slight pressure of the testes surface makes the semen flow out of the spermiduct, which is a definite indication of the stage 3 testes.

Stage 4 testes are still almost as large as stage 3 testes, but they are flaccid and empty-like with only conjunctive tissue devoid of spermatogenesis activity that is characterized by empty tubules or lobules (not shown). This stage is indicative of a male just after spermiation (spent stage).

In multiple-spawners, the testes return to stage 3 after spermiation to complete another breeding event, whereas single-spawners return to an early stage 2 during the resting period. A resting male is difficult to distinguish from an early maturing (stage 2) male except at the beginning of the resting period, when the testes are still relatively flattened compared to a classic stage 2 testes. On histological sections these testes show all stages of spermatogenesis, but with low spermatozoa content and generally with empty spermatic ducts in the medio-central and posterior part of the testes.

Discussion

Among the many maturation scales reported in the literature, particularly those for Neotropical fishes

(Bazzoli and Godinho 1991; Ferreira et al. 1996; Magalhães et al. 1996; Bazzoli et al. 1997; Ricardo et al. 1998; Brito and Bazzoli 2003; Fonseca Ratton et al. 2003; Gonçalves et al. 2006), that of Ferreira and Godinho (1990), subsequently used by Teles and Godinho (1997), was relatively similar to the scale developed here for multiple-spawners—always disregarding semantic differences (such as the misuse of “resting” for immature fish). However, this maturation scale did not consider the case of single-spawners and differed from the scale presented here for males in that it recognized only three stages and lacked precision (it did not distinguish between maturing and ripe testes). Although developed without histological support, the maturation scales proposed by Loubens et al. (1984) and later improved by Loubens and Aquim (1986) on several Amazonian species were the most precise and closest to the scale proposed here. Unfortunately, owing to the nature of these publications (reports), their distribution was likely too limited to have influenced subsequent studies. The maturation scale proposed in this study is slightly simpler than that of Loubens et al. and relies on key physiological processes (estimated by histological analyses) describing oogenesis and spermatogenesis dynamics. A well-documented analysis of a cytological and cytochemical study during the oogenesis of ten Brazilian species (Bazzoli and Rizzo 1990) described four oocyte developmental stages, as proposed here. However, the correspondence between oocyte and ovarian stages was not established, and the terminology used was different.

Concerning the terminology, stage II oocytes are generally termed “vitellogenic oocytes”. However, it is important to mention that from a physiological point

of view, the term vitellogenesis should be used to describe exogenous vitellogenesis, i.e. the incorporation of vitellogenin and its processing and storage within the ooplasm of the growing oocyte by specific receptors (Nuñez Rodriguez et al. 1996), which represents more than 80% of the oocyte content in ripe oocytes (Wallace 1985; Tyler and Sumpter 1996). Considering that in many species the cortical alveoli stage is considered to be a vitellogenic stage (Bazzoli and Godinho 1994; Gomes et al. 2007) endogenously produced (Selman and Wallace 1989; Tyler and Sumpter 1996), the term “endogenous vitellogenesis” may be confusing, as cortical alveoli are not vitellogenic substances. Our observations in Tilapia (*Oreochromis niloticus*) have established the concomitance of both endogenous (oil droplets) and exogenous vitellogenesis (yolk granules and globules) (Ndiaye et al. 2006), which seems to be a general pattern of marine and freshwater fish oocytes (Selman and Wallace 1989; Le Menn et al. 2000). As previously mentioned, the physiological state of a previtellogenic and vitellogenic female are very different, and cortical alveoli and lipid droplets are clearly visible during this stage when observed by photonic microscopy (Selman and Wallace 1989). However, yolk vesicles and small yolk granules are already present in the cortical ooplasm at the time of the large distribution of cortical alveoli and lipid droplets at the very beginning of stage II oocyte. Based on these observations, we propose “vitellogenic” or “maturing” be considered to describe the females for which the lipid droplets and cortical alveoli are visible in the ooplasm by photonic microscopy, taking into account that small yolk vesicles or granules are also present at this time, but not as predominant as in stage III oocytes and are difficult to observe with photonic microscopy. The synthesized glycoproteic material stored in the cortical alveoli is not yolk reserves since it is produced by the oocyte and released between the chorion and oolema during fertilization to avoid polyspermy and to harden the chorion (see Whitaker 2006 for review). Nevertheless, stage II oocytes indicate the initiation of the reproductive cycle. In practical terms, for researchers who have no access to histological analyses, females can be considered to be “sexually mature” or reaching maturity from the ovarian stage 2 onwards.

Stage IV oocytes radically differ from stage III oocytes since they indicate the end of vitellogenin uptake after oocyte maturation (Selman and Wallace

1989). Their appearance corresponds to a very different and short-lived physiological stage just before spawning, explaining the rarity of this stage when field sampling is not extensive enough.

We have pointed out the necessity of making a precise determination of the onset of vitellogenesis (stage 2) or spermatogenesis (stage 2), since these parameters enable the correct determination of the size and age of the animal at first sexual maturity. The average size at maturity is usually estimated as the size at which 50% of the individuals (males or females) in the population (here the sampled individuals, assuming that the sampling is representative of the population) are at an advanced stage of the sexual cycle during the breeding season (Legendre and Ecoutin 1989; Barbieri et al. 1994; Duponchelle and Panfili 1998; Duponchelle et al. 2007). In practice, this is the size at which 50% of the individuals (males or females) have reached stage 2 of the maturation scale proposed in this study. However, in order to avoid classifying resting individuals (stage 5-1 for females) as immature, it is most important to use individuals collected during the height of the breeding season, when resting individuals are scarce. Indeed, in females of some species, distinguishing between immature (stage 1) and resting (stage 5-1) specimens outside the breeding season may prove difficult. Inaccurately classifying resting females as immature will lead to an overestimation of the size at maturity. This frequently translates in many ups and downs in the correct size of the logistic curve of maturity (i.e. the size above the 50% level of mature individuals) instead of a smooth curve. To avoid such classically occurring pitfalls, the size and age at first maturity must be determined on samples collected during the height of the breeding season whenever possible. Otherwise, these important parameters will likely be overestimated.

A correct estimation of the duration of the breeding season, another important life history trait, also depends on the accurate determination of the maturation stages. As this parameter is usually determined mostly on females, we will consider only females in the discussion. Commonly observed mistakes in the literature are (1) not to consider the fish that have just spawned and (2) to consider maturing females (stage 2 of the present scale) to define the breeding season, because the maturation scale used is not precise enough and does not

distinguish between maturing and ripe females. However, when initiating a vitellogenic cycle, a female may be from at best several weeks (such as in tilapias; Duponchelle and Legendre 2001) up to months (such as in *C. macropomum* or *Pseudoplatystoma* spp.; unpublished data) from the spawning event that actually defines the breeding season. A correct estimation of the stages just after spawning (stage 5 in single-spawners and 5-2 in multiple-spawners) is also crucial in determining the breeding season as these states indicate that a spawning event just occurred and that another one is initiating (in multiple-spawners: stage 5-2). Therefore, a precise determination of the breeding season will consist of an analysis of the relative cumulated percentage of stages 3A, 4A and 5 (for single-spawners) or stages 3B, 4B and 5-2 (for multiple-spawners) of the adult population (fish in which the size is equal or above the average size at first maturity) at monthly intervals. Taking into account the immature specimens in the analysis, which is often observed in the literature, does not make sense and leads to an underestimated proportion of breeding individuals in the population.

Batch fecundity (i.e. the number of oocytes laid per spawning event), hereafter referred to as fecundity, is another important life history trait whose precise estimation relies on a correct maturation scale. The scale proposed in this study distinguishes between maturing females (stage 2), females at the end of their vitellogenic cycle (stage 3) and ovulated females (stage 4), which is a frequent omission in many published scales. As stage 4 characterizes females with ovulated eggs (oocytes free in the ovarian lumen) that are easily expelled from the oviduct by gentle pressure on the abdomen, the manipulation associated with the fish's capture almost always leads to a loss of ovulated eggs. Consequently, a mean fecundity assessed on stage 4 females is likely to be an underestimation. Inversely, estimating the fecundity on stage 2 females will lead to an over-estimation for the following reasons: (1) the oocytes that will be spawned are not yet fully grown and are therefore very difficult to separate from the other oocytes that constitute the next batch (in multiple spawners); (2) atresia occur regularly during the vitellogenic cycle (Hunter and Macewicz 1985; Rizzo and Bazzoli 1995; Tyler and Sumpter 1996; Miranda et al. 1999) and the number of vitellogenic oocytes in a stage 2 gonad is superior to

the number that will actually be spawned, including for a single-spawner. Instead, we suggest the use of stage 3 females, with larger oocytes (end of stage III and early stage IV), which represent those that will be released at the next spawn, usually a few days later.

The synthetic maturation scale proposed in this study is based on the integrated physiological microscopic (oocytes and spermatogenesis stages) and macroscopic (ovarian and testes) dynamics of a variety of economically important African and Amazonian species and accounts for the main spawning strategies encountered in oviparous teleosts—annual single-spawners and multiple-spawners. The accuracy of the macroscopic scale has been confirmed by histological observations of the testes and ovaries, which allowed us to minimize the number of critical maturation stages and make this scale relatively simple, yet precise enough to likely be applicable to most oviparous teleost fishes. This scale has been successfully applied to several fish species pertaining to some of the most important African and Neotropical taxa: Characiformes, Siluriformes, Osteoglossiformes and Perciformes. In addition to the need to define a “universal” macroscopic maturation scale for oviparous teleosts that would facilitate the standardization of protocols in field studies and make inter-specific comparisons easier, another important goal of this work was to provide a maturation scale precise enough to accurately determine some of the most important life history traits in population and stock assessment studies or ecological studies at the community level.

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