Evolutionary transitions, environmental correlates and life-history traits associated with the distribution of the different forms of hermaphroditism in fish

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Tesis doctoral

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La doctoranda
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To my mother

Agraïments / Acknowledgements / Agradecimientos

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Abstract

A sexual system defines the pattern of distribution of male and female function that exists in a given species, which can occur in separate individuals (gonochorism) or in the same individual (hermaphroditism). In hermaphrodites, male and female function can happen simultaneously or sequentially: male first (protandry) or female first (protogyny). Understanding the evolutionary transitions among the different sexual systems as well as the biotic and abiotic correlates that explain their distribution among taxa is a major challenge in evolutionary biology.

The incidence, distribution and evolutionary transitions among the different sexual systems were studied in actinopterygian (ray-finned) fishes, the only group of vertebrates where hermaphroditism is present. To this end, information was compiled on the sexual system, environmental correlates and life-history traits of 10,875 ray-finned fishes. Then, modern phylogenetic methods were applied to reconstruct the ancestral sexual state.

The number of hermaphrodite species identified to date is 552 out of the >35,000 fish species currently known, with a maximum estimate of 1,500-2,000 species. Gonochorism is the ancestral sexual state and constitutes an evolutionary stable strategy. Simultaneous hermaphroditism and, to a lesser extent, protogyny, is also stable but protandry is not. Protogyny associates particularly with tropical regions, with species living usually in coral reefs but also hard-bottom substrates that may favor the establishment of the social structures typical of protogyny. Protogynous species mature at the same time but live longer than gonochoristic relatives. Protandry is less habitat specific. The male gonadosomatic index was used as a proxy to study sperm competition in sparids and revealed that, while gonochoristic and protogynous species support the predictions of the size-advantage model, which explains the fitness advantages of sequential hermaphrodites, protandrous species do not. Thus, small males of protandrous species have to invest disproportionally more in sperm production, not only when spawning in aggregations with high levels of sperm competition but also when spawning in pairs due to the need to fertilize highly fecund females, much larger than themselves. Simultaneous hermaphroditism is a derived, rare character that has evolved independently from gonochorism and protogynous hermaphroditism, but not from protandry. Simultaneous hermaphrodites have a wide latitudinal distribution and are present in deep and shallow waters in essentially similar proportions. Simultaneous hermaphrodites have lower maximum body size than gonochoristic relatives only when the mating system allows sufficient egg production by other means to compensate the lesser fecundity associated with smaller females.

These results are of interest not only to ecology and evolutionary biology but also to applied biology, as they can help predict possible changes in the distribution and phenology of hermaphrodites in a context of global change. Results can also contribute to better management and exploitation practices, since many hermaphrodite species are of economic importance for fisheries, aquaculture and the ornamental fish trade.

Resum

Un sistema sexual és defineix com el patró de distribució de la funció masculina i femenina que existeix en una espècie donada, que pot tenir lloc en individus separats (gonocorisme) o al mateix individu (hermafroditisme). En els hermafrodites, la funció masculina i femenina pot ocórrer simultàniament o seqüencialment: masculí primer (proteràndria) o femení primer (protogínia). Comprendre les transicions evolutives entre els diferents sistemes sexuals, així com les correlacions amb factors biòtics i abiòtics que expliquen la seva distribució entre els taxons, és un gran desafiament en la biologia evolutiva.

Es va estudiar la incidència, la distribució i les transicions evolutives entre els diferents sistemes sexuals en peixos actinopterigis, l'únic grup de vertebrats on l’hermafroditisme és present. Per a això, es va recopilar informació sobre el sistema sexual, les correlacions ambientals i els trets del cicle vital en 10.875 peixos. Posteriorment, es van aplicar mètodes filogenètics moderns per reconstruir l'estat sexual ancestral.

S’han identificat fins a la data 552 espècies hermafrodites de les més de 35.000 espècies de peixos conegudes. El gonocorisme és l'estat sexual ancestral i constitueix una estratègia evolutiva estable. L’hermafroditisme simultani i, en menor mesura, la protogínia, també són estables, però la proteràndria no. La protogínia s'associa principalment amb zones tropicals i esculls de coral, però també amb substrats de fons dur que poden afavorir les estructures socials típiques de la protogínia. En canvi, la proteràndria és menys específica de l’habitat. Les espècies protògines maduren al mateix temps però viuen més que els seus parents gonocoristes. L'índex gonadosomatic es va utilitzar com un indicador indirecte per estudiar la competència espermàtica i va revelar que, mentre que les espècies gonocoristes i protògines donen suport a les prediccions del model de l’avantatge per la mida, que explica les avantatges dels hermafrodites seqüencials, les proteràndriques no ho fan. Així, mascles petits proteràndrics han d'invertir més en la producció d'espermatozous, no només quan fresen en agregacions amb alts nivells de competència espermàtica, sinó també quan fresen en parelles a causa de la necessitat de fertilitzar femelles molt més grans que ells mateixos. L'hermafroditisme simultani és un caràcter poc comú i derivat que ha evolucionat del gonocorisme i de la protogínia però no de la proteràndria. Els hermafrodites simultanis tenen una àmplia distribució latitudinal i són presents en aigües someres i profundes en proporcions essencialment similars. A més, tenen una mida màxima menor que els parents gonocoristes només quan el sistema d'aparellament permet una producció d'ous suficient per altres mitjans per compensar la menor fecunditat de les femelles més petites.

Aquests resultats són d'interès no només per l'ecologia i la biologia evolutiva, sinó també per a la biologia aplicada, ja que poden ajudar a predir possibles canvis en la distribució i fenologia dels hermafrodites en un context de canvi global. També poden contribuir a millorar la gestió i explotació, ja que moltes d'aquestes espècies són d'importància econòmica per a la pesca, l'aqüicultura i la aquariologia.

Prologue

This Ph.D. thesis was carried out at the Group of Biology of Reproduction (GBR), of the Department of Renewable Marine Resources, Institute of Marine Sciences (ICM-CSIC) in Barcelona, under the supervision of Dr. Francesc Piferrer, and under the Ph.D. Programme in Aquaculture of the Universitat Autònoma de Barcelona during the years 2014 to 2019. The aim of this thesis was to investigate the evolutionary transitions among sexual systems, the abiotic environmental correlates such as latitude, salinity and habitat, and important life-history traits such as longevity, maturity and body length associated with the distribution of the different forms of hermaphroditism in fish, specifically focusing on the teleosts.

The thesis is divided into four chapters:

> *Chapter 1.* Hermaphroditism in Fish: Incidence, Distribution and Associations with Abiotic Environmental Factors.
>
> *Chapter 2.* Switches, stability and reversals: the evolutionary history of sexual systems in fish.
>
> *Chapter 3.* A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae).
>
> *Chapter 4.* Simultaneous hermaphroditism in fish: Incidence, evolutionary transitions and selective pressures constraining body size.

Each chapter corresponds to an article either submitted or in preparation for submission as of September 16, 2019:

> Pla, S., Maynou, F., Piferrer, F. Hermaphroditism in Fish: Incidence, Distribution and Associations with Abiotic Environmental Factors. *Submitted.*
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> Pla, S., Capellini, I., Benvenuto, C., Piferrer, F. Switches, stability and reversals: the evolutionary history of sexual systems in Teleosts. *In preparation.*
>
> Pla, S., Benvenuto, C., Capellini, I., Piferrer, F. A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae). *Submitted.*
>
> Pla, S., Piferrer, F. Simultaneous hermaphroditism in fish: Incidence, evolutionary transitions and maximum body size. *Submitted.*

Part of the results from Chapter 1 were published in preliminary form in the following article:

> Pla, S., Maynou, F., Piferrer. F. 2013. La distribució de l'hermafroditisme als peixos del món. Actes de la XIII Jornada de la Biologia de la Reproducció (SCB), pp 11-14, Barcelona (Spain) 18 June.

Part of the results from Chapter 4 were published in preliminary form in the following article:

> Pla, S., Piferrer, F. 2017. La distribucció de l'hermafroditisme en la família Serranidae segons variables ambientals. Actes de la XV Jornada de la Biologia de la Reproducció (SCB), [15] pp 9-12, Barcelona (Spain) 21 June.

Results obtained in this thesis have been presented at the following scientific meetings:

> XIII Jornada de la Biologia de la Reproducció. Societat Catalana de la Biologia. Barcelona, Spain. 18 June 2013.
>
> 16th Congress of the International Society for Behavioral Ecology. Exeter, United Kingdom. 28 July – 3August 2016.
>
> XV Jornada de la Biologia de la Reproducció. Societat Catalana de la Biologia. Barcelona, Spain. 21 June 2017.
>
> Sex in the oceans: from molecules to macroevolution. Strömstad, Sweden. 15-17 May 2018.
>
> Simultaneous and Sequential Hermaphroditic Organisms Workshop. Salford, United Kingdom. 25-26 April 2019.

During the course of this research, I spent three months at the School of Environment and Life Sciences of the University of Salford, United Kingdom, under the supervision of Dr. Chiara Benvenuto (expert in behavioral ecology) with a Santander Universities Travel Award Grant 2016/2017. I also spent some days at the School of Biological and Environmental Science at the University of Hull, United Kingdom, under the supervision of Dr. Isabella Capellini (expert in phylogenetic comparative methods) to obtain training in phylogenetic analysis comparative methods.

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General introduction

1. Sexual reproduction

1.1 Definition and origin

Sexual reproduction is a process that involves the combination of gametes (a germ cell capable of unite with another germ cell; eggs and sperm) to form descendants that inherit the genetic material of both parents. Thus, sexual reproduction involves the alteration of diploid and haploid states during the life cycle of an organism that reproduces sexually (Beukeboom and Perrin, 2014).

Ancestral eukaryotes are usually small unicellular organisms, with monomorphic or isogamic and mobile gametes. These unicellular organismes eventually evolved into multicellular organisms, giving rise to anisogamy (Bulmer and Parker, 2002, Leonard, 2010, Lehtonen and Kokko, 2011). Anisogamy or different male and female gametes, is thus widely distributed in multicellular eukaryotes such as metazoans, plants and some fungi (Bell, 1982, Leonard, 2010, Beukeboom and Perrin, 2014). Anisogamy, despite high costs in the production of offspring or in energy requirements associated to mating, has contributed to the great sexual diversity existing today (Lehtonen et al., 2016a, Smith and Maynard-Smith, 1978). This is compensated by an improvement of offspring fitness in the long-term (Beukeboom and Perrin, 2014).

To understand the origin of the two sexes, one must understand the morphological change of the gametes between isogamy and anisogamy (Lehtonen et al., 2016a) and its evolution (Parker, 1982) (**Fig. 1**). A more specialized type of anisogamy is oogamy, often used instead of anisogamy to refer two sexes in animals (Leonard, 2010), which involves, among other changes, flagellum loss in female gametes, which greatly reduces their mobility (da Silva, 2018).

1.2 Males and females

In anisogamy, female and male sexes are defined for the first time (Beukeboom and Perrin, 2014) by the morphological and behavioural differentiation of their gametes (**Fig. 1**). Therefore, a male is the sex that produces a high amount of small gametes called sperm. In contrast, a female is the sex that produces fewer and large gametes called ova (Beukeboom and Perrin, 2014). Thus, different sex roles arise from the divergence between the two types of gametes (Lehtonen et al., 2016b).

1.3 Sexual selection and Bateman’s principles

Sexual selection is a mode of natural selection originated by differences among individuals of the same sex in the ability to compete for securing mates of the opposite sex. It is, in many aspects, an evolutionary force much more powerful than “regular” natural selection in the sense that it can favour the appearance of traits with no apparent adaptive value unless they are viewed exclusively from their value in increasing reproductive fitness. For example, bright coloration of a male will serve to attract females but it will also make the male more visible and thus prone to be captured by predators. Darwin realized this apparent contradiction with the theory of natural selection (Darwin, 1859), in which most of the adaptive traits that allow a greater survival are inherited to the offspring. In this context, the evolution and prevalence of anisogamy was the subject of long debate. At one point it was believed to be a prerequisite for sexual selection (Bateman, 1948, Parker et al., 1972); however, nowadays it is believed to be a result of sexual selection, specifically, with the dimorphism between gametes seen as a consequence of sexual roles (Lehtonen et al., 2016b, Hanschen et al., 2018) (**Fig. 1**).

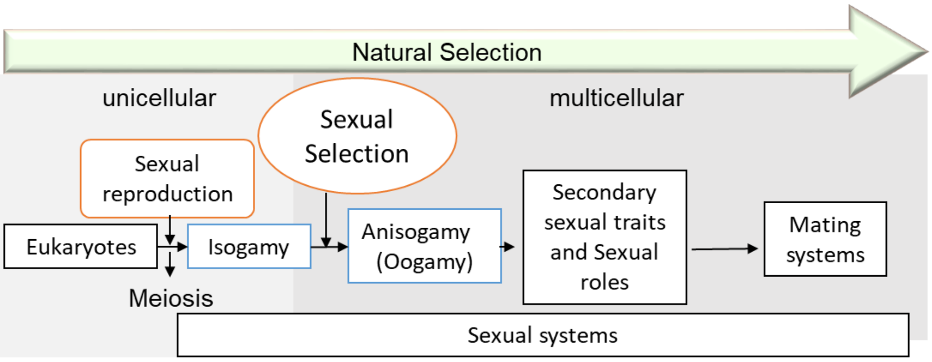


Figure 1. Evolution of sex and sexual systems.

Sexual selection has two mechanisms called intrasexual and intersexual (Darwin, 1871). First, the *intrasexual mechanism* is the type of sexual selection in which individuals of the same sex compete among them to mate with members of the opposite sex. In experiments with *Drosophila*, Bateman (1948) was the first to demonstrate that competition mainly occurs between males. Therefore, Bateman's (1948) principles explains how the asymmetry in male vs. female gametes eventually led to different sex roles and opposite selection pressures. Briefly, Bateman's principles state that, due to the smaller cost of producing sperm when compared to eggs: (i) male reproductive success increases with mate number whereas female reproductive success does not (**Fig. 2**); (ii) males have greater variance in reproductive success than females due to the high probability of failure in mating or attracting females, and (iii) the sex with the greater variance in reproductive success undergoes stronger sexual selection (Tang-Martínez, 2012). Therefore, males have the opportunity to increase their fitness by increasing the number of matings, but females cannot increase their fitness mating more times, so they can only choose the best male (see intersexual selection below) (**Fig. 2**). As this unbalanced amount of gametes occurs in all species with anisogamy, the principle can be applied in all anisogamous animals, including hermaphrodite species (Bateman, 1948).

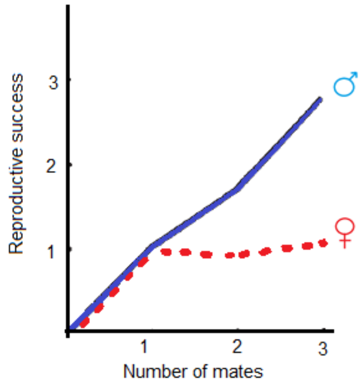


Figure 2. The relationship between the number of mates and the reproductive success of the male and female in *Drosophila melanogaster*. Code: males (blue) and females (red). Adapted from Bateman (1948).

Bateman’s principle have been used to test the strength of sexual selection across sexes in gonochoristic and simultaneous hermaphroditic species although whether they really apply to the latter is currently highly debated (Leonard, 2005, Tang-Martínez, 2012, Tang-Martínez, 2016). Charnov (1979), the evolutionary biologist who developed the theory of sex allocation (see below), realized that in simultaneous hermaphrodites the difference of fitness in each sex produces a sexual conflict that limits male reproductive success. This goes against Bateman’s principles that consider that males are the sex with the most variability in reproductive success. However, this inconsistency in the application of Bateman's principles to simultaneous hermaphrodites can be explained if one considers that natural selection can act on the within-generation variance in offspring number, which constitutes Gillespie’s principle (Leonard, 1999). Gillespie's principle (Gillespie, 1974, Gillespie, 1977) establishes that a reduction in the variance in offspring number increases the fitness of a given genotype. Thus, the preferred sex role, according to Bateman's principle, should be the females since is the sex with less variance in reproductive success. Although Gillespie’s and Bateman’s principles assign the preferred role to different sexes, the sex roles preferences depend on the taxa since both preferences have been determined (Leonard, 2010).

Sequential hermaphrodites, on the other hand, present a different sort of challenges. Sexual selection has been recognized to be also important due to the existence, by the nature of sex change, of populations with heavily skewed sex ratios. Thus, based on Bateman’s principle, the most abundant sex —males in protandrous species and females in protogynous species— should be the sex where sexual selection is strongest (Leonard, 2006). Surprisingly, this has not yet been formally tested.

Second, the *intersexual mechanism* is the type of sexual selection in which females are the ones who select the males with whom to mate, having developed different strategies to do so (Bourne et al., 2003). Females may choose males by paying attention to phenotypic traits such as body size and colour, ornaments, courtship behaviour, etc.

(Gonçalves-de-Freitas et al., 2009), obtaining direct benefits such as nuptial gifts or help in parental care. However, females can also achieve benefits by selecting the male with the perceived best genotype to be passed to their offspring. There are three explanations from a genetic point of view: 1) *Fisher hypothesis*, in which the female is interested in some trait (e.g., a long tail) and expects her offspring to inherit that trait (Fisher, 1930). 2) *Good genes hypothesis* or *Handicap principle*, where the presence of some male specific, in principle costly sexual traits indicates their good overall quality of its genes due to the high effort it makes to survive despite the cost inherent to that trait (Zahavi, 1975, Zahavi, 1977). 3) *Compatible genes hypothesis*, where genes compatible with different feminine genetic traits are looked for to achieve a heterozygous descent (Sayol and Ferrandiz-Rovira, 2018). In addition, females do not always prefer dominant males that could indicate good genes in the offspring. Thus, females can also choose males who care for the offspring or are simply passive. However, dominant males may impede subordinate males to be chosen by females. Therefore, females cannot always choose (Gonçalves-de-Freitas et al., 2009).

2. Sexual systems

2.1 Definition

A sexual system, according to Leonard (2018a), is defined by the pattern of distribution of male and female function that exists among the organisms of a given species. There are several major sexual systems being gonochorism and hermaphroditism the two main ones. Among them there are mixed systems such as androdioecy, gynodioecy and trioecy, and finally unisexuality (Leonard, 2018b).

2.2 Gonochorism

Is one of the most common sexual system present in all taxa and is usually referred to dioecism in plants. In gonochoristic species, individuals reproduce exclusively either as females or as males during their entire lifetime. Thus, a given individual has ovaries and is a female or has testis and is a male. Gonochorism is characteristic of many phyla and classes of plants and animals (Leonard, 2018b).

2.3 Unisexuality

Unisexuality is a sexual system where all individuals of one species reproduce through parthenogenesis, and are only females. Nevertheless, and despite much debate, it is considered by many —and also by us— a type of sexual reproduction since there is meiosis and, therefore, recombination, the hallmark of sexual reproduction (Mable, 2007). There are basically two types of unisexual reproduction: gynogenesis and hybridogenesis. In gynogenesis, sperm of an unrelated species activate the eggs although it does not contribute genetically to the zygote and thus a female clone is generated. On the other hand, hybridogenesis involves hybrids, whereby the unisexual all-female species excludes one of the parental genomes and produces gametes containing only one of the parental genomes. Unisexuality is present in poikilothermic species such as amphibians, reptiles and fish (Piferrer, 2018).

2.4 Hermaphroditism

Hermaphroditism is defined as the sexual system where individuals of a given species expresses both the male and female function during their lifetime, either sequentially or simultaneously. Thus, the same individual can produce both sperm and eggs during its lifetime.

Hermaphroditism is assigned to a species when all or the vast majority of its members express both sexual roles. Some species can have individuals containing both sexual tissues, e.g., a bisexual gonad many times called ovotestis. This can be due to errors during gonadal development or the presence of contaminants, for example. In these cases, individuals are called intersexes (Piferrer, 2001; (Abdel-moneim et al., 2015) but this does not qualify the species to be considered hermaphrodite (Sadovy de Mitcheson and Liu, 2008). Therefore, a species is considered hermaphrodite only when it functions as both sexes, evidenced by a rigorous study of its gonadal histology in several individuals of different sizes at various periods (Sadovy and Shapiro, 1987). Particularly in fish, bimodal size-frequency distributions and biased population sex ratios should not be considered as evidence for hermaphroditism (Sadovy and Shapiro, 1987).

Hermaphroditism is present in major taxonomic divisions of plants, representing ~90% of angiosperms (Barrett, 2002) and 34% of gymnosperms (Walas et al., 2018), and it is common in several phyla and class of Metazoans (Leonard, 2013). In animals, a rough estimate of the number of hermaphroditic species is 65,000, or about 5–6% of animal species, which increases to 30% if insects are excluded (Jarne and Auld, 2006). Therefore, it is present in half of the 85 classes (Eppley and Jesson, 2008) and in 22 of 32 phyla of invertebrates (69%), being common or prevalent in 14 of them, including Porifera, Cnidaria, Platyhelminthes, Mollusca, Urochordata (ascidians), and Annelida.

2.4.1 Sequential hermaphroditism

Sequential or consecutive hermaphroditism occurs in species where individuals functionally mature as one sex first, female or male, and then functionally change to the opposite sex once during their life cycle. According to the initial functional sex, species can be classified as protogynous (*proto* = first + *gynous* = female), where individuals begin as females and change sex to male, or protandrous (*proto* = first + *androus* = male), where individuals begin as males and change sex to female (Devlin and Nagahama, 2002).

Sequential hermaphroditism is found in many families of teleost fish and in some invertebrates such as polychaetes, gastropods, and some groups of shrimps. It has also been found in angiosperms.

Within protogyny and protandry there are two subtypes. Thus, in some protogynous species, all individuals undergo sex change and thus all males derive from functional females. These males are called secondary males in a terminal phase and this subtype of protogyny is called monandry (*mon-* = one + *andry* = male, meaning a single male origin). In other protogynous species, there are males that derive directly from larval or juveniles called primary males because they never experience sex change. These males have the appearance of females in the initial phase and may later undergo a morphological and behavioural change towards secondary males in the terminal phase. Thus, in addition to secondary males that derive from functional females, there are primary males. The protogynous population that express both development pathways is called diandry (*di-* = two + *andry* = males, two different origins of the male sex) (**Fig. 3A**). Likewise, within the protandrous species, there is monogyny (*mono* = one + *gyny* = female, meaning a single female origin), where all functional, terminal phase females provided from functional males, while two development pathways in protandrous is called digyny (*di-* = two + *gyny* = female, two different origins of the female sex (Reinboth, 1967, Sadovy and Shapiro, 1987) (see **Fig. 3B**).

The differentiation of these primary or secondary sex in fish populations in the literature is scarce. However, some species have been detailed (e.g. Sadovy de Mitcheson and Liu (2008), Walker and McCormick, (2009)).

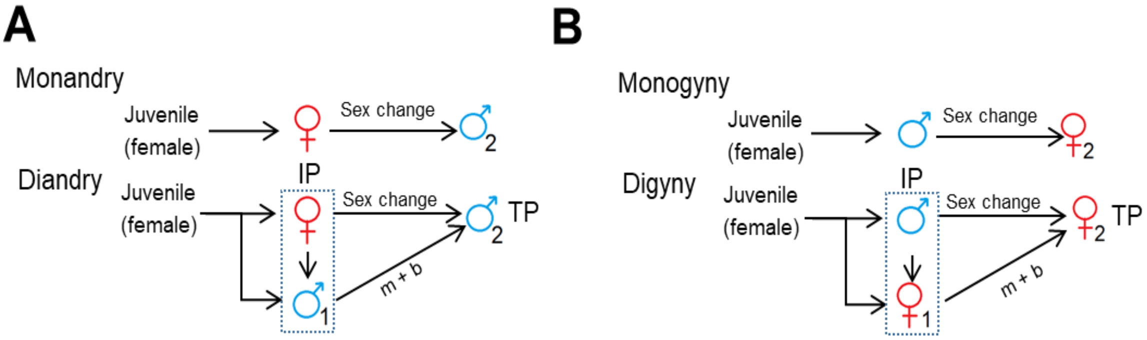


Figure 3. Sequential hermaphrodite development pathways. A) Protogyny. B) Protandry. Numbers indicate whether it is primary sex (1) or secondary sex (2). Abbreviation: IP, initial phase (in dashed box); TP, terminal phase; m, morphological; b, behaviour.

2.4.2 Simultaneous hermaphroditism

Simultaneous or synchronous hermaphroditism, also called true hermaphroditism, refers to individuals who produce gametes of both sexes (sperm and eggs) at the same time or within a short period of time. Simultaneous hermaphrodites can reproduce either by outcrossing, whereby an individual can mate with another individual in both roles in a single reproductive event or season (Leonard, 2018a), or selfing, whereby the eggs of an individual are fertilized by its own sperm. Outcrossing in simultaneous hermaphrodites is characteristic of many large taxa of plants and animals, while self-fertilization is rarer (Leonard, 2018b).

2.5 Androdioecy

Androdioecy is a sexual system that consists of populations consisting of two types of individuals: hermaphrodites that produce gametes of both sexes simultaneously or males, but never females. Androdioecy is restricted to a few taxa, such as 50 plants and 115 animals species, which are mainly crustaceans (Pannell, 2002, Weeks et al., 2006, Weeks, 2012).

2.6 Gynodioecy

Gynodioecy is a mixed sexual system that consists of populations consisting of hermaphrodites that produce gametes of both sexes simultaneously or females but never males. It is common in angiosperms but it is very rare in animals (Leonard, 2010, Weeks, 2012, Leonard, 2018b).

2.7 Trioecy

Trioecy is a mixed sexual system that consists of populations where individuals can be males, females, or simultaneous hermaphrodites. It is a quite rare condition but has been found in both plants and animals (Weeks, 2012, Leonard, 2018b).

3. Sex allocation theory

3.1 Definition of sex allocation and questions

Sex allocation theory deals with the assignment of resources to male and female function and was proposed by Eric Charnov (1982). Sex allocation theory addresses questions such as what allocation of resources to males and females is favoured in gonochoristic species, when and in which direction to change sex in sequential hermaphrodites, how much energy should be devoted to male vs. female function in simultaneous hermaphrodites, and under what ecological conditions these different sexual systems are evolutionarily stable strategies (Charnov, 1982).

Sex allocation theory synthesizes several theoretical (concepts and mathematical models) and empirical predictions or hypothesis (Charnov, 1982) that allow understanding the way parents provide resources to male vs. female offspring, assuming that natural selection will favour those parents who alter this distribution of resources to increase their fitness (Fisher, 1930, Charnov, 1982). Darwin (1871-1874) was the first to realize that some species had highly skewed sex ratios, i.e., the relationship between the number of males and females in a population, but could not understand why. Famous are his thoughts about this phenomenon: “Nevertheless, there are certain animals (for example, fishes and cirripedes) in which males largely preponderate and it is by no means obvious how this male-producing tendency could have been acquired. I formally thought that the tendency to produce both sexes in the same numbers would follow from natural selection, but I now see that the whole problem is so intricate that is safer to leave the solution for the future” (Darwin, 1871). Fisher (1930) explained the reason of equal sex ratio (male: female) by his *theory of equal investment*, where the cost, the invested effort and genetic contribution to their offspring, is considered equal in both sexes, because all offspring have a mother and a father. Hence, if the sex ratio is unbalanced, frequency-dependent natural selection will straighten the sex ratio towards 1:1. The theory was tested in birds and mammals, which naturally have a 1:1 sex ratio (Williams, 1979, Charnov, 1982, Clutton-Brock, 1986) due to their sex determining mechanisms (West, 2009). However, the sex ratio may not be equal in response to environmental conditions. In fish, temperature-induced sex ratio shifts in one generation evolved towards 1:1 in the subsequent generations once the environmental perturbation ceased (Conover and Van Voorhees, 1990, Conover et al., 1992).

To resolve unequal costs between the sexes, an evolutionary stable strategy (ESS), a concept developed by Maynard Smith (1978), explains how a common behaviour can persist in evolutionary time if it is profitable and has no external alterations.

3.2 Sex allocation in gonochorism

In gonochorism, sex allocation refers to the allocation of resources to the male and female functions in individuals who will retain the same sex throughout their lives. Based on empirical evidence, it is now known that parents can adjust offspring sex ratio accordingly to maximize their reproductive success. In disagreement with Fisher (1930), Hamilton (1967) was the first to consider that competition could induce sex ratio deviations and formulated the *Local Mate Competition* (LMC) process, where when one sex competes for mates or resources, selection diverts the sex ratio to the opposite sex. For example, if females compete for resources, selection favours a sex ratio shift to males. This theory finds empiric support in numerous taxa (West et al., 2005) such as wasps, mites, nematodes, flowering plants (Charnov, 1982), fig wasps (Hamilton, 1979), ants

(Hamilton, 1972), flatworms (Schärer and Ladurner, 2003) and fish (Fischer, 1981, Fischer, 1984, Petersen, 1991). Later Clark (1978a) described two more models: *Local Resources Competition* (LRC), in which daughters compete with their mothers for resources and sex ratio in population is male-biased. It was tested in birds and mammals. In contrast, *Local Resources Enhancement* (LRE), in which selection favours the productions of more individuals of the sex that cooperates or helps. For example, daughters cooperate with mothers for resources and sex ratio in population is female-biased to increase this cooperation (Trivers and Hare, 1976, Trivers and Willard, 1973). It was tested in primates with ambiguous results. Finally, Trivers and Willard (1973), in agreement with Fisher's principle, considered that the environment can adjust the sex ratio through various factors without dependence on parents, through environmental mechanisms and sex change. It was a successful theory because it provided qualitative and quantitative support for the analysis of the factors of interest although not all could be included because of the difficulty in considering the possible interactions (West, 2009). Komdeur (2012) determined how parental care might modify the sex ratio in vertebrates through the cooperation or competition from offspring and quality of the territory. This had a major impact because it was previously thought that vertebrates (birds) could not manipulate the sex ratio (West, 2009). Examples of environmental factors that could alter the sex ratio are male quality in birds and lizards, host size in wasps, maternal quality in primates, and age (or size) and temperature in fishes (West, 2009).

3.3 Sex allocation in hermaphroditism

In hermaphroditism, sex change can occur by two ways: 1) in a fixed manner, only occurring provided a given size or age is reached (Warner, 1975, West, 2009). Many fish have indeterminate growth following the Von Bertalanffy curve (Kozłowski, 1996) so size and age are correlated. 2) By environmental information, that responds to social-ecological conditions based on inhibition and stimulation mechanisms. Inhibitor or suppressor mechanisms occur, for example, in protogynous species with a male dominant in the harems of females, when they prevent sex change to a candidate fish; whereas stimulation or induction mechanisms occur when sex change does not occur automatically when the dominant one is lost or removed of the group (Ross, 1990). In addition, sex change can respond evolutionarily to demographic parameters such as fertility, mortality and growth (Warner, 1975). Thus, sex allocation theory already included demographic traits that favour sex change (Charnov, 1982).

3.3.1 Sequential hermaphrodites: The size-advantage model

The best-known and supported theoretical framework to suggest when to sex change is the *size-advantage model*, which it is analogous to the Trivers and Willard (1973) hypothesis mentioned above but with the variables size or age. This model suggests that sex change is due to the increase of reproductive success by body size, favouring the first sex that matures slowly and then sex change when the second sex achieves a greater fitness at a larger body size (Ghiselin, 1969, Warner, 1975, Warner et al., 1975, Leigh et al., 1976, Charnov, 1982). Therefore, natural selection would favour the sex in which individuals achieve a greater reproductive success by increasing body size (**Fig. 4**).

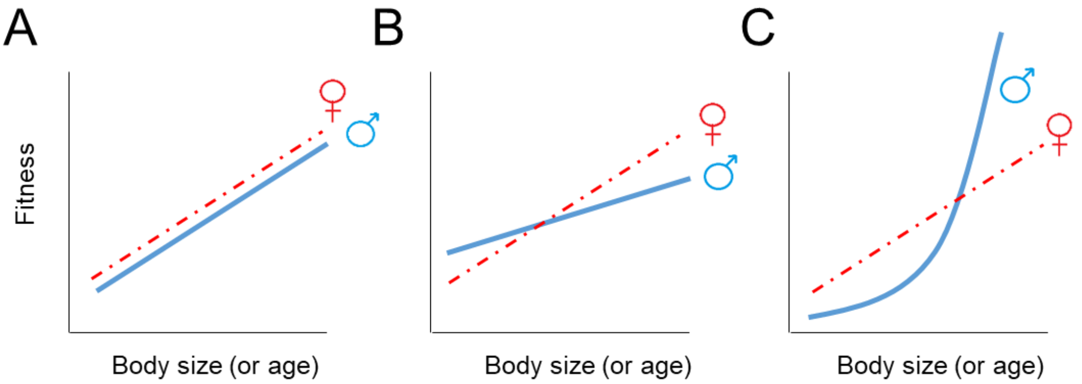


Figure 4. Interpretation of the size-advantage model according to each sexual system. The female has always a constant increase of fitness while that of males varies. A) Gonochorism; both male and female have the same fitness with increasing age/size, B) Protandry, females fitness increases faster than males with age/size C) Protogyny, male fitness greater increases with age/size. Modified from Warner (1984).

Empirical studies support this model in several fish families, predicting quantitatively the size or age at which individual should change sex with life-history dimensionless, i.e. regardless of biological details. A particular study that when it was published got a lot of attention found that in sex changing animals, including fish, and regardless of group sex change invariably occurs when they attain 80% of their maximum body size, or 2.5 times their age at maturity" (Allsop and West, 2003). This is an example of what are called life-history invariants, defined as characteristics that do not change in the life-history of animals (Charnov, 1993). However, these results were questioned in species with populations subdivided into breeding groups (Munday et al., 2006).

The size-advantage model also predicts non-Fisherian sex ratios in sequential hermaphrodites. Thus, male-biased sex ratios are typically observed in populations of protandrous species, where individuals reproduce first as males, change sex and then reproduce as females, which are larger, and hence more fecund than both smaller females and large males. In contrast, female-biased sex ratios are observed in populations of protogynous species, where individuals are born females and later switch to males, which have higher fitness than both smaller males and large females (Shapiro, 1987, Warner, 1975). These predictions have been confirmed in an study involving 121 species of five phyla: fish, arthropods, annelids, molluscs and echinoderms was tested (Allsop and West, 2003, Allsop and West, 2004). Recently, it has been shown that in sequential hermaphrodites, particularly in protogyny, reproductive success is higher in the second sex and that, overall, results in a reduction of effective population size. Thus, the direction of sex change influences the demography and resilience of sex-changing fish (Benvenuto et al., 2017).

Empirical studies show that not only size or age influence sex change, but that various reproductive strategies and tactics can respond to ecological and social conditions and affect the process of sex change. There are several situations of dominance in a social structure that allow them to sex change (Munday et al., 2006). Thus, in practice sex change will only occur when there is a net increase in the reproductive success of an individual (Warner, 1988a); otherwise it will not take place (Munday et al., 2006). One study strongly supported this focusing in the general predictions of the size-advantage model (Munoz and Warner, 2003, Munoz and Warner, 2004). Thus, it was shown that larger individuals do not always change sex, as one would expect, since there are some aspects, such as sperm competition environments or size-fecundity bias, that could benefit a large female and the one who will change sex will be a smaller female (Munoz and Warner, 2003, Munoz and Warner, 2004).

There are three models or hypothesis that may explain bi-directional sex change in sequential hermaphrodites. 1) The *risk of movement model*, which states that instead of moving to another area in search of a partner, an individual may change sex, stay in the same area and thus avoid being preyed. Support for this model was gathered in the gobid *Gobiodon histrio* (Nakashima et al., 1995, Munday et al., 1998). 2) The *growth-rate model,* which states that both sexes benefit from the size of the female because it increases faster than a male. Therefore, when a pair of the same sex find each other, according to their size, the smallest fish will change to female while the bigger one to male (Kuwamura et al., 1994, Nakashima et al., 1996). This model was tested considering four different situations in *G. histrio*: a pair of females, a pair of males, a pair where the male was larger than female and a pair where the female was larger than male. Sex change occurred in the first three scenarios (Munday, 2002). 3) The *loss of dominance hypothesis,* which applies when dominant male losses their privileged position in a polygamous system and it becomes a non-reproducing subordinate because another male has taken his place. In this situation, the former dominant male can change sex back to female. There is evidence of such behaviour in the gobid *Trimma okinawae* in field experiments (Sunobe and Nakazono, 1990, Sunobe and Nakazono, 1993).

3.3.2 Simultaneous hermaphrodites: The low-density model

In the development of the sex allocation theory, a simple mathematical model was devised to predict when gonochorism was favored over hermaphroditism taking into account the fitness (also called the reproductive success) between the male and female function (Charnov, 1982, Charnov et al., 1976). According to this model, gonochorism is favored when the success of one sex is compensated with the other sex, i.e., is negatively correlated to the success of the other sex. The factors that can favour it (concave curve in **Fig. 5**) making hermaphroditism unstable, can be for example, high fixed costs in reproductive structures (Heath, 1977) or the high population density and partner search (Puurtinen and Kaitala, 2002, Leonard, 2010).

Hermaphroditism is favoured when the success of one sex is positively correlated with the success of the other sex. Hermaphrodites, who divide their resources between the two sexual functions link the success of one role to the success of the other one. Factors that may favour hermaphroditism (convex curve in **Fig 4**) are, for example, the low probability of finding a mate (Ghiselin, 1969).

The traditional model aimed to explain the existence of simultaneous hermaphroditism has been the so-called low-density model (Ghiselin, 1969). This model states that simultaneous hermaphroditism is likely to evolve in species living in low-density or isolated populations because of the small probability of finding a mate. These circumstances constitute a handicap, especially for the male function since individuals have the potential of obtaining higher reproductive success specializing as males and the lack of enough females can saturate their fitness (Charnov, 1979, Charnov, 1982). Simultaneous hermaphroditism is expected to occur in some sessile organisms, such as tunicates; in parasites, such as trematodes; or in deep-sea organisms, such as some fishes (Ghiselin, 1969). However, the low mobility and availability of mating partners are not sufficient to ensure the appearance of simultaneous hermaphroditism since some organisms such as, for example, some gastropods exhibit very little mobility, yet they are not simultaneous hermaphrodites but gonochoristic (Leonard, 2013). Furthermore, there are simultaneous hermaphrodites that live in populations in which it does not seem to be a problem to find a co-specific, suggesting that there may be alternatives to the low-density model.

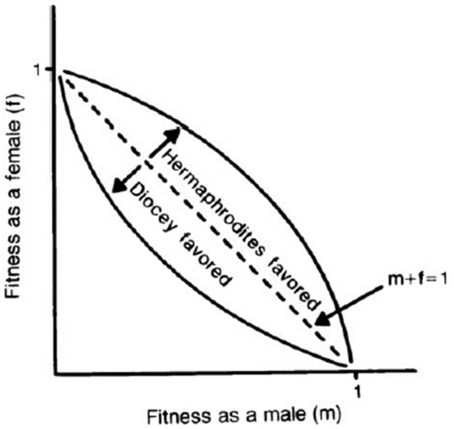


Figure 5. Advantage of be dioecy (concave) and hermaphroditism (convex) through trade-off between male and female fitness. Taken from Charnov (1982).

It has been stated that sex allocation theory addresses the question about how much energy should be devoted to male vs. female function in simultaneous hermaphrodites. This can lead to both sexual conflict, i.e., how to allocate resources in the ovotestis

(Fischer, 1981, Fischer, 1984), and to local mating competition, which can, in turn, lead to sperm competition (Schärer et al., 2014). Nevertheless, sex allocation must still be flexible enough, as simultaneous hermaphrodites must decide how optimally to allocate resources to male and female tissues within the same gonad or behaviour within the same individual (Schärer and Janicke, 2009).

Studies in serranids have been carried out to test the predictions of the sex allocation theory regarding simultaneous hermaphrodites (Fischer, 1981, Fischer, 1984, Fischer and Petersen, 1987, Petersen, 1991, Petersen and Fischer, 1996, Petersen, 2006). These predictions include female-biased sex ratios when there are limited opportunities to mate and male-biased sex ratios with an increasing number of mates (see (Schärer and Janicke, 2009), for review). On the other hand, a density experiment demonstrated how sex allocation in simultaneous hermaphrodites was phenotypically plastic. In low densities, the body size increases and invests in male allocation, showing a trade-off between male function and growth in *Serranus tortugarum* (Hart, 2016).

4. Transition among sexual systems

There have been several attempts to reconstruct the evolution of sexual systems in animals, where simultaneous hermaphroditism was suggested to be the ancestral state that evolved to gonochorism rather than the other way around (Iyer and Roughgarden, 2008). However, other studies found mixed support between simultaneous hermaphroditism and gonochoristic as ancestor of animals was reported (Eppley and Jesson, 2008, Sasson and Ryan, 2017), suggesting that transitions from gonochoristic to simultaneous hermaphroditism were more likely (Eppley and Jesson, 2008).

Recently, Leonard (2013; 2018b) stated that a plausible evolutionary sequence for transitions between gonochorism and simultaneous hermaphroditism in animals was lacking, and proposed that selection for decreasing phenotypic plasticity in sexual phenotype could explain the transition between simultaneous hermaphroditism and gonochorism through intermediate stages of environmental sex determination and sequential hermaphroditism (**Fig. 6**).

Several studies in a variety of taxa from plants to invertebrates, have contributed to our knowledge about the evolution of sexual systems (Baeza et al., 2009, Jarne and Auld, 2006, Weeks et al., 2009, Eppley and Jesson, 2008).

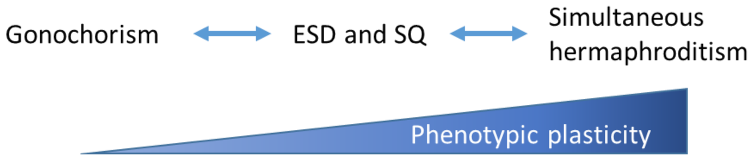


Figure 6. Gradient from gonochorism to simultaneous hermaphroditism. Abbreviations: ESD, environmental sex determination mechanism; SQ, sequential hermaphroditism. Adapted from Leonard (2018b).

4.1 In plants

In plants, there is a well-developed body of theory concerning the evolutionary pathways from simultaneous hermaphroditism to gonochorism or the other way around (Delph, 2009), being gonochorism a derived and a less frequent condition (Barrett, 2002, Sasson and Ryan, 2017) and both are evolutionary stable conditions. Thus, for example, in angiosperms, gonochorism evolved independently several times from a simultaneous hermaphrodite ancestor (Bawa, 1980).

The evidence of intermediaries between gonochorism and hermaphroditism systems only have been determined in plants (Charlesworth and Charlesworth, 1978). In flowering plants, gynodioecy was suggested as such an intermediate and it has been documented in >500 species in 50 families (Saur Jacobs and Wade, 2003) rather than androdioecy (Charlesworth and Charlesworth, 1978, Charlesworth, 2006).

4.2 In invertebrates

In contrast to the situation in plants, simultaneous hermaphroditism is generally considered a condition evolved from gonochorism in animals (Ghiselin, 1969), as it occurs, for example, in molluscs (Collin, 2013), although it should be stated that the opposite has been documented in barnacles (Kelly and Sanford, 2010). The evidence of intermediaries between gonochorism and hermaphroditism systems is rare in animals, with no evidence of gynodioecy. However, androdioecy has been suggested in some invertebrates and some fish (Lorenzi and Sella, 2008, Barrett, 2010, Avise, 2011, Weeks, 2012) while trioecy in a few cnidarians (Weeks, 2012, Leonard, 2018b).

4.3 In vertebrates

Fish is the only group of vertebrates where hermaphroditism and sex change is found. The diversity of sexual systems across the phylogenetic tree of fishes suggests multiple evolutionary origins and transitions, thus making them a key group to study the evolution of different forms of hermaphroditism (see ‘5.2 Transition among sexual systems in fish’ section below). On the other hand, reptiles present gonochoristic and parthenogenetic sexual systems and the latter are believed to evolve from the hybridization of two gonochoristic species (Crews et al., 1986).

4.4 Williams' Paradox

The predominant ideas in the 70s about the benefits of hermaphroditism over gonochorism suggested that the sexual systems should be very sensitive to ecological parameters and, in the case of simultaneous hermaphroditism, particularly to the chances of finding mates (Williams, 1975). However, a large amount of empirical data shows that the best predictors of sexual systems distribution at large-scale in plants and metazoans is phylogeny, i.e., phylum or class (Leonard, 2013; 2018b), not ecology, because their evolution from these taxonomic levels are considered ancient and stable in spite of diverse ecological environments (Leonard, 2018b). Based on the difference between theory predictions and actual data Leonard (2018b) called this discrepancy the "William's paradox" (Leonard, 2013; 2018b). Nevertheless, it is true that at least in fish closely related species may have different sexual systems, thus supporting William (1975) assertion, and thus recently Leonard questioned whether Williams's paradox was really a paradox (Leonard, 2018b).

A phylogenetic study in a variety of hermaphoritic families showed multiple evolutionary pathways among the different sexual systems within single lineages. Therefore, the distribution of sexual systems are influenced by exogenous factors, showing, in principle, little evidence of phylogenetic inertia (Erisman et al., 2013).

Since George Williams published his work in 1975, progress has been made in understanding sexual systems, although we still lack a body of theory that can explain the actual distribution of sexual systems in animals. Further, we still do not know the evolutionary transitions between hermaphroditism and gonochorism and for that and regarding Williams' postulates, it is necessary to better know the influences that abiotic factors may have. Thus, to relate whether the distribution of different types of hermaphroditism in fish is due to abiotic factors, it would be necessary to test some of the most common associations found recurrently in the literature, e.g., and just to name a recurring one, the presumed lack of hermaprodite fish in freshwater (Wootton and Smith, 2014, Pavlov et al., 2009).

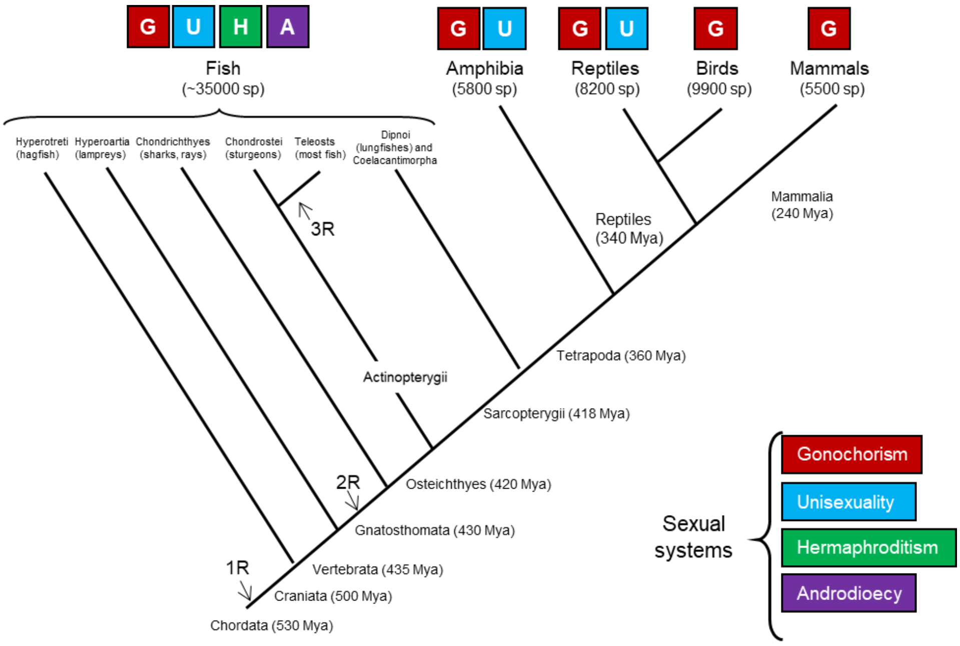
5. Sexual system in fish

The great reproductive diversity of fishes is reflected in their sexual systems. Actinopterygian fishes is the only group that includes all known reproduction types in vertebrates: gonochorism, unisexuality, various forms of hermaphroditism and androdioecy (Atz, 1964, Yamamoto, 1969, Beukeboom and Perrin, 2014, Smith and

Wootton, 2016, Costa et al., 2010) (**Fig. 7**). Unisexual species in fish account less than 1% of all fish species for which their sexual system is known (Piferrer, 2018). There are two types of unisexuality, gynogenesis and hybridogenesis. A classical example of gynogensis is the Amazon molly, *Poecilia formosa*, typical of Southern United States and Mexico (Darnell and Abramoff, 1968), whereas a typical example of hybridogenesis are members of the live-bearing clearfin livebearer, *Poecilospsis lucida* (McKay, 1971), common in Central America.

Within sequential hermaphroditism, besides protogyny and protandry there are some species capable of changing sex in both directions several times during their lifetime, which is called bi-directional sequential hermaphroditism or both-way hermaphroditism (Devlin and Nagahama, 2002). Bi-directional sex change is rare in the wild and thus it has been found naturally only in gobies (Kuwamura et al., 1994, Nakashima et al., 1996). However, bi-directional sex change can be induced in the laboratory, something that testifies the capacity for sex change in fish and facilitates its study at the social and molecular levels (Sunobe and Nakazono, 1993, Kojima et al., 2008).

In simultaneous hermaphrodites, outcrossing is the predominating mating system (Avise, 2011) since it facilitates genetic recombination (Maynard Smith, 1978). However, as an exception to this predominance, there are two species, *Kryptolebias marmoratus* and *K. hermaphroditus* of the family Rivulidae (Harrington, 1961, Costa et al., 2010, Leonard, 2013), which can self-fertilize. However, it has been argued that in fact they are androdioecious, suggesting that this system may be advantageous in some circumstances (Lubinski, 1993). Nevertheless, in general, gynodioecy and androdioecy conditions are only rarely reported in fish (Petersen and Fischer, 1986, Robertson and Justines, 1982), indicating that these mixed systems do not constitute a solid evolutionary stable strategy (Charnov et al., 1976) in fish.



5.1 A brief description of fish evolution

Fish are the most primitive group of vertebrates but in terms of number of species represent a bit more than half of all known species of vertebrates, while amphibians, reptiles, birds and mammals together account for approximately the other half (**Fig. 6**). The evolution of fish took place after the first whole genome duplication (WGD), noted as 1R, when the Agnatha (myxini and lampreys) emerged during the late Ordovician period (~440 Mya). Later the Gnathostomata appeared from another WGD (2R) (Holland et al., 1994) and the Chondrichthyes (cartilaginous fishes) emerged in the Silurian period (~430 Mya). Finally, between the late Silurian and the early Devonian period, the Actinopterygii (ray-finned fishes) originated and diverged from the sarcopterygian, lobe-finned fishes (Smith and Wootton, 2016). In turn, from the Actinopterygii evolved the Chondrostei and the Teleost in 250 Mya, or 200 Mya according to Meyer and Van de Peer (2005). [Note: estimated divergences may greatly vary among different sources]. The teleost are considered the most derived group of fishes that emerged in the late Triassic (~220 Mya) after a third WGD (3R) (Van de Peer et al., 2003). This genomic duplication has facilitated their great biological diversity, radiation and colonization of many different habitats (Meyer and Van de Peer, 2005). Multiple WGDs have been related to their phenotypic complexity (Hoegg et al., 2004), offering opportunities for diversity, even in reproduction. However, the correlation between 3R and biological diversity has also been questioned on the ground that only existing species were considered rather than including fossils as well (Donoghue and Purnell, 2005).

Hermaphroditism in teleost fish has evolved several times independently in some clades (Avise and Mank, 2009), suggesting that all teleost individuals have the potential to express hermaphroditism (Adolfi et al., 2018). Therefore, teleosts are second to none among vertebrates as a suitable group where to study the plasticity of sexual systems. Specifically, (Adolfi et al., 2018) showed how hermaphroditism in teleost fish could evolve thanks to the loss of the Müllerian duct, with the consequence that gamete release became totally independent of the urinary system, making anatomic and developmental preconditions which were optimal for the emergence of physiological sex change (Adolfi et al., 2018). This means that the disposition of the male and female gonoducts can facilitate the transition between sex roles. Furthermore, Amh/Amh2 (anti-Müllerian hormone and its receptor) signalling had a preeminent role in both germ cell maintenance, proliferation and sex differentiation (Adolfi et al., 2018).

Figure 7 (previous page). Evolution of teleost fish and the distribution of sexual systems in the major groups of vertebrates. The ‘R’ indicate each genome duplication, three in total. Modified from Piferrer (2018).

5.2 Transition among sexual systems in fish

Based simply on analysis of its distribution in fish, gonochorism is believed to be the ancestral condition from which all other sexual systems evolved because of its high presence in all basal lines as well as in other vertebrate lineages (Atz, 1964, Ghiselin, 1969, Policansky, 1982, Cole, 2010). The different systems of hermaphroditism evolved independently in several fish lineages (Ross, 1990, Mank et al., 2006), showing to be an evolutionarily labile system (Smith, 1975). Therefore, hermaphroditism is a polyphyletic sexual system because of its multiple origins among the clades, derived from a gonochoristic ancestor (Avise and Mank, 2009). Studies have been focused on specific families such as Pomacentridae, Gobiidae and several families of the Aulopiformes, where it was shown that gonochorism is the ancestral state from where hermaphroditism evolved (Erisman et al., 2013, Cole, 2010, Davis and Fielitz, 2010). In contrast, in other families such as Labridae, Serranidae and Epinephelidae protogynous was found to be the ancestral sexual system (Kazancioglu and Alonzo, 2010, Erisman et al., 2013). However, Sparidae, it is not yet resolved (Erisman et al., 2013) and because of its great diversity of sexual systems it would be an interesting family to study. However, determination of the ancestral sexual system in teleosts or Actinopterygians as a whole has not yet been formally tested, a situation no longer tenable taking into account all modern phylogenetic methods that are currently available.

The rates of transitions among different sexual systems in fish have also not been determined on a large scale either considering all Actinopterygian or focusing just on the teleosts. However, in Serranidae (ancestor = protogyny) it was shown how gonochorism evolved once in the genus *Paralabrax,* and how simultaneous hermaphroditism once evolved a lineage with several genera. However, the evolutionary transitions leading to simultaneous hermaphroditism and gonochorism could not be well resolved (Erisman and Hastings, 2011). Further, the androdioecy suggested in *Serranus baldwini* and *Serranus psittacinus* was considered as a possible intermediary state between a simultaneous and a sequential hermaphrodite by its haremic system (Hastings and Petersen, 1986, Sadovy and Domeier, 2005, Avise, 2011). However, it was finally determined that it was not related to protogyny, suggesting that transitions between sexual systems do not require intermediaries (Erisman and Hastings, 2011, Erisman et al., 2013). The transition from simultaneous hermaphroditism to androdioecy seems to occur in Rivulidae family (Costa et al., 2010). Therefore, the evolution of androdioecy from the simultaneous hermaphroditism would contradict the hypothesis that simultaneous hermaphroditism is an endpoint (Erisman et al., 2013, Leonard, 2013).

In the Aulopiformes, whose ancestor is gonochorism, simultaneous hermaphroditism seems to have evolved once during the early Cretaceous, representing the earliest evolution of simultaneous hermaphroditism (Davis and Fielitz, 2010). In addition, in Gobidae, whose ancestor is believed to be gonochoristic, some bi- directional genera such as *Trimma* and *Priolepis* genera evolved directly without protogynous intermediaries (Sunobe et al., 2017)

Thus, an overall study of the evolutionary history of sexual systems, the transitions rates between sexual systems and which of them are stable or unstable in teleosts is lacking. A good understanding of the evolution of sexual systems is relevant for evolutionary studies of adaptations and speciation.

5.3 The incidence of the different types of sexual systems

Over the years, several studies have determined the incidence of hermaphrodites in fish. It was determined in 2% of 25,000 fish species (Pauly, 2004) distributed in more than 20 families in nine orders (Mank et al., 2006). The most recent study on the distribution of hermaphroditism in teleost fishes confirms that functional hermaphroditism occurs in 6% of all teleost families, corresponding to 7 orders, 27 families and 94 genera (Sadovy de Mitcheson and Liu, 2008). Currently, more than ~35,000 species of fish grouped in 520 families have been documented (Fricke et al., 2019). This represents a 25% increase with respect to the number of species present when the incidence of hermaphroditism was last assessed in fish. Thus, the incidence of hermaphroditism is currently unknown, Therefore, a comprehensive and updated account on the phylogenetic distribution of the sexual systems in Teleost fishes is urgently required.

Until now, the distribution of each sexual system within teleosts showed that sequential hermaphroditism predominated in four orders and fifteen families while simultaneous hermaphrodites was presnt the four orders of nine families (Sadovy de Mitcheson and Liu, 2008) since the presence of opposing systems and antagonism pathways in the same body is complex (Bull and Charnov, 1985). Within sequential hermaphroditism, protogynous predominates in three orders and eleven families (Sadovy de Mitcheson and Liu, 2008) present especially in wrasses (Labridae; (Nakamura et al., 1989), groupers (Epinephelinae;(Shapiro et al., 1993) and porgies (Sparidae: (Buxton and Garratt, 1990). On the other hand protandry is rarer (Avise and Mank, 2009) but representing the second most common type of hermaphroditism in fish . It is distributed in three orders and six families (Sadovy de Mitcheson and Liu, 2008) and it is present, for example, in anemone fish (Pomacentridae; (Godwin, 1994) and in some porgies (Sparidae; (Wu et al., 2010). Third, bi-directional sequential hermaphroditism occurs in only six families of perciforms, mainly in gobies (Tanaka, 1990, Hioki, 1996, Nakashima et al., 2000, Wittenrich and Munday, 2005). Examples of fish that change sex naturally are the redhead goby, *Paragobiodon echinocephalus* (Nakashima et al., 1995) and the okinawa rubble goby, *Trimma okinawae* (Manabe et al., 2007). Examples simultaneous hermaphroditism in fish include the belted sandfish, *Serranus subligarius* (Clark, 1959), the paintspotted moray, *Gymnothorax pictus* (Fishelson, 1992) and the shortnose greeneye, *Chlorophthalmus agassizi* (Mead, 1959).The androdioecy system was not considered in the Sadovy de Mitchelson and Liu (2008) review.

5.3.1 Families where hermaphroditism is present in fish

As mentioned above, the incidence of hermaphroditism occurs in 6% of all teleost families (Sadovy de Mitcheson and Liu, 2008). Some hermaphrodites have a high economic value in fisheries and aquaculture. Here, we show some of the families that contain hermaphrodite species of high commercial interest for fisheries or aquaculture, such as Serranidae, Sparidae, Labridae. In addition, some hermaphroditic species important for aquariology such as Gobiidae and Pomacentridae.

The Serranidae, named globally as sea basses, is one of the largest Perciform families with around 75 genera and 549 species (Froese and Pauly, 2018). Currently, they are classified in five subfamilies: Serraninae or sea basses, Anthinnae or anthiines, Epinephelinae or groupers, Grammistinae or soapfishes and Liopropominae species (Fricke et al., 2019). They attain up to 3 m maximum length and 400 kg of weight. They are widely distributed in all salinities and latitudinally comprising from the tropics to temperate regions. In general, species can reach depths of 100 m but some groupers up to 500 m (Froese and Pauly, 2018). It is a family with species of high commercial and nutritional value (Froese and Pauly, 2018). For example, aquaculture production of *Epinephelus coioides* (**Fig. 8A**) in Asia and the Pacific is 317 Tm, while *Epinepheus aeneus* (**Fig. 8B**) is of great economic importance for fisheries. In the Mediterranean and west coast of Africa capturers reached 6,837 Tm in 2016 (Fisheries, 2016).

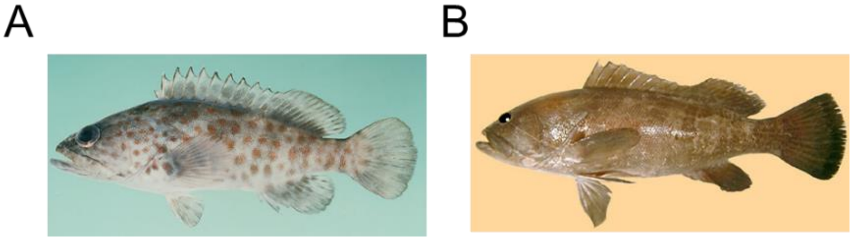


Figure 8. Serranidae (Epinephelinae subfamily) representative species. A) Orange-spotted grouper, *Epinephelus coioides* and B) White grouper, *Epinephelus aeneus*. Pictures taken from Randall, J.E. (1993) and Dammous, S. (1998), respectively, from FishBase.

The Sparidae, commonly named porgies or seabreams, is one of the most diverse Perciform families with around 38 genera and 159 species (Froese and Pauly, 2018). They comprise a wide geographical distribution, mainly in tropic and temperate coastal regions (Chiba et al., 2009) and they are present in all salinity habitats. They attain up to 2 m maximum length and 80 kg of weight (Froese and Pauly, 2018). Most seabreams are excellent food and thus they are of high commercial importance. Few example, such speceis present in the Mediterranean Sea include *Pagellus bogaraveo* (**Fig. 9A**) with a captures of 194 Tm and *Diplodus sargus* (**Fig. 9B**), with captures of 4,078 Tm and global aquiculture production of 15 Tm. Finally, the gilthead seabream, *Sparus aurata* (**Fig. 9C**) with global captures of 9,430 Tm and global aquiculture production of 185,980 Tm (Fisheries, 2016).

Figure 9. Sparidae representative species in the Mediterranean Sea. A) Blackspot seabream, *Pagellus bogaraveo*, B) White seabream, *Diplodus sargus* and C) Gilthead seabream, *Sparus aurata*. Pictures taken from Dammous, S (1999), Patzner, R.A. (1992) and Fisheries (FAO, 2016), respectively.

The Labridae, commonly named as wrasses, is the third largest Perciform family with around 71 species and 548 species (Froese and Pauly, 2018). They are exclusively found in saltwater, comprising form tropical to temperate regions. In general, species can reach depths of 120 m but some up 500 m (Froese and Pauly, 2018). They can attain up to 1.7 m maximum length and 191 kg of weight. This family contains species of high commercial value (Froese and Pauly, 2018). For example, *Labrus bergylta*, (**Fig. 10**), distributed between Norway to Morocco from 64ºN to 27ºN latitudes is fished with, for example, total captures of 149 Tm in Norway and 13 Tm in Portugal in 2011 (Froese and Pauly, 2018).

Figure 10. Labridae representative species. A) Ballan wrasse, *Labrus bergylta*. Taken from Østergaard, Thorke A.S. (2000) in FishBase.

The Gobiidae, simply referred to as gobies, is the largest family of vertebrates 258 genera and 1,843 species (Froese and Pauly, 2018). Currently, it is considered not as part of the order Perciformes but inside the order Gobiiformes (Nelson, 2006). They are widely distributed in marine and brackish waters and rarely in freshwater. They mostly live in tropical and subtropical regions (Froese and Pauly, 2018). In general, species can reach depths of 80 m. They attain up to 0.8 m maximum length and 0.6 kg of weight (Froese and Pauly, 2018). This family has species important for acuariology, such as *Gobiodon okinawae* (**Fig. 11A**).

Finally, the Pomacentridae, commonly named the damselfishes, is a perciform family with around 29 genera and 400 species (Froese and Pauly, 2018). They are widely distributed in tropical and few temperate regions in saltwater but are rare in brackish water and freshwater. In general, species can reach depth of 60 m (Froese and Pauly, 2018). They attain up to 20 cm maximum length. Damselfishes are valued aquarium fishes, although some of them such as *Amphiprion ocellaris* (**Fig. 11B**) are extremely aggressive (Froese and Pauly, 2018).

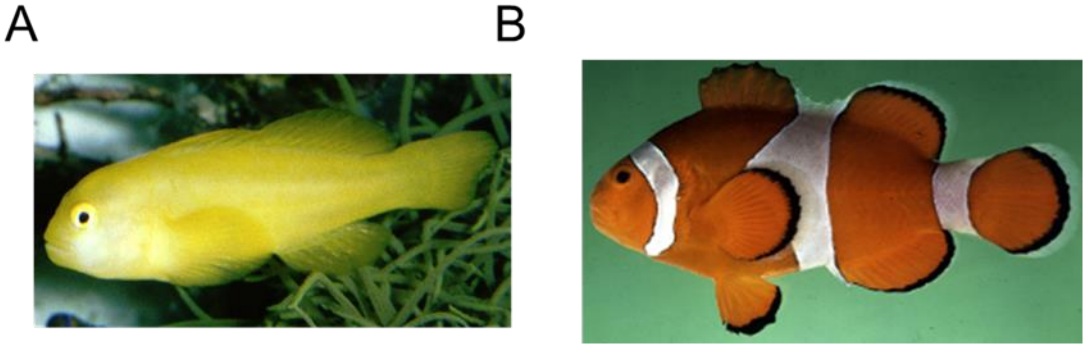


Figure 11. Aquarium representative species of Gobiidae and Pomacentridae, respectively. A) Okinawa goby, *Gobiodon okinawae* and B) Clown anemonefish, *Amphiprion ocellaris*. Taken from Randall, J.E. (1997) and Kockzius, M. (2000) in FishBase.

6. Ultimate causes for the existence of hermaphroditism in fish

In biology, ultimate causes is a term employed to define the evolutionary forces that favour the appearance of a given trait, in this case a particular sexual system such as hermaphroditism. Next, we explain the ultimate causes that traditionally have been viewed as promoting the existence of hermaphroditism in Metazoa but we will focus the discussion on the situation in fish.

6.1 Social structure

Here, we define social structure as the organization of a species in a given space considering whether this grouping is temporary or permanent. In the literature, the term social structure or social systems is often confused with mating systems (explained below). First, most fish make large aggregations, consisting of groups of individuals in high densities in order to reproduce. They usually do so at the same place and time of the year. The most common are resident aggregations, where individuals move over short distances and reproduce frequently, while in transition aggregations individuals move long distances and reproduce few times a year. Examples of aggregation species are groupers and snappers (www.SCRFA.org).

Second, the haremic-polygynous is a type of social structure whose grouping is permanent and where a large dominant male monopolizes mating access to several females, controls a territory and provides shelter and protection to these females (Brennan, 2010). Examples of haremic systems are redband parrotfish, *Sparisoma aurofrenatum*, sea goldie, *Pseudanthias squamipinnis*, dusky grouper, *Epinephelus marginatus*, and bluestreak cleaner wrasse, *Labroides dimidiatus* (Allsop and West, 2003). This social structure occurs in the serranid lantern bass, *Serranus balwini* and barred serrano, *S. psittacinus*, simultaneous hermaphrodite that lost its ovarian tissue, becoming a functional large male that monopolizes several small simultaneous hermaphrodites (Hastings and Petersen, 1986, Fischer and Petersen, 1987, Petersen, 2006, Erisman and Hastings, 2011, Erisman et al., 2013).

Finally, and in relation to intersex selection, the pre-mating strategy that allows females to select the best males are the male aggregations that form 'leks' (Brennan, 2010). A lek is the temporary aggregation of males visited by females singly or en masse, who select the males with whom they mate. Once mating is accomplished, the females leave the lek (Pandian, 2011). Examples of species that make leks are the slippery dick, *Halichoeres bivittatus*, leopard coralgrouper, *Plectropomus leopardus*, redfin parrotfish, *Sparisoma rubripinne,* and bluehead wrasse, *Thalassoma bifasciatum* (Allsop and West, 2003).

6.2 Mating systems

Mating systems is the pattern of sexual interactions that take place considering the number of males and females involved in reproduction (Leonard, 2018). Most sexual systems except protogyny exhibit size-assortative mating, which means that individuals of one sex tend to mate with individuals of the other sex and of equivalent size or at least of equivalent size range, thus expecting that individuals chose the larger available partner within their range (Fischer and Petersen, 1987; Warner, 1988).

Teleosts exhibit mainly two mating systems (Patzner, 2008, Wootton and Smith, 2014) classified according to the number of males and females present in a mating event. First, monogamy, a permanent couple consisting of a male and a female with some long-term commitment. An example is *Serranus tigrinus* (Fischer and Petersen, 1987, Petersen, 2006). However, species engaging in this system can sometimes spawn with an extra-partner (Wootton and Smith, 2014). Second polygamy, which occurs between several individuals but there is a certain selection of pairs in each sexual act, distinguishing two types; when a male reproduces with several females (polygyny) or when a female reproduces with several males (polyandry) (Smith and Wootton, 2016). In addition, there is the promiscuous system, which can be considered within the polygamy system or not, where several males can fertilize several females and vice versa, which complicates the selection of pairs (Smith and Wootton, 2016). Intrasexual selection evolved mating systems that have an unequal number of sexes such as polygyny, polyandry and promiscuity, with polygyny being the most common while monogamy is not effective because it is composed of a single pair (same number of sexes) (Bateman, 1948). Therefore, the mating systems with unequal number of sexes predominated, and intrasexual selection determines the mating systems and not the other way around, as Darwin (1871) thought (Bateman, 1948).

Finally, the conditional reciprocity (or serial monogamy) is a reproductive behaviour caused by a sexual conflict between simultaneous hermaphrodites. The first evidence of this system in the serranid black hamlet, *Hypoplectrus nigricans*, where members alternate the male and female roles sequentially, releasing eggs in a daily "egg trade" (Fischer, 1980, Fischer, 1981, Fischer and Petersen, 1987, Warner, 1984). This cooperative behaviour occurs mainly in pairs, although some individuals may spawn with other partners in each breeding season.

In systems where non-dominant males cannot mate due to the presence of a dominant male, alternative mating strategies appear. This is possible because most fishes present external fertilization, giving the opportunity to develop several strategies such as sneaker males, female mimics and satellite males (Gross and Shine, 1981, Patzner, 2008). However, about 500-600 species present internal fertilization (Smith and Wootton, 2016). The male sneaker is the strategy where small males sneak during a spawning event involving the dominant males and deposit their sperm to fertilize the eggs of the haremic females. Female mimicry is the strategy where a small male develops a female-like appearance and enters camouflaged the harem to spawn with the true females. In wrasses, this is facilitated because small males have the same body colour as females, unlike large males (Warner and Robertson, 1978, Warner, 1984). Finally, satellite males consist of individuals that are allowed to stay in the harem and participate in reproduction with some females in exchange for their cooperation in the defence of the harem. An evolutionary study of alternative strategies in fish showed that the sneaker is the most ancestral and from it derives the female mimicry and cooperative behaviour of satellites as more complex strategies (Mank and Avise, 2006). Their appearance is correlated with the presence of dominant male sex character to avoid monopolization rather than fleeing parental care (Mank and Avise, 2006, Sayol and Ferrandiz-Rovira, 2018). Most teleost fishes have no parental care after fertilization. However, in some species, there is a parental care, in others maternal care and still in others both (Smith and Wootton, 2016).

6.3 Spawning mode

Spawning mode refers to the reproductive process in which the male releases or deposits his sperm to fertilize the female's eggs in the water. Encompassing the social structures and mating systems described above, it has been grouped into two main spawning modes.

First, group spawning, in which several males spawn with one or more females, and compete for parenthood. In addition, post-mating strategies can be developed, related to increasing the gonad size in males or females can prevent male ejaculation from morphological structures (Brennan, 2010). The social and mating systems corresponding to this type of spawning are the long aggregations with polygamy and promiscuous systems. Second, pair spawning, where a single male spawns with a single female, being the only one that produces the offspring. Pair spawning is related to the social structure of the harem system where females reproduce mainly with the dominant male.

6.4 Sperm competition

Due to allometric growth, individuals can increase their gonads and produce more gametes as they grow. Thus, in fish it is common to increase the size of the testes even more to produce more sperm (Birkhead and Møller, 1998). A relevant factor in several mating systems is sperm competition between males (intrasexual selection). To estimate the intensity of competition, one can measure testis weight (Stockley et al., 1997). However, a preferred measure is the the gonadosomatic index (GSI), defined as the relationship between gonad weight and total body weight in percent (Birkhead and Møller, 1998), is usually used as a better indicator of gamete production. GSI is High GSI values (> 3%) are typically observed in species spawning in large aggregations or group spawning (Buxton and Garratt, 1990, Tucker Jr et al., 1993), where males compete to fertilize the eggs of females. In contrast, in harem-polygyny systems, where a dominant territorial males control a set of females, male-male competition is very low, so the GSI is low (Erisman et al., 2009, Molloy et al., 2007). A study comparing the relationship between sexual and social systems according to the size-advantatge model in a phylogenetic approach in the Epinephelidae family, determined that the evolution of protogynous species was linked to the haremic system, which spawn in pairs and therefore have low sperm competition (Erisman et al., 2009, Walker and McCormick, 2009). Thus, a reduction in post-mating male competition is manifested in the reduced size of the testis of protogynous species compared to their gonochoristic relatives (Warner, 1975; Stockley et al. 1997; Taborsky, 1998; Molloy et al. 2007). Gonochorism is typically related to group spawning, which led to high sperm competition and loss of sex change capacity (Molloy et al., 2007, Walker and McCormick, 2009, Erisman et al., 2009, Erisman et al., 2013). Taking as a basis just a few species of different families, a comparative analysis showed using independent contrasts phylogenetic methods that the GSI of protogynous species are significantly smaller than their gonochoristic congeners (Molloy et al. 2007). However, it would be interesting to determine if this relationship between sperm competition and GSI values holds when current phylogeny trees and modern evolutionary methods are applied to a sufficiently bigger number of teleost species.

In small groups where mating occurs in random or monogamous pairs, sperm competition should not be high (Warner, 1988a) and, therefore low GSI values should be expected. However, the protandrous sparids exhibit much higher GSI values on average than is expected, suggesting that they face more intense sperm competition than previously thought according to the sex allocation predictions (Erisman et al., 2013). Therefore, it would be interesting to investigate the evolutionary history of sexual systems and test predictions of the size-advantage model in protandrous sparids combining information on sexual systems, mating systems, sperm competition and the principles of the sex allocation theory, while accounting for allometric effects and current state-of-the-art phylogeny.

In simultaneous hermaphrodites, sexual conflict can lead to sperm competition (Schärer et al., 2014). In environments where males compete (high sperm competition) resources are allocated to male function, whereas in environments with low sperm competition resources are mainly allocated primarily to female function (Petersen, 1991). This dynamic asymmetry of resources between male and female function contributes to the stabilization of simultaneous hermaphroditism (Petersen, 2006).

6.5 Correlates with other life-history traits

It has been argued that in order to understand the distribution and adaptive value of the different sexual systems, a more detailed and sophisticated understanding of how life-traits parameters interact with the sexual system is required (Leonard, 2013).

In many sequential hermaphrodites, particularly protogynous species living in coral reefs, there is a well identified social control of sex change. This has been linked with the fact that in these species there are many opportunities for mating and complex social systems. It has been speculated that these complex social interactions, therefore, could favour longevity (Sadovy de Mitchelson and Liu, 2008). Longevity is a crucial life-history trait that is influenced by both ecology and evolutionary history as well as growth patterns and mortality rates (Hubble, 2003). However, to the best on knowledge, whether protogynous hermaphrodites live longer than gonochoristic relatives has not been formally tested.

Size at maturity is a crucial trait of an individual's fitness, which is influenced by longevity and selective pressures such as mortality (Roff, 1992, Stearns, 1992, Promislow and Harvey, 1990). It is usually assumed that in fish, there is a positive relationship between age at first maturity and longevity. Thus, short-lived species mature earlier due to strong selective pressure due to high mortality than long-lived ones, which may exhibit initial slower growth but progressively increase the production of eggs with advancing age. Thus, long-lived species tend to be bigger with slow growth, at least initially, but showing a larger period of reproduction (Das, 1994). However, it has been reported that in reef-associated labrids, smaller fish mature at a larger size relative to their maximum adult size than larger species do (Hubble, 2003). Based on the above, it has been suggested, therefore, that hermaphrodites could mature later than gonochoristic relatives (Leonard, 2013), an aspect that until now, to the best on knowledge, never been formally tested.

In sequential hermaphroditism fish, size determine fitness, since a larger body size means, in principle, higher reproductive capacity (Barneche et al. 2018). However, in simultaneous hermaphrodites small body size has been associated in some invertebrates when compared to their gonochoristic relatives because it is associated by the parasitic or interstitial life, although numerous exceptions do exist (Clark, 1978b). A trade-off in energy allocation possibly due to the cost of egg production could lead to lower sizes (van Straalen et al., 2000, Baeza, 2006). However, in fish, the possible link with small body size and simutltaneos hermaphroditism is unknown.

7. Proximate mechanisms

Proximate causes or mechanisms explain biological functions in terms of immediate physiological or environmental factors.

7.1 Sex determination: An overview

Sex determination is the developmental process by which the bipotent gonad establishes the ovary or testis by genetic or environmental factors (Bull, 1983). Fish have labile sex-determination mechanisms, but once established, they experience no change throughout their lives, except in hermaphrodite species that can change sex after maturation (Devlin and Nagahama, 2002). Teleost fishes are an interesting model for the study of sex determination and differentiation because their mechanisms are very varied and plastic (Mank et al., 2006).

The two main types of sex determination mechanisms in gonochoristic vertebrates are genetic sex determination (GSD), where sex is determined by either i) major sex factors (genes) located in sex chromosomes (chromosomal sex determination), an example is blue tilapia, *Oreochromis aureus* (Campos-Ramos et al., 2001), ii) by major sex factors plus secondary loci as European seabass, *Dicentrarchus labrax* (Vandeputte et al., 2007) and cichlids (Oldfield, 2005), or iii) by a combination of sex determine loci distributed exclusively among the autosomes (polygenic sex determination, PSD) as the Atlantic silverside, *Menidia menidia* (Conover and Kynard, 1981). GSD is the dominant sex determining mechanism, is present in all vertebrates, and tend to produce a balanced sex ratio (1:1), although in PSD sometimes the sex ratio may vary due to certain combinations of sex-linked loci. (Kallman, 1984). Although it does not normally occur in wild, some “pure” GSD species, such as *Oryzias latipes,* may have environmental influences if their magnitude is above a certain threshold (Piferrer, 2017). The second major type of sex determination mechanisms is environmental sex determination (ESD), where sex is determined during early sensitive periods after fertilization (Penman and Piferrer, 2008) by external factors and there are no consistent genetic differences between the sexes (Bull, 1983), which provides high plasticity among fish (Mank et al., 2006). This mechanism is not present in birds or mammals (Piferrer, 2017, Piferrer, 2018). However, they are found in reptiles and gonochoristic fish, and the factors influencing the underlying sex determination mechanisms are in principle exclusively abiotic (Piferrer, 2018). Environmental factors that can alter the phenotype of the final sex are basically temperature and pH (Baroiller et al., 2009, Ospina-Alvarez and Piferrer, 2008). Temperature is the most common factor in ESD, where three patterns are exhibited in reptiles (Valenzuela and Lance, 2004) and only one in fish: more males with increased temperature (Ospina-Alvarez and Piferrer, 2008).

Currently, GSD and ESD rather than being mutually exclusive are considered to be at two ends of a continuum (**Fig. 12**) (Sarre et al., 2004, Heule et al., 2014), providing a wide range of possibilities for sex determination mechanisms in fish (Mank et al., 2006).

Sex differentiation is defined as the process by which an undifferentiated gonad develops either into a testis or into an ovary, i.e., the phenotypic or gonadal sex. (Piferrer and Guiguen, 2008). Molecular and physiological mechanisms control the process that will imply morphological changes in the gonad. Morphological and endocrinology aspects are well understood and fairly conserved among fish (Devlin and Nagahama, 2002).

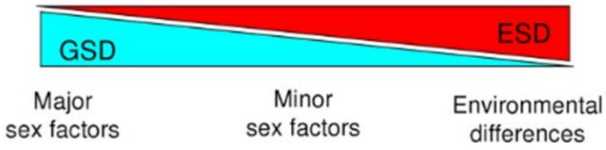


Figure 12. The three major components on the sex determination mechanism. Abbreviations: GSD, genetic sex determination; ESD, environmental sex determination (Penman and Piferrer, 2008).

Over the years, the perception of sex determination has changed before it was seen as a system where a master switch at the top of a hierarchical cascade triggered sex determination (Ohno, 1967, Herpin and Schartl, 2015). However, recently in mammals, mice and some fish it has been demonstrated that there might be different initiating switches that act as interconnected factors (Hodgkin, 1992, Capel, 2017). These networks show antagonistic patterns (Ungewitter and Yao, 2013, Windley and Wilhelm, 2015). Sex determination thus is initiated and then is maintained through strong feedback loops that stimulate the expression of genes associated with a particular sex while silencing or repressing the genes associated with the opposite sex (Munger et al., 2009, Munger et al., 2013, Matson et al., 2011, Capel, 2017). Thus, a continued action of genetic and environmental factors tips the scale toward testis or ovarian differentiation. These networks act at the morphological, physiological and behavioural level, and their structure allows rapid transitions (Capel, 2017).

7.2 Gonadal anatomy and morphology

Most teleost fish have a pair of generally symmetrical bilateral gonads, which may be partially or fully fused, except for primitive fish such as hagfish and lamprey, which have individual gonads. The gonad is the reproductive gland that produces hormones and sex cells or gametes (eggs in females or sperm in males). In males, the sexual gonad is called testes, and in females, it is called ovary. The gonads are dorsally located inside the body cavity of the near the kidneys. Most males have two similar testes wrapped in a tunica albuginea and inside there are seminiferous tubules with germ cells, which give rise to sperm. Most female teleost have two ovaries. The lumen of the ovary and the oviduct are continuous, so germ cells called oocytes will be released from the oviduct into the body cavity (Guimarães-Cruz et al., 2005).

7.2.1 Pattern of sex differentiation in gonochorism

According to the process of development of the gonad in fish, two patterns of sex differentiation are classified in gonochorism (Yamamoto, 1969). First, the differentiated species, whereby an undifferentiated gonad is converted directly into either ovaries or testicles (**Fig. 13A**). Examples are the japanese ricefish medaka, *Oryzias latipes* (Yamamoto, 1969) and the European seabass, *Dicentrarchus labrax* (Bláquez et al., 1998). Secondly, the undifferentiated species, where all individuals initially develop an ovary-like gonad and then, a half remain as females and the other half undergo tissue degeneration by apoptosis to subsequently and immediately undergo testicular differentiation (**Fig. 13B**). Sometimes it is called a false hermaphrodite or juvenile hermaphrodite because the same gonad can contain both ovarian and testicular tissue. However, it is incorrect because the transition to the ovary-like gonad to male sex occurs before the differentiation of the gonad. Examples include the European eel, *Anguilla anguilla* (Grandi and Colombo, 1997), and the zebrafish, *Danio rerio* (Takahashi, 1977).

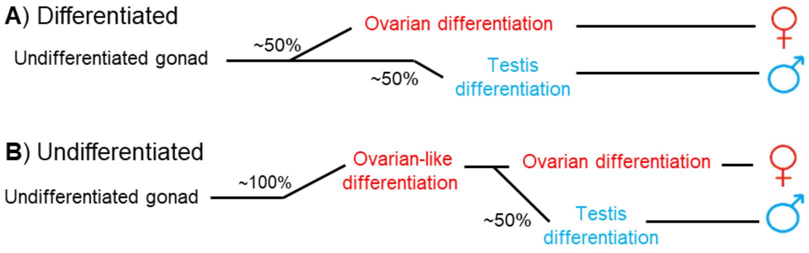


Figure 13. Classification of gonochoristic species based on the pattern of gonadal sex differentiation. **A**) Differentiated species, **B**) Undifferentiated species. Modified from Piferrer (2001).

7.3 Types of gonad morphology in hermaphrodites

Hermaphrodites can present three main types of gonadal morphology according to the disposition of the germinal cells in the gonad (Sadovy and Shapiro, 1987). First, the delimitated type of gonad, where female and male tissue are separated by a membrane of connective tissue. An example is the gonads of members of the family Sparidae, whose male tissue is located in the ventral region, topologically separated from the female tissue. Second, the undelimited type I gonad, in which male and female tissues are separated but do not have any barriers. This is typical of some simultaneous serranids as *Hypoplectrus puella*. Finally, the undelimited type II gonad, whose tissues are completely mixed as it occurs in in Epinephelidae, Labridae and Scaridae (Reinboth, 1962, Reinboth, 1975, Smith, 1965) (**Fig. 14**).

7.4 Gonadal morphology during sex change

A long-held belief, which is probably true but that has never been formally tested, is that plasticity in gonadal morphology in teleost fishes occurs because, in contrast to the rest of vertebrates, where the embryological gonads have two components: cortex and medulla, whereby testes derive from the medulla and ovaries derive from the cortex, in fish, gonads contain only the cortex equivalent from where both ovaries and testes derive (Atz, 1964, Smith and Wootton, 2016). This, presumably, would facilitate sex change.

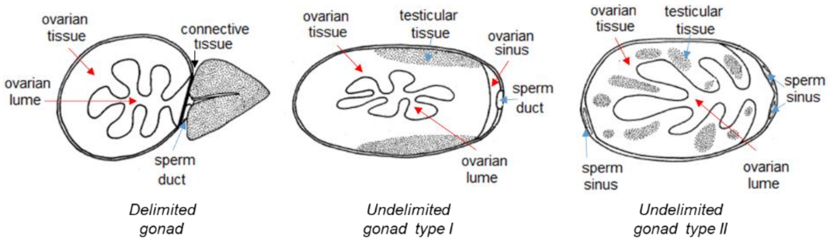


Figure 14. Three gonad morphologies in hermaphroditism fish. Colours of arrows indicate different gender tissues; male (blue) and female (red). Adapted from Sadovy and Shapiro (1987).

In sex-changing species, the gonad undergoes important morphological and behaviour changes, which begin with the degeneration of the germ cells of the first sex, ample gonadal reprogramming and reorganization of the ducts (Hattori and Casadevall, 2016).

The gonad transition in protogynous fish starts from a regressive ovary with previtellogenic follicles that degenerate (ovarian atresia) to the proliferation of spermatogonies until the cells of the spermatogenic cyst predominate (**Fig. 15A**). In the male tissue from an undelimited gonad, certain remnants or traces of the first sex such as ovarian lumen, lamellar structures and atretic follicles are present (Reinboth, 1962, Shapiro, 1987, Hattori and Casadevall, 2016). The presence of female features in the second sex shows evidence that the male was derived from a female (Reinboth, 1980). However, in the delimitated gonad of the sparids, males do not have ovarian remains within the male portion of the gonad. However, ovarian rudiments may be found next to the developed testis (Sadovy and Shapiro, 1987).

In diandry, the gonadal morphology of primary and secondary males differ. Primary males have a simple sperm ducts similar to those of gonochoristic species and do not have ovarian features (Reinboth, 1962, Reinboth, 1970). In contrast, the tissue of secondary males shows sperm sinuous ducts in the gonad walls, a central membrane in the testis cavity and remnants of the ovary lumen of female (Reinboth, 1962, Reinboth, 1970, Chan and Yeung, 1983, Shapiro and Rasotto, 1993). These features constitute morphological evidence of sex-change and include the presence of atretic bodies, sperm sinuses and a membrane-lined central cavity (Sadovy and Shapiro, 1987).

In protandrous species, male tissue degenerate as oocytes develop and mature until the testicular tissue disappears (Hattori and Casadevall, 2016) (**Fig. 15B**). The female with the resulting undelimited gonad rarely contains traces of male tissue (Shapiro, 1987). However, in the delimitated gonad of the sparids, the females may present a small sterile testicular lobe (Reinboth, 1962, Reinboth, 1970, Pollock, 1985, Hattori and Casadevall, 2016) while males, the first sex, show immature ovarian tissues (Sadovy and Shapiro, 1987).

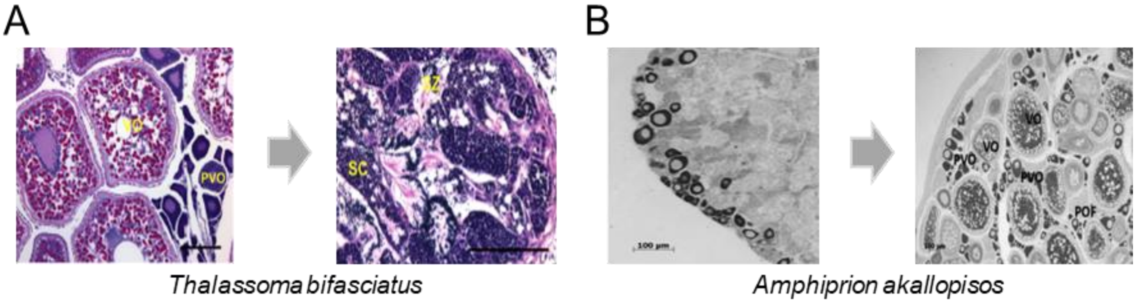


Figure 15. Histological changes in teleost gonads during the process of sex reversal. **A**) Protogynous and **B**) Protandrous hermaphroditism. Pictures are taken from Todd et al. (2016).

Connecting with the unique embryological origin of teleosts gonads as compared to the rest of vertebrates and mentioned above, Francis (1992) suggested that in teleost all gonads initially develop as females and therefore female can be considered the "default" sex. Therefore, oocytes and immature ovarian tissue can be present even in males of gonochoristic species, as is the case of the European sea bass, *Dicentrarchus labrax* (Piferrer et al., 2005). However, the presence of female traces such as oocytes in a male individual is not a faithful indicator of hermaphroditism (D'Ancona, 1949, Shapiro, 1987). Because of this, the protandrous (first male sex) is difficult to be diagnosed (Sadovy and Shapiro, 1987).

7.5 Neuroendocrine control of sex change

Reproduction in vertebrates has a neuroendocrine control that consists of a complex neurochemical interactions between the brain, the pituitary and the gonads (Bieniarz and Epler, 1992, Yaron, 1995, Trudeau, 1997), integrating the nervous and endocrine system (Weltzien et al., 2004) and coordinating physiological responses during sex change (Liu et al., 2017). Environmental stimuli during sex determination in gonochoristic individuals are abiotic such as temperature or pH (or photoperiod at the onset of puberty), whereas in sex-changers environmental stimuli are typically of a biotic nature such as the presence of congeners or social interactions (Capel, 2017). In sex-changing fish, it is believed that the perception of external stimuli takes place through a visual signal, but it is not yet clear how this is integrated into the brain and provides physiological responses (Todd et al., 2016, Capel, 2017) (**Fig. 16**). It was suggested that in protogynous species, behavioural and gonadal changes are produced by independent neuroendocrine pathways (Nakamura et al., 1989, Warner and Swearer, 1991, Godwin et al., 1996).

7.5.1 The hypothalamus-pituitary – gonad (HPG) axis

In vertebrates, reproduction is controlled by the brain-pituitary-gonadal (BPG) axis or also called hypothalamus–pituitary–gonad (HPG) axis (Godwin, 2010), whose signal is initiated in the brain through external information. It is a preserved regulatory system (Maruska and Fernald, 2011). There are three main key components in HPG axis. 1) Gonadotropin-releasing hormone (GnRH) produced in the hypothalamus; 2) gonadotropins (GtHs) such as the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) produced in the pituitary, and 3) sexual steroids produced in the gonads, with multiple positive and negative fee-back loops (Smith and Wootton, 2016). (**Fig. 16**)

Recently, the hypothalamic-pituitary-interrenal (HPI) or hypothalamic-pituitary-adrenal (HPA) axis, which controls the stress response has been considered the most important pathway and the main trigger of sexual transition in protogynous wrasses (Todd et al., 2019). In addition, the HPI axis contributes to modulate important processes such as growth and behaviour (Wada, 2008, Solomon-Lane et al., 2013). The integration of the HPG and HPI axes makes sense but understanding their coordinated roles during gonadal sex differentiation and sex change in fish requires still a lot of research, likely to come in the years to come.

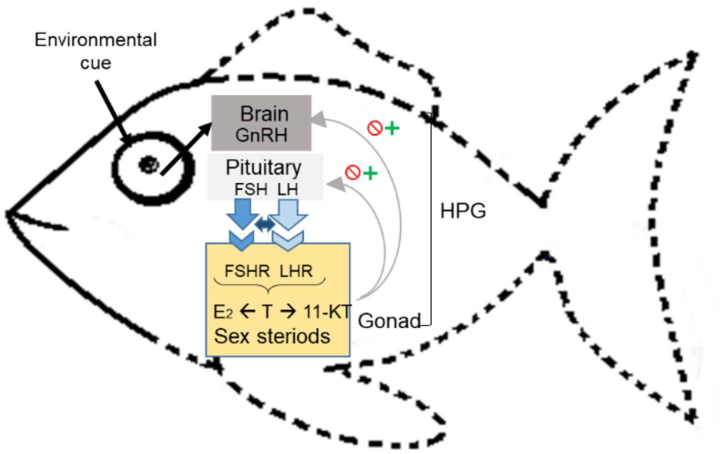


Figure 16. Simplified illustration depicting the integration of environmental cues through the Central Nervous System and their effects on the synthesis of gonadal sex steroid. Only some of the major players are shown. Abbreviations: HPG, hypothalamus-pituitary – gonad axis; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, 17β-estradiol; T, testosterone; 11-KT, 11-ketotestosterone.

7.5.2 Hypothalamic neuropeptides and monoamines

On the HPG axis, neuropeptides and monoamines regulate sexual behavioural change (Godwin and Thompson, 2012, Lamm et al., 2015). The most important hormone peptide that controls and is the main regulator of the initiation of sex change in the gonad from multiple sites on the HPG axis is GnRH. It is secreted in the hypothalamus and acts directly on pituitary gland where it stimulates the synthesis and secretion of GtHs. In the hypothalamus, through GnRH signaling as well as the contribution of other neurohormones, control of FSH and LH synthesis and secretion takes place, allowing in sex-changing species a rapid change in sexual behaviour in addition to changes in the structure of the gonad (Zohar et al., 2010, Godwin and Thompson, 2012, Maruska and Fernald, 2013).

One of the main regulators of GnRH is dopamine (DA), an inhibitor that stimulates the sex change of the gonad when it is decreased allowing the release of GnRH and, consequently, the secretion of LH (Larson, 2011, Zohar et al., 2010). Other peptides that influence the release or inhibition of GtHs are kisspeptin (Kiss1/2), which stimulate GnRH secretion (Elizur, 2009, Mechaly et al., 2013) and gonadotropin-inhibitory-hormone (GnIH) (Moussavi et al., 2013), respetively. However, their exact influences on sex-changing fish remains uncertain.

Serotonin (5-HT) and arginine vasotocin (AVT) stimulate protogynous species to sex change. AVT is associated with male behaviour, courtship and aggressiveness (Godwin and Thompson, 2012, Semsar et al., 2001, Semsar et al., 2004), however, there is a variety of patterns among species and it requires further research (Todd et al., 2016). A detailed description of how neuropeptides may be involved in sex change in protogynous species is found in Liu et al. (2017). On the other hand, melatonin (MEL), secreted in the pineal gland of the brain is the hormone responsible for controlling circadian rhythms and photoperiod effects in reproduction (Falcón et al., 2007, Falcon et al., 2010). Although the relationship between MEL and GnRH in fish is only becoming understood, MEL and NE are the first neurochemicals in the brain that act when the sex change is initiated, increasing LH and resulting, for example, in a rapid coloration in the protogynous bluehead wrasses (Aspengren et al., 2003).

7.5.3 Gonadotropin (GtH) signaling

The GtHs are peptide hormones secreted by the pituitary gland that control steroid hormone levels in the BPG axis (Devlin and Nagahama, 2002, Lubzens et al., 2010). In fish, like in the rest of vertebrates, there are two GtHs, FSH and LH. Both target the gonads where they bind into their specific receptors fshr and lhr, respectively (Bogerd et al., 2005) and regulatesteroidogenesis and gametogenesis (Levavi-Sivan et al., 2010). Thus, the GtHs regulates steroidogenesis by stimulating ovarian follicular cells (vitellogenesis) in females or Leydig somatic cells (spermatogenesis) in males testis (Devlin and Nagahama, 2002, Weltzien et al., 2004).

Specifically, FSH regulates steroidogenesis while LH regulates gametogenesis (maturation of oocytes and sperm) (Swanson et al., 1991, Devlin and Nagahama, 2002, Lubzens et al., 2010). In seabream, FSH is important only in spermatogenesis, while LH regulates gametogenesis in both sexes. In contrast, the specific roles of GnRH and GtH is difficult to determine because different patterns have been found in nearby species. For example, the fshr receptor expresses even contradictory patterns in a protogynous grouper species (Alam et al., 2010).

7.5.4 Molecular endocrinology of sex change

The genes related to sexual differentiation are conserved in vertebrates and are involved in controlling the expression of steroidogenic enzymes and morphological changes in the gonads. Research has shown that the same genes that control gonad differentiation in gonochoristic species are involved in the regulation of sex change in hermaphroditic species (Piferrer and Guiguen, 2008; Todd et al., 2019).

7.5.4.1 Transcription factors

Transcription factors regulate the expression of target genes by binding to their regulatory elements, usually in the promoter region. The most relevant transcription factors for gonadal development in fish are *dmrt1* and *foxl2*, which are related to gonadal development in male and female, respectively.

In ovaries of sequential hermaphrodites there is high *foxl2* expression (Liu et al., 2015). However, during the female-to-male transition *foxl2* lower expression is acompanied by an increase in *dmrt1* (Alam et al., 2008). The decline of *foxl2* occuring during the last stages of gonadal transition (Bhandari et al., 2003) is not a trigger for sex change but, rather, a response to decreased sex steroids through feedback (Liu et al., 2017, Guiguen et al., 2010). An intermediate phase was observed between the transition of the female and male gonads, in which male genes are activated (Todd et al., 2019). Therefore, *foxl2* and *dmrt1* are not the initial triggers of sex change but are relevant later during the process (Todd et al., 2019).

7.5.4.2 Steroidogenic enzymes and steroid hormones

Steroidogenic enzymes are synthesized but, in contrast to GtHs, which can be synthesized, stored and later secreted, cannot be stored. Thus, gene expression of steroidogenic enzymes usually matches the amount and type of sexual steroids present in the gonads. The main steroidogenic enzyme is aromatase, coded by the *cyp19a1* gene in fish (Piferrer and Blázquez, 2005), which converts androgens to estrogens. It is found in the granulosa cells of the ovarian follicle.

Aromatase inhibition causes genotypic females to develop as fertile, functional males even in gonochoristic species (Piferrer et al., 1994). It is a key enzyme in gonadal differentiation in fish because is correlated with female development (Piferrer and Guiguen, 2008) thus being essential for ovarian differentiation (Li et al., 2019). Its suppression, in contrast, can activate male development (Devlin and Nagahama, 2002, Li et al., 2019). The main androgens in teleost fish are testosterone (T) and 11-ketotestosterone (11-KT), while the main estrogen is 17β-estradiol (E_2), (Baroiller et al., 2009, Devlin and Nagahama, 2002, Guiguen et al., 2010).

The relative abundance among the two main types of sex steroids influences sex. Thus, it could be said that in fish sexual fate depends on the balance between E2 and 11-KT, and changes in this balance can lead to changes in the direction of sex differentiation in both gonochoristic and hermaphroditic species (Devlin and Nagahama, 2002, Guiguen et al., 2010).

In a protogynous (female-to-male), E_2 levels decrease and consequently the ovary degenerates, followed by a sex change and a gradual increase in production of 11-KT at the beginning of spermatogenesis (Nakamura et al., 1989, Bhandari et al., 2003, Bhandari et al., 2006, Ohta et al., 2008, Muncaster et al., 2013) (**Fig. 17A**). A central aspect of protogynous sex change is the downregulation of *cyp19a1* (Liu et al., 2017, Li et al., 2019, Higa et al., 2003, Nozu et al., 2009) and a concomitant increase in *dmrt1* (Liu, 2016).

It has recently been determined that estrogens are the key to controlling sex change (Li et al., 2019), but not only does the sexual steroid appear to allow gonad change, but molecular change is also required to maintain hormone production and, consequently, sex change (Todd et al., 2016). Thus, not all aspects of sexual differentiation are dependent on steroids, as in medaka and gobies, where the primordial germ cells are independent of steroids (Devlin and Nagahama, 2002) and E_2 follows a pattern but 11-KT shows no association between sexual steroid and gonadal development, respectively (Todd et al., 2016).

The opposite pattern with respect to the situation described above for protogynous species occurs in protandrous sex change, which suppresses male steroids (11-KT) and stimulate enzymes that synthesize estrogen (Godwin and Thomas, 1993, Lee et al., 2001, Devlin and Nagahama, 2002) (**Fig. 17B**). Estradiol is a consequence of the process and not a cause because during protandrou sex change androgen levels decrease. However, the E_2 levels only increase once the ovarian formation is well underway, but not before (Godwin, 1994).

Interestingly, in bidirectional gobies, changes 11-KT are different according to the species, thus the role of 11-KT is unclear. Therefore, sexual steroid levels show no apparent association with gonadal development in these species. It has been argued that the low concentration of 11-KT reflects the lack of secondary traits in males and facilitates the flexibility for faster phenotype changes (Kroon et al., 2009, Godwin, 2010). In simultaneous hermaphroditic species, unfortunately, and despite its interest, there has been little progress on the uderlying mechanisms regulating hormonal production and two sexual roles in the same individual at the same time.

Related to the HPI axis, evidence has recently accumulated showing that cortisol can be one of the main glucocorticoids that plays a role in the regulation of sex change in sequential hermaphroditism (Todd et al., 2016). Recently, cortisol has been considered the trigger of gonad transition in protogynous species because it modulates gene expression during gonadal sex change by initiating a change in steroidogenesis (Todd et al., 2018). Their high levels in the early stage of protogynous sex change were observed coinciding with aromatase downregulation and ovarian atresia (Todd et al., 2018). Thus, a long-term stressed fish may be masculinized due to inhibition of aromatase expression, thus decreasing $E_{2,}$ and increased 11-KT production in both gonochoristic (Fernandino, 2012; 2013) and protogynous gobies (Solomon-Lane et al., 2013). Further, acting at the two main neuroendocrine pathways (HPG and HPI axis), cortisol may also increase in response to high levels of NE (Godwin, 2010; Lamm et al., 2015), increased arginine vastocin (Baker et al., 1996; Gesto et al., 2014) and by stimulation of melatonin (Liu et al. 2016).

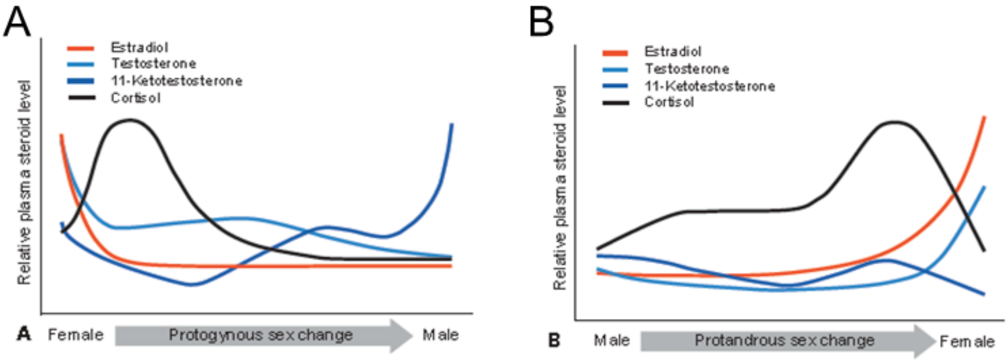


Figure 17. Changes in the level of sex steroids and cortisol in plasma during sex change in hermaphroditic fish. A) Protogynous sex change and B) Protandrous sex change. Taken from Todd et al. (2016).

7.5.4.3 Epigenetic regulation of sex change

Epigenetics is defined as mitotically (and even meiotically) heritable changes in gene expression that do not depend on changes in the DNA sequence (Deans and Maggert, 2015) DNA methylation and histone modification are two of the most well known epigenetic mechanisms regulating gene expression and thus capable not only of determining the fate of pluripotent cells during their development but also of maintaining their function and identity during adulthood (Lee et al., 2014, Atlasi and Stunnenberg, 2017). In the genome, DNA methylation typically occurs in when a cytosine is found followed by a guanine, called a CpG dinucleotide. . When methylated CpGs are located in the promoter region of a gene, this gene tends to be silenced and its expression is downregulated (Todd et al., 2019), although nowadays other gene parts such as the first intron and first exon are known to have the same or even more importance across vertebrates, from fish to mammals (Anastasiadi et al., 2018).

In the last years, the contribution of epigenetics to sex determination and differentiation across taxa has emerged (reviewed in Piferrer (2013)). Furthermore, recent evidence shows that the epigenetic mechanisms controlling sexual development in gonochoristic species are the same that control sex change in hermaphroditic species (Piferrer et al., 2019). An example of how DNA methylation can cause a change in sex differentiation in gonochoristic species was provided in the European seabass (*Dicentrarchus labrax*). When European seabass is subjected to high temperatures during early development, the aromatase gene *cyp19a1a* promoter is significantly methylated, its expression drops and fish that otherwise would develop as females because of their genetic constitution end up developing as males (Navarro-Martín et al., 2011). Thus the early development may have lasting influences, but recently it has been shown that effects can even begin long before fertilization (Danchin et al., 2019a). Therefore, when there is an environmental change that remains stable over several generations, epigenetic changes are inherited and can eventually be assimilated into genetic changes (Danchin et al., 2019b)

So far the role of epigenetics has been studied only in a few hermaphrodite species (Piferrer et al., 2019, for review). The genes that fulfill the expected inverse relationship between DNA methylation and gene expression are, remarkably, *dmrt1* and *cyp19a1a*. However, sex-related (but not inverse correlation) consistent differences across species have also been found for amh and *foxl2*. Among sequential hermaphrodites species examined so far include the protogynous ricefield eel (*Monopterus albus*), and the protandrous black porgy (*Acanthopagrus schlegelii*). Finally, a studied example of simultaneous hermaphroditism is the mangrove killifish (*Kryptolebias marmoratus*), in which epigenetic mechanisms control sexual identity and behaviour based on regulated genes modulated by environmental factors (Ellison et al., 2015). Epigenetics, by definition, deals with the integration of genomic and environmental information to bring about a given phenotype. Epigenetics is at the basis of phenotypic plasticity. Among the living world, one of the most stricking and beautiful examples of phenotypic plasticity is provided by the sexual phenotypic plasticity, where sex-changing hermaphrodites are second to none. Thus, although it is beyond the scope of this thesis, the importance of integrating epigenetics in the study of the regulation of sex change in hermaphrodites in the years to come needs no further apology.

Aims

Based on what has been discussed in the general introduction and some of the most important identified gaps in knowledge, the **overall objective of this thesis** was to study of the evolutionary transitions, environmental correlates and life-history traits associated with the distribution of the different forms of hermaphroditism in fish. To fulfull this objective, the thesis is arranged in four chapters with **specific objectives** each, namely:

1. To provide a comprehensive and updated account on the quantitative incidence and phylogenetic distribution of the different types of sexual systems in fishes, paying special attention to hermaphroditism, and to test some of the most common associations recurrently found in the literature regarding environmental variables but, to the best of our knowledge, never formally tested before (Chapter 1).

2. To reconstruct the ancestral sexual state of actinopterygian as well as to determine evolutionary rate transitions among the different sexual systems, and to test some important life-history correlates in the evolution of protogyny (Chapter 2).

3. To test the predictions of the size-advantage model stating that protogynous and protandrous hermaphrodites should exhibit lower gonadosomatic index values due to expected lower sperm competition (Chapter 3).

4. To clarify the number and distribution of simultaneous hermaphroditism in fishes and to study their evolutionary transitions and to find out whether there is an inherent reduction of body size associated with simultaneous hermaphroditism (Chapter 4).

Results

Chapter 1

Hermaphroditism in Fish: Incidence, Distribution and Associations with Abiotic Environmental Factors

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Abstract

Social structure and mating behavior have been traditionally considered important biotic factors explaining the evolution and incidence of functional hermaphroditism in fishes. However, no quantitative assessment of the association of hermaphroditism with abiotic environmental factors exists. Here, we compiled information on the sexual system of 10,875 fishes. Hermaphroditism is a rare condition in fish and is found so far only in 552 teleost species. Also, sequential hermaphroditism predominates over simultaneous hermaphroditism at a ratio ~9:1 and, within the former type, protogyny (female-to-male sex change) predominates ~3:1 over protandry (male-to-female). We also collected environmental information for 9,037 of the above species (latitude, temperature, salinity, habitat, depth, and substrate). Protogyny associates particularly with tropical regions, with species living typically at <200 m depth in coral reefs but also hard-bottom substrates that may favor the establishment of the social structures typical of protogyny. Protandry is less habitat specific and does not predominate in environments where hermaphroditism is rare such as soft-bottoms of the continental shelf and in estuarine brackish waters. Finally, simultaneous hermaphroditism has a wide latitudinal presence and tends to increase with depth but is present in deep and shallow waters in essentially similar proportions. In summary, this work both confirms and clarifies some of the long-held beliefs on the associations of hermaphroditism with environmental variables, while amending and correcting others. Therefore, this study can serve as the basis for further research in specific groups for different purposes, including ecological and evolutionary issues as well as conservation and management of exploited species.

Keywords: ecological correlates, protandry, protogyny, sex allocation, sexual systems, Williams' paradox.

1. Introduction

Hermaphroditism, defined as the presence of the two sexes in the same individual, either simultaneously or sequentially, is present in the major taxonomic divisions of plants and is common in several phyla and classes of Metazoans (Lewis, 1942, Leonard, 2013). In animals, a rough estimate of the number of hermaphroditic species is 65,000, or about 5–6% of animal species, a figure that increases to ~30% if insects are excluded (Jarne and Auld, 2006). Hermaphroditism is present in half of the 85 classes (Eppley and Jesson, 2008) and in 22 of 32 phyla of invertebrates, being common or prevalent in 14 of them, including Porifera, Cnidaria, Platyhelminthes, Mollusca, Urochordata (ascidians), and Annelida.

The best-known and supported theoretical framework within sex allocation theory (Charnov, 1982) to explain when sequential hermaphroditism is favored, is the size-advantage model, which predicts that sex change will occur when the reproductive success of one sex increases more rapidly with size (or age) than the reproductive success of the opposite sex (Ghiselin, 1969, Warner, 1975, Warner, 1984). On the other hand, the low-density model was proposed to explain when simultaneous hermaphroditism is favored, a sexual system associated with the low probability of finding a partner since in isolated populations the lack of females can saturate male fitness (Ghiselin, 1969, Charnov, 1979, Charnov, 1982).

The above mentioned models have been very good at providing testable hypothesis on the evolution of different sex allocation systems. However, a major drawback is that these models do not explain the distribution of sexual systems in metazoans. Thus, the classical ideas of the benefits of hermaphroditism over gonochorism suggested that sexual systems should be very sensitive to ecological parameters and, in the case of simultaneous hermaphroditism, particularly to the chances of finding mates (Williams, 1975). However, a large amount of empirical data shows that the best predictors of sexual systems distribution at large-scale in plants and metazoans is phylogeny, i.e., phylum or class (Leonard, 2013, Leonard, 2018b), not ecology. Thus, evolution and maintenance of a particular sexual system at these taxonomic levels is considered ancient and stable in spite of the diverse ecological environments that the different constituting species of that taxa may be subjected to (Leonard, 2018b). Based on the difference between theory predictions and actual data, Leonard (2018b) named this discrepancy the "William's paradox" (Leonard, 2013, Leonard, 2018b).

In vertebrates, fish are the only group where hermaphroditism is present. Thus, fish express all sexual systems known in vertebrates, which are gonochorism, unisexuality, hermaphroditism and androdioecy (Atz, 1964, Yamamoto, 1969, Beukeboom and Perrin, 2014, Smith and Wootton, 2016). There are two main types of hermaphroditism in fish: 1) Sequential hermaphroditism, where individuals of a given species can: a) first mature as females and later change sex to males and function as males for the rest of their lives (protogyny), or b) mature first as males, then change sex to females and function as females for the rest of their lives (protandry), or c) alternate both sexual roles throughout life (bi-directional sequential hermaphroditism), with multiple sex-change episodes (Sunobe and Nakazono, 1993, Manabe et al., 2013). 2) Simultaneous hermaphroditism, where both male and female gametes are produced by the same individual, either at the same time or within a very short period of time (Atz, 1964, Yamamoto, 1969), and thus sex change does not occur.

The number of species with different types of hermaphroditism in fish in not known precisely despite the fact that some hermaphroditic species are of high economic importance for fisheries (e.g., *Epinephelus aeneus, Labrus bergylta, Pagellus bellottii,* etc.) or aquaculture (e.g., gilthead seabream, *Sparus aurata*; white seabream *Diplodus sargus*, etc.). Over the years, several studies have attempted to determine the incidence of hermaphroditism in fish. Based on FishBase it was estimated to occur in 2% of 25,000 fish species (Pauly, 2004). Later, based on published literature it was concluded to be distributed in 20 families in 9 orders but without any estimation of actual number of species (Mank et al., 2006). The classification of the different types of hermaphroditism in fish is mainly based on histological analysis of the male and female tissues in the gonads. However, proper diagnosis of hermaphroditism can be complicated by cases of false hermaphroditism (also called juvenile hermaphroditism) in gonochoristic species of the undifferentiated type, or by the presence intratesticular oocytes (ovotestis), also in gonochoristic species (Piferrer, 2001). Sadovy and Shapiro (1987) set a series of clear criteria for the proper diagnosis of hermaphroditism, in which histological confirmation of the hermaphrodite gonad in different individuals at different ages is a must. Finally, in a very comprehensive study, functional hermaphroditism was confirmed in 7 orders, 27 families and 94 genera (Sadovy de Mitcheson and Liu, 2008).

In fish, the most important ecological parameters explaining the presence of functional hermaphroditism are the social structure and mating behavior (Warner, 1988a, Munday et al., 2006). Thus, for example, protogyny is favored in species where large dominant males monopolize matings in a harem of females (Walker and McCormick, 2009, Erisman et al., 2009). Ample support for the size-advantage model has been found over the years focusing in particular groups of sequential hermaphrodites, e.g., Labridae (Kazancioglu and Alonzo, 2010), Serranidae (Erisman et al., 2009, Erisman et al., 2013). Likewise, support for the low-density model has been suggested in several taxa (Ghiselin, 1969). However, in fish it has been related to species that inhabit the deep sea, such as several families of Aulopiformes, although it is also present in shallow-water serranid species, where the mating system contributes to maintain simultaneous hermaphroditism as an evolutionary stable strategy (Pla and Piferrer, submitted). Social structure and mating system are biotic factors. However, one of the major conclusions of the Sadovy de Mitcheson and Liu (2008) study was the need to further investigate environmental correlates, particularly environmental ecological or abiotic factors explaining or contributing to explain the distribution of hermaphroditism in fish (Sadovy de Mitcheson and Liu, 2008). A recent study of the evolution of hermaphroditism in various fish lineages concluded that, at a low taxonomic level, while some groups were influenced by phylogeny others showed only low evidence of phylogenetic inertia, indicating strong external influences (Erisman et al., 2013). Thus, in many fish families such as Serranidae and Sparidae closely related species may exhibit different sexual systems. Consequently, if different ecological conditions lead to different sexual systems in closely related species, this would provide, at least at low taxonomic levels, support for William's (1975) statement.

When (Sadovy de Mitcheson and Liu, 2008) published their comprehensive study more than 10 years ago, the number of known fish species at that time was ~28.000, a number that currently has increased to ~35.000 (i.e., a significant 25% increase) grouped in 520 families (Fricke et al., 2019). Further, nowadays there is not only contrasted information on the sexual system of more species but also data about many environmental variables related to those species.

Hence, the first aim of this study was to provide a comprehensive and updated account on the phylogenetic distribution of the sexual systems in Actinopterygian fishes (**Table 1**) where teleosts predominate (**Fig. 1**) and a quantitative estimate of the incidence of hermaphroditism in Actinopterygian fishes (**Table 1**) and of teleosts at the species level (**Fig. 2**). We also tested some of the most common associations recurrently found in the literature regarding environmental variables and the distribution of the different types of hermaphroditism in fishes —and identified as particularly worth further exploring in the Sadovy de Mitcheson and Liu (2008) review— but, to the best of our knowledge, never formally tested before, namely:

1) The association of the different sexual systems between tropical and temperate marine environments among related families and species, which offers considerable potential for comparative studies of ecological and geographical traits. Specifically, we first identified the latitudinal distribution of hermaphrodite species with respect to gonochoristic species in teleost fishes (**Fig. 3A**). Then, we identified whether the distribution of the different sexual systems varied between tropical and temperate marine environments in teleost families selected to contain different forms of hermaphroditism (**Fig. 3B**) and we tested the probability of finding each sexual system, specifically protogyny, in tropical or temperate latitudes in Actinopterygian fishes (**Table 2**).

2) The association of the different sexual systems according to water salinity, which provides the possibility to test hypotheses of possible adaptive significance of variations in sexual systems. Specifically, we identified and tested the frequency of the different sexual systems of teleost fish in freshwater, brackish water and saltwater (**Fig. 4**).

3) The association of different sexual systems with different marine habitats (**Fig. 5A**). Then, we tested whether different types of sequential hermaphroditism in perciform fishes associated with shallow tropical marine coral reefs, for being stable habitats that favor the social interactions necessary for the evolution of protogyny **(Fig. 5B**). Specifically,

whether protogyny perciforms is indeed associated with reefs, particularly coral reefs (**Fig. 5C**).

4) The association of sequential hermaphroditism, particularly protandry, with rare habitats for hermaphroditism. Specifically, we tested whether protandry was indeed the prevailing sexual system among hermaphrodites in estuaries (**Fig. 6A**) and soft bottom grounds of the continental shelf (**Fig. 6B**).

5) The association of simultaneous hermaphroditism with teleost species from the deep-sea has been made numerous times (Ghiselin, 1969, Baldwin and Johnson, 1996, Davis and Fielitz, 2010), although, as stated by Sadovy de Mitcheson and Liu (2008), "a few are found in shallow waters". Here, we analyzed the distribution of simultaneous hermaphroditism as a function of depth to determine their relative frequency (**Fig. 7**).

2. Material and methods

2.1 Creation of an ad hoc comprehensive database

We collected information from FishBase (www.fishbase.com; (Froese and Pauly, 2018) on the sexual system for a total of 10,912 Actinopterygian species, of which 10,875 were teleosts. We also collected information on environmental variables from Fishbase. We ended up with a database containing information for both sexual system and environmental variables for 9,037 Actinopterygian species (9,005 teleosts). This database contained 552 hermaphrodite species, all of them teleosts.

2.2 Validation of the database

Of the 552 teleost hermaphroditic species present in our database, we could verify, on a one-by-one basis, the sexual system for 380 of them against the primary literature. The main overall references used were: (Sadovy de Mitcheson and Liu, 2008, Devlin and Nagahama, 2002, Allsop and West, 2003) but reviews on specific families were given priority, e.g., Sparidae (Buxton and Garratt, 1990), Labridae (Kazancioglu and Alonzo, 2010) and Serranidae (Erisman and Hastings, 2011). Of the 380 species checked, 360 (94.7%) matched, i.e., the sexual system assigned to each one of them in FishBase coincided with the one assigned in the primary literature, while 20 species (5.3%) did not match. In case of discrepancy between FishBase and the primary literature in terms of sexual system, we took as valid that of the primary literature. Assuming a similar level of discrepancy in the remaining 172 species for which we could not verify their sexual system, we should have at most nine species in absolute terms with wrong assignment of the sexual system, which represent 1.6% of the total of 552 species (**Fig. S1**). We consider this level of error low and acceptable.

For taxonomic classification higher than genus and species (order, family) as well as the most current and most accurate counts of valid species, we used Eschmeyer's Catalog of Fishes database, which takes into account revisional studies on an almost daily basis: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. For actual species relationships and phylogenetic analysis, we used the most comprehensive molecular, fully bifurcating and dated phylogenetic tree of Actinopterygian fishes to date (Rabosky et al., 2018).

2.3 Statistical analysis

In this study, the *sexual system* was considered a nominal categorical variable that can take different values (gonochorism, protogyny, protandry, bi-directional, simultaneous hermaphroditism) and abiotic environmental factors (*latitude*, *latitude class*, *mean sea surface temperature*, *salinity*, *habitat*, *substrate* and *depth*) can be of different types (**Table S1**). Among them, the binary variables can take the values of 0 (absence) or 1 (presence) and, for a given species, individuals can take more than one value per variable. For the categorical nominal variables *latitude class* and *habitat*, statistical analyses were carried out. In the first case, to model the probability of predominance of a given sexual system in tropical or temperate latitudes, a generalized linear mixed phylogenetic model in a Bayesian context, MCMCglmm (Hadfield, 2010) was used. Latitude was categorized as tropical and temperate as independent binary variable coded as 0 and 1 and the sexual system as the dependent categorical variable with the states: gonochorism, protogyny, protandry, bi-directional and simultaneous hermaphroditism coded as 0 and 1 each. We tested the probability of finding each sexual system in tropical or temperate latitudes in 3,179 Actinopterygian species present in Rabosky et al. (2018) tree, focusing on the probability of protogyny being associated with the tropical region, taking protogynous and gonochorism sexual system as a reference level. We considered largely uninformative priors (mu= 0 and variance = 10^8), for the fixed factor (sexual system) and a chi-squared prior for the phylogeny as random factor (Hadfield, 2010, Villemereuil et al., 2013). The residual variance was fixed to 1 (V=1), MCMC chains were run for 1 billion iterations with a burn-in of 15,000.

In the second case, to test the association of *habitat* as a function of sexual system, a multinomial generalized linear model was applied. *Habitat* was categorized into six values (bathydemersal, bathypelagic, benthopelagic, demersal, pelagic and reef-associated) as independent nominal categorical variable and the hermaphroditic sexual systems as the dependent categorical variable with the states: protogyny, protandry, bi-directional and simultaneous hermaphroditism. We tested whether hermaphroditic species are associated to specific *habitat*, and whether protogynous are associated with reefs, particularly coral reefs. Multinomial generalized linear models were also applied to test for the association between sexual systems and *salinity*, using the R library *nnet*

(Ripley et al., 2016). The Chi-squared test was used to analyze binary data, i.e., presence or absence of a given sexual system in a given habitat.

Finally, to analyze the distribution of simultaneous hermaphroditism as a function of depth, we examined the four depth data points provided by FishBase, namely: *depth shallow,* the shallowest depth at which a given species is found; *depth common shallow,* the mean upper depth at which a given species is commonly found; *depth common deep,* the mean bottom depth at which a given species is commonly found; and *depth deep,* the maximum depth reported for a given species. To test the probability of being simultaneous hermaphrodite with the depth, a Logit generalized linear model was used. We considered the depth as independent variable and the sexual system as the dependent variable. A summary of all specific questions tested in this study, description of variables used and statistical tests applied can be found in **Table S1**.

3. Results and Discussion

3.1 Incidence and distribution of hermaphroditism in fishes

The distribution of the different sexual systems among Actinopterygian fishes at different taxonomic levels, indicating absolute and relative numbers is shown in **Table 1**. Fifty out of the 55 orders of fish (i.e., 90.90%) have at least one species for which its sexual system is known. All 50 orders contain gonochoristic species, while only 13 orders (26%) have hermaphroditic species: Anguilliformes (1 family/6 genera), Cupleiformes (1/1), Cypriniformes (1/2), Stomiiformes (1/3), Aulopiformes (10/23), Perciformes (20/99), Acropomatiformes (1/1), Centrarchiformes (1/6), Scorpaeniformes (2/6), Gobiiformes (1/14), Synbrachiformes (1/3), Blenniformes (1/1), Cichliformes (1/1) (**Fig. 1**). Therefore, our study confirms hermaphroditism in thirteen orders, of which six (Anguilliformes, Aulopiformes, Perciformes, Scorpaeniformes, Stomiiformes, Synbranchiformes) match with the seven orders confirmed by Sadovy de Mitcheson and Liu (2008), while for the seventh (Cyprinodontiformes) androdioecy rather than hermaphroditism is the sexual system present (see below). In previous studies, hermaphroditism was not reported for Scorpaeniformes (Devlin and Nagahama, 2002, Avise and Mank, 2009). However, its presence was subsequently confirmed (Sadovy de Mitcheson and Liu, 2008; present study).

Of the 514 families of Actinopterygian fishes, we collected information on the sexual system of 317 of them (61.7%). Hermaphroditism is present in 42 families (13.2%), all of them of teleosts. A complete list of all teleost families with their respective orders, where hermaphroditism is present, and the numerical distribution of each sexual system is shown in **Table S2**. Likewise, out of the 4,899 genera, we collected information on the sexual system of species belonging to 2,692 (54.95%) of these genera and hermaphroditism is present in 164 (6.09%). This represents a significant increase of an additional 15 families and 70 genera with respect to the 27 families and 94 genera in (Sadovy de Mitcheson and Liu, 2008).

In Perciforms —the order where hermaphroditism has the highest incidence— we found hermaphroditism in 20 out of 143 teleost families vs. 16 teleost families in the previous study (Sadovy de Mitcheson and Liu, 2008), followed by the Aulopiformes with 10 (vs. 5 before) teleost families, and then the rest of orders with very low presence of one family at the most. This low incidence could be explained by the taxonomic reorganization.

Of the 33,787 teleost species of fish listed in the most updated version of Eschmeyer's catalog of fishes (Fricke et al., 2019), we could compile information on the sexual system of 10,875 teleost species, i.e., one third of the total (Note: these include gonochoristic and hermaphrodite species, but not 3 androdioecious and 2 unisexual species). Of the 10,875 species, the majority (10,323 or 94.9%) are gonochoristic. Hermaphroditism is present in its various forms in a total of 552 teleost species (5.1%) **(Fig. 2**). This figure is different from the 2% estimated fifteen years ago based on the number of hermaphrodite species and the total number of species known at the time, ~25,000, regardless of whether there was information on their sexual system or not (Pauly, 2004). Using the same principle, we come up with a similar figure: 1.63% (552 out of 33,787, **Table S2**). In any case, if we take into account only the species for which there is information on the sexual system, hermaphroditism with its different manifestations, clearly is a rare condition in fish, involving ~5% of the teleost species.

An estimation of the maximum number of hermaphrodite species in fish is possible. Assuming that hermaphrodites are restricted to teleosts, we have that the 42 teleost families that contain hermaphrodites have a total of 9,136 species. Within these families, we have information on the sexual system of 3,315 species (2,763 gonochoristic, 552 hermaphrodite). Extrapolating the same proportion to the 9,136 species, we obtain an estimate of 1,521 hermaphrodite species but doing on a family by family basis and weighing for the different number of species in each family we obtain a value of 2046 species. Thus, it can be said that the maximum number of hermaphrodite species in fish may be between 1500 and 2000, which means that roughly between one third and one fourth of the maximum number of estimated hermaphrodite species has been identified so far.

Focusing only on the two major types of hermaphroditism, sequential hermaphroditism (487 species; 88.2%) predominates in front of simultaneous hermaphroditism (65 species; 11.8%), thus in sharp contrast with the situation in plants and invertebrates (Leonard, 2013). Even if we added an additional 124 species of simultaneous hermaphrodites of the order Aulopiformes mentioned elsewhere (Baldwin and Johnson, 1996, Erisman et al., 2013) but for which we did not find explicit evidence or confirmation, sequential hermaphroditism would still be predominant. The reason for the low numbers of simultaneous hermaphrodites in fish is not known but it could be related to the difficulty of handling hormones of opposite sexes in the same gonad with two functional roles at the same time (Devlin and Nagahama, 2002), and also the possible problems of inbreeding depression due to self-fertilization.

Table 1. Distribution of the different sexual systems among fishes (Actinopterygii) at different taxonomic levels, indicating absolute and relative numbers

	Counts	Percent[1]	Percent[2]	Percent[3]	Percent[4]
All orders	54*	100%	-	-	-
Orders with data for sexual system	49	90.74%	-	-	-
Gonochorism	49	-	100%	-	-
Parthenogenetic	2	-	4.08%	-	-
Androdioecy	1	-	2.04%	-	-
Hermaphrodites	**13**	-	26.53%	-	-
Sequential hermaphrodites	12	-	-	92.30%	-
Protogyny	9	-	-	-	75%
Protandry	6	-	-	-	50%
Bi-directional	4	-	-	-	28.57%
Simultaneous hermaphrodites	5	-	-	38.46%	-
All families	514*	100%	-	-	-
Families with data for sexual system	317	61.67%	-	-	-
Gonochorism	300	-	94.63%	-	-
Parthenogenetic	2	-	0.63%	-	-
Androdioecy	1	-	0.31%	-	-
Hermaphrodites	**42**	-	13.24%	-	-
Sequential hermaphrodites	32	-	-	76.19%	-
Protogyny	24	-	-	-	75%
Protandry	11	-	-	-	34.37%
Bi-directional	7	-	-	-	21.87%
Simultaneous hermaphrodites	15	-	-	35.71%	-
All genera	4,899ǂ	100%	-	-	-
Genera with data for sexual system	2,692	54.95%	-	-	-
Gonochorism	2,035	-	75.59%	-	-
Parthenogenetic	2	-	0.07%	-	-
Androdioecy	1	-	0.03%	-	-
Hermaphrodites	**164**	-	6.09%	-	-
Sequential hermaphrodites	138	-	-	84.14%	-
Protogyny	108	-	-	-	78.26%
Protandry	26	-	-	-	18.84%
Bi-directional	14	-	-	-	10.14%
Simultaneous hermaphrodites	30	-	-	18.29%	-
All species	33,822*	100%	-	-	-
Species with data for sexual system	10,912	32.26%	-	-	-
Gonochorism	10,355	-	94.89%	-	-
Parthenogenetic	2	-	0.02%	-	-
Androdioecy	3	-	0.03%	-	-
Hermaphrodites	**552**	-	5.06%	-	-
Sequential hermaphrodites	487	-	-	88.22%	-
Protogyny	393	-	-	-	80.70%
Protandry	68	-	-	-	13.96%
Bi-directional	26	-	-	-	5.34%
Simultaneous hermaphrodites	65	-	-	11.78%	-

Notes:

1.- Counts based on: * = Eschmeyer Catalog of Fishes (Fricke et al. 2019) , ǂ FishBase (Froese and Pauly, 2018).

2.- [1]Percent: Total number of counts for each taxonomic level. [2]Percent: Major sexual systems with respect to data available. [3]Percent: Sequential or simultaneous hermaphrodites within hermaphrodites. [4]Percent: Within sequential hermaphrodites.

3.- At the order, family and genera levels values should not necessarily add 100 since within the same taxonomic level there might be more than one type. However, at the species level all values with in a given column add to 100.

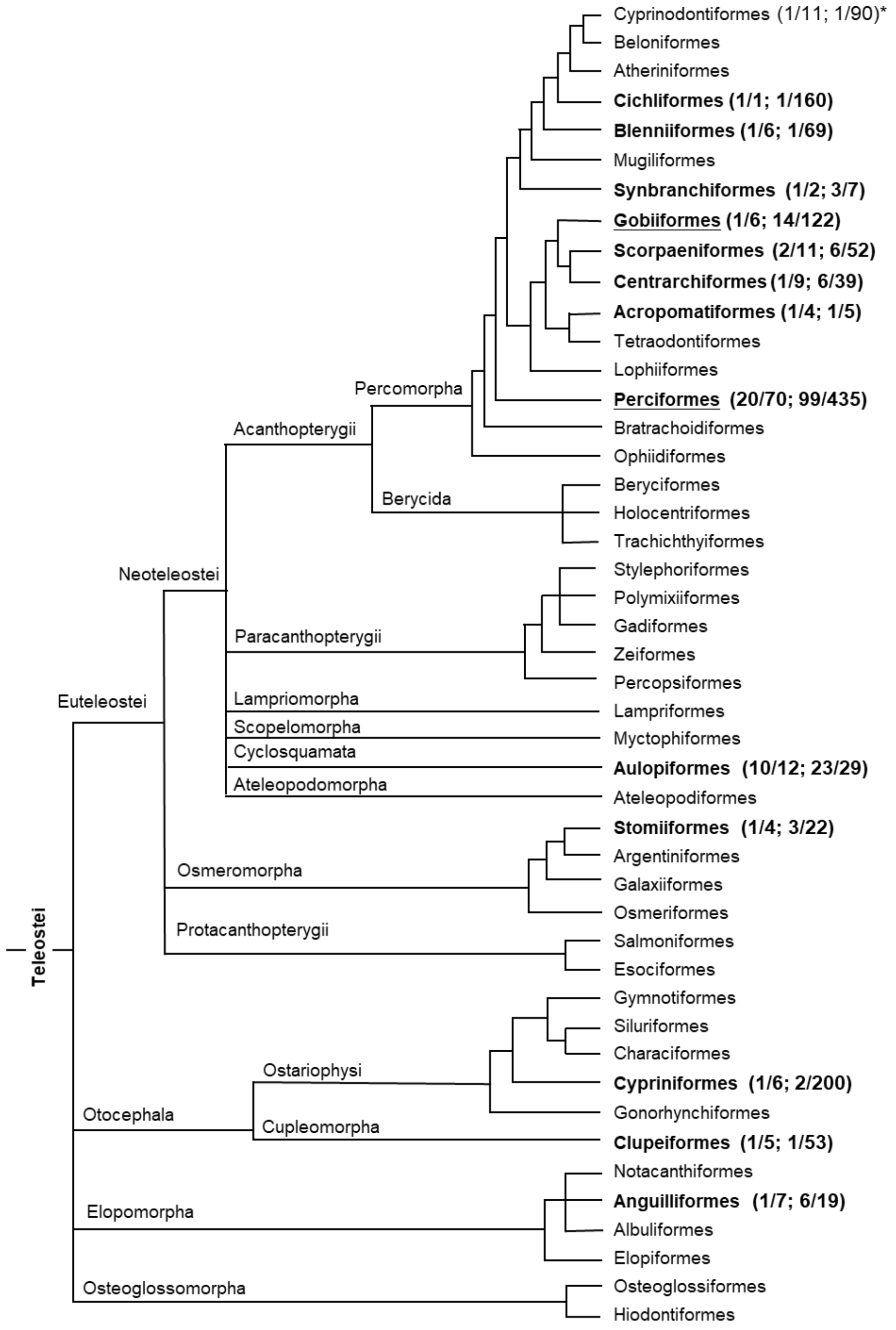

Figure 1. Phylogenetic distribution of the occurrence of hermaphroditism among the teleosts. In the orders where it is present (highlighted in bold) the first and second fractions within parenthesis indicate the number of families with hermaphrodite species with respect to the total number of families, and the number of genus with hermaphrodite.

Focusing only on the two major types of hermaphroditism, sequential hermaphroditism (487 species; 88.2%) predominates in front of simultaneous hermaphroditism (65 species; 11.8%), thus in sharp contrast with the situation in plants and invertebrates (Leonard, 2013). Even if we added an additional 124 species of simultaneous hermaphrodites of the order Aulopiformes mentioned elsewhere (Baldwin and Johnson, 1996, Erisman et al., 2013) but for which we did not find explicit evidence or confirmation, sequential hermaphroditism would still be predominant. The reason for the low numbers of simultaneous hermaphrodites in fish is not known but it could be related to the difficulty of handling hormones of opposite sexes in the same gonad with two functional roles at the same time (Devlin and Nagahama, 2002), and also the possible problems of inbreeding depression due to self-fertilization.

Within sequential hermaphroditism, protogyny clearly predominates (393 species; 80.70%) followed by protandry (68 species; 13.96%) and bi-directional sequential hermaphroditism (26 species; 5.34%) (**Fig. 2**). Bi-directional sex change is found naturally only in the F. Gobiidae (26 species), probably linked to their short life-cycle, small size and elevated predation risk (Patzner et al., 2011). However, in additional cases evidence has been obtained in laboratory settings after hormonal or social induction of bi-directional sex change as reported, for example, in several species of the family Pomacanthidae (Hioki, 1996, Sakai et al., 2003), Epinephelidae (Tanaka, 1990), Labridae (Nakashima et al., 2000, Kuwamura et al., 2002, Ohta et al., 2003, Kuwamura et al., 2007) and Pseudochromidae (Wittenrich and Munday, 2005), but it is not known if it can also occur in the wild. Adding the species where evidence is gathered only in the laboratory, the incidence of bi-directional sequential hermaphroditism would rise from 5.3% to 8.42%. A complete list of the 552 hermaphrodite species with their appropriate sexual system is shown in **Table S3**.

Androdioecy, where individuals in a population can be simultaneous hermaphrodites or males, but never females, has so far only been described in three species of the genus *Kryptolebias* (*K. ocellatus, K. marmoratus* and *K. hermaphroditus*) of the Rivulidae family (Costa et al., 2010, Costa, 2016). The latter two species are the only known vertebrates in which self-fertilization occurs (Harrington, 1961, Costa et al., 2010). There are two species of *Serranus* (S. *baldwini* and *S. psittacinus*, the latter often referred to as *S. fasciatus* in previous studies) that have also been considered androdioecious (Erisman et al., 2013). Unlike *Kryptolebias*, however, populations consist of simultaneous hermaphrodites where a dominant individual will end up losing its female function and behave as a male in a harem of hermaphrodites (Petersen and Fischer, 1986, Petersen, 1990). Although the populations of the species of the genus *Kryptolebias* and *Serranus* referred to above can end up consisting of males and simultaneous hermaphrodites, in this study, S. *baldwini* and *S. psittacinus* were considered simultaneous hermaphrodites, not androdioecious, as done before by Petersen and Fisher (1986).

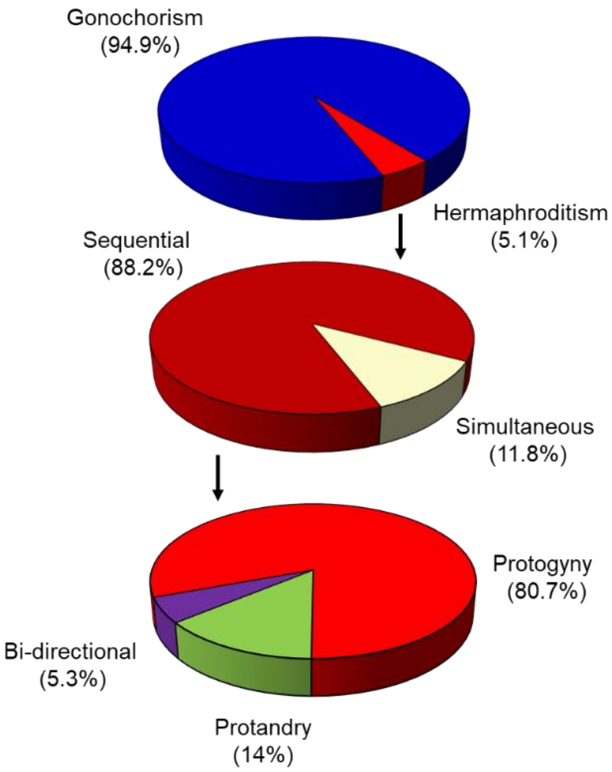

Figure 2. Graphical representation of the relative abundance of the major sexual systems among teleost fishes. Sample sizes: Gonochorism (n = 10,323 sp.), Hermaphroditism (n = 552 sp.), Sequential hermaphroditism (n = 487 sp.), Simultaneous hermaphroditism (n= 65 sp.), Protogyny (n= 393 sp.), Protandry (n= 68 sp.) and Bi-directional hermaphroditism (n= 26 sp.). Parthenogenesis (unisexuality, n= 2), androdioecy (n= 3) were not included due to the very low number of species.

3.2 Association of the different sexual systems between tropical and temperate marine environments

The study of the association of the different sexual systems between tropical and temperate marine environments among related families and species offers considerable promise for comparative analyses of ecological and geographical traits. Here, we first identified the latitudinal distribution of hermaphrodite species with respect to gonochoristic species in teleost fishes. Then we tested whether the distribution of the different sexual systems varied between temperate and marine environments in families selected to contain different forms of hermaphroditism.

The majority of species including hermaphrodite species are found in tropical and subtropical latitudes, with mean sea surface temperatures in the range of 16.8ºC–25.8ºC (**Fig. 3A**). A total of 303 gonochoristic species (11.8% of all gonochoristic species for which we had data on latitudinal distribution range; n = 2,561) are found at high-latitudes, of which 16 species live in polar waters. On the other hand, 18 hermaphrodite species

(3.8% of all hermaphrodite species for which we had data on latitudinal distribution, n = 473) live at high latitudes, which correspond mainly to deep-sea simultaneous hermaphrodites of the order Aulopiformes (e.g., *Alepisaurus brevirostris, A. ferox, Scopelarchus analis* and *Benthalbella infans*) or some protogynous species that have a wide latitudinal distribution such as *Labrus bergylta, L. mixtus* or *Cyclothone microdon*. No sequential hermaphrodites are found outside the latitudinal range of 71°N–59°S while no simultaneous hermaphrodites are found outside the latitudinal range of 84°N–72°S. The fact that hermaphrodite species are particularly abundant approximately in the 30°N–30°S latitude range is likely because this is also the range with the highest abundance of species, but it could also be due to a possible physiological limitation of sex change at high latitudes due to growth and reproduction influences as well as habitat distribution (Sadovy de Mitcheson and Liu, 2008, Trip et al., 2011). So far, studies in fish have shown how latitude can affect their reproduction (Morato et al., 2003, Lek et al., 2012) by altering spawning time and declining relative fecundity at high latitude (Kock and Kellermann, 1991). Latitude also likely influences demographic traits such as growth and mortality (Warner, 1975).

In teleost families with species in both tropical and temperate regions, we found an increase in the diversity of the sexual system and the number of species in tropical regions in Gobiidae, Pomacentridae, Serranidae and Sparidae but not in Labridae (**Fig. 3B**). Further, the MCMCglmm analysis showed that gonochorism is significantly more likely to be present in temperate regions than the protogynous species (Post.mean= -2.5171; pMCMC < 0.001) and the opposite for protogyny, with increased probability in tropical latitudes (Post.mean= 2.2838; pMCMC = 0.002; **Table 2**). However, protandry, like gonochorism, was more associated with temperate regions (Post.mean= -3.23; pMCMC = 0.03). Finally, simultaneous hermaphroditism was equally associated with both regions (Post.mean= -1.0878; pMCMC = 0.52; **Table 2; Fig. S2**). In conclusion, our analysis confirms the higher probability of finding protogynous species in tropical regions when compared to gonochoristic and protandrous species, which are more likely found in temperate regions, and no clear latitudinal preference for simultaneous hermaphrodites.

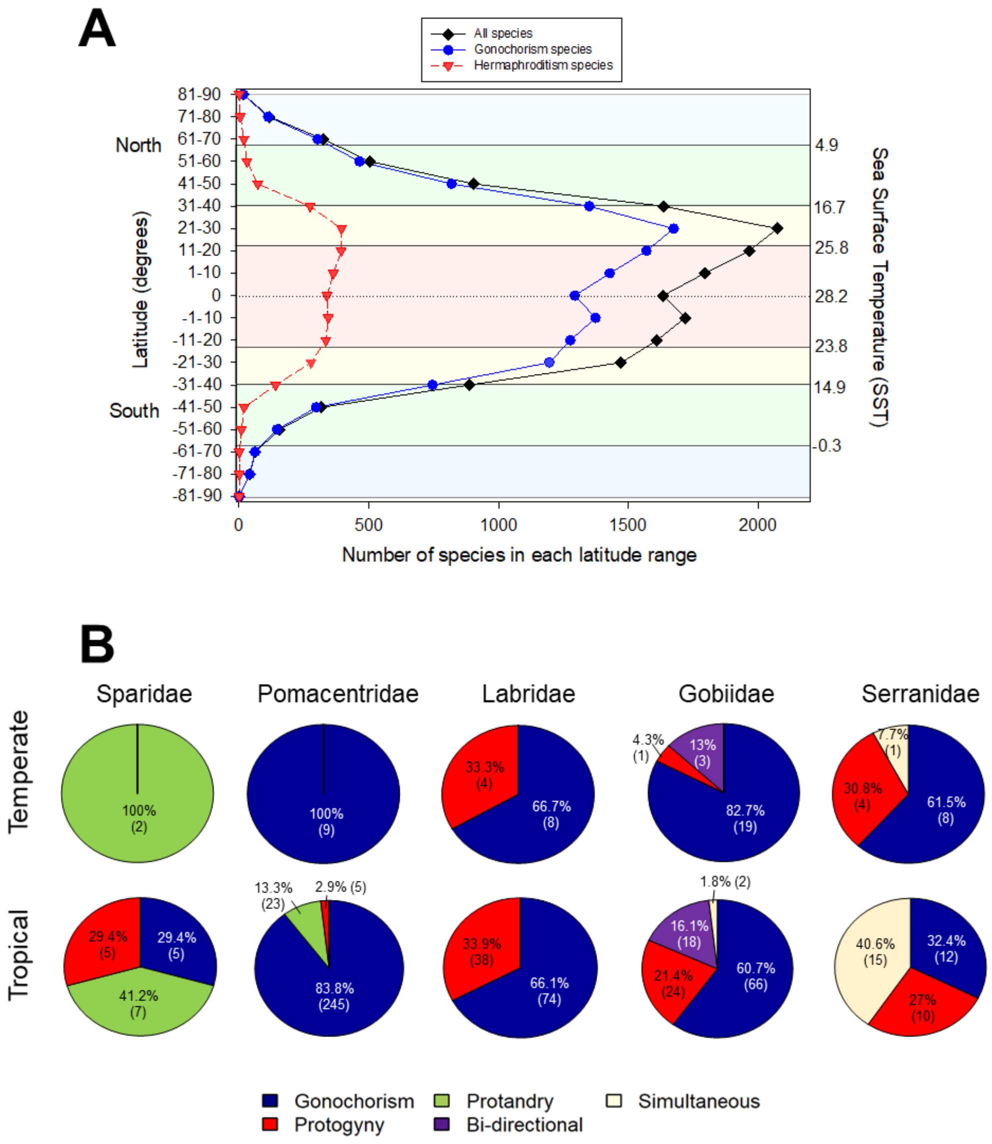

Figure 3. Latitudinal distribution of teleost fish according to their sexual system. (A) Distribution of 3,041 teleost species, of which 2,561 are gonochoristic and 466 hermaphroditic, according to latitude and mean Sea Surface Temperature (SST). Data sources: latitude, FishBase; SST, NOAA online database (www.nsof.class.noaa.gov) for the year 2015. For SST we took the mean of the 1st and 15th day of each month of the year. Number of species (x-axis) are not exclusive for each latitude range. According to FishBase the ranges are distributed in tropical (23°N to 23°S, pink), subtropical (23° to 35°, yellow), temperate (35°–66.5°, green) and boreal and polar (67°-90°, blue). (B) Comparison of sexual systems of teleosts between temperate and tropical marine environments at the level of species present in both regions in some of families with higher abundance of hermaphroditic species. The number in parenthesis represents the absolute number of species. Latitude ranges were described according to FishBase as follows: Tropical (preferred water temperature > 20°C) and Temperate (tolerate minimum water temperatures < 10°C and may have viability problems in > 15°C).

Table 2. Results of the posterior MCMCglmm values to test the probability of finding the different sexual systems, specifically protogyny, in tropical or temperate latitudes. In the first three rows, gonochorism (G) is taken as a reference level, while, to confirm that protogyny (PG) increases in tropical latitudes in absolute terms rather than just as compared to gonochorism (G), in the last three rows protogyny (PG) is taken as a reference level. Positive post.mean values indicate higher probability of the test sexual system to be found in tropical habitats, while negative post.mean values indicate higher probability of the test system (PG-G in the 4th row) in temperate habitats

Source	**Post.mean**	**Lower CI**	**Upper CI**	**ESS**	**pMCMC**
G - PG	2.2838	0.8696	3.8668	999	0.002 ******
G - PA	-0.8263	-3.1760	1.6286	792.7	0.523
G - SH	1.2783	-1.7549	4.1730	999	0.404
PG - G	-2.5171	-4.1923	-1.0321	999	**<0.001 ****
PG - PA	-3.2343	-6.0478	-0.3875	1327	**0.032 ***
PG - SH	-1.0878	-4.2887	2.1838	999	0.521

Significance equivalents: *= 0.05; ** = 0.01; *** = 0.001.
Iterations = 15001:9995001; Thinning interval = 10000

Code: G, gonochorism; PG, protogyny; PA, protandry and SH, simultaneous hermaphroditism. Post.Mean, posterior mean is equivalent to regression coefficient estimates; CI, 95% credible intervals are equivalent to 95% confidence intervals; ESS, estimated sample size and pMCMC posterior probability values are comparable to standard *P*-values.

3.3 The distribution of different sexual systems according to water salinity

Knowledge on the association of the different sexual systems according to water salinity could provide opportunity to test hypotheses of possible adaptive significance of variations in sexual systems (Sadovy de Mitcheson and Liu, 2008). However, to the best of our knowledge, a clear picture of the distribution of the different sexual systems in different water salinities has never been addressed. Specifically, we tested the distribution of the different sexual systems in freshwater, brackish water and saltwater. Here, we compiled information on the habitat in terms of salinity for a total of 9,005 teleost species (**Fig. 4**).

Of the 8,450 gonochoristic species for which we had information on salinity preference, 4,014 (47.50%) are exclusively found in saltwater, 419 (4.96%) both in saltwater and brackish water, 18 (0.21%) exclusively in brackish water, 320 (3.79%) in saltwater, brackish water and freshwater, 348 (4.11%) both in brackish water and freshwater, and 3,331 (39.42%) exclusively in freshwater (**Fig. 4A**). In contrasts, of the 378 protogynous species for which we had information on salinity preference, 339 (89.68%) are exclusively found in saltwater, 31 (8.20%) both in saltwater and brackish water, 0 (0%) exclusively in brackish water, 1 (0.26%) in saltwater, brackish water and freshwater, 4 (1.06%) both in brackish water and freshwater, and 3 (0.79%) exclusively in freshwater (**Fig. 4B**). Regarding the 68 protandrous species, 43 (63.24%) are exclusively found in saltwater, 15 (22.06%) both in saltwater and brackish water, 0 (0%) exclusively in brackish water, 10 (14.70%) in saltwater, brackish water and freshwater, 0 (0%) both in brackish water and freshwater, and 0 (0%) exclusively in freshwater (**Fig. 4C**). Finally, of the 69 simultaneous hermaphrodite species for which we had information on salinity preference, 65 (94.20%) are exclusively found in saltwater, 3 (4.35%) both in saltwater and brackish water, 0 (0%) exclusively in brackish water, 0 (0%) in saltwater, brackish water and freshwater, 0 (0%) both in brackish water and freshwater, and 1 (1.45%) exclusively in freshwater (**Fig. 4D**).

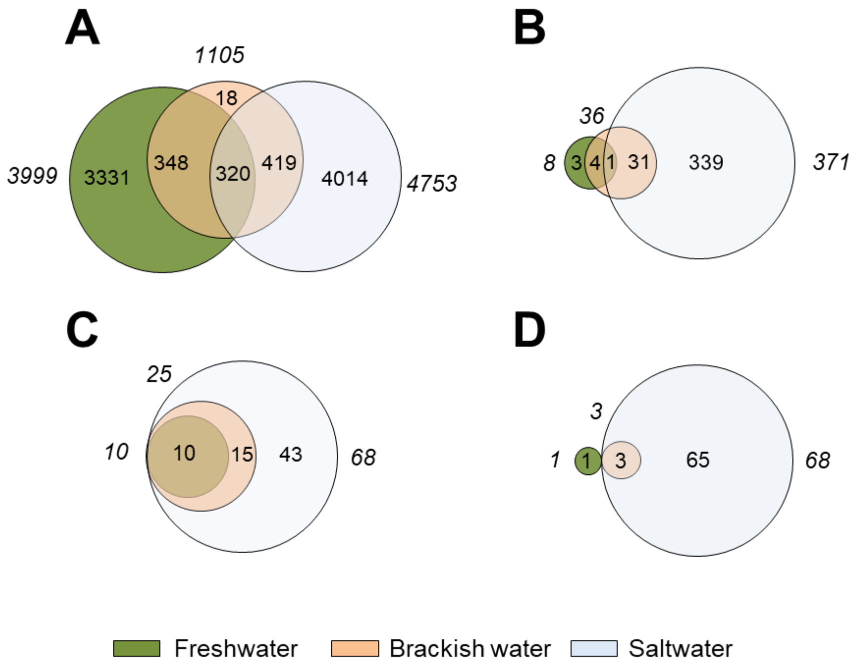

Figure 4. Abundance of the different sexual systems according to water salinity. Represented is the distribution of 9,005 of teleost species with different or shared salinity habitats in Venn diagrams (approximate size) (http://bioinfogp.cnb.csic.es/tools/venny/) whose FishBase definitions are freshwater (<0.5 grams per liter of total dissolved mineral salts, ppt), brackish water (> 0.5 and < 30 ppt) and saltwater (> 30 ppt). Numbers in italics outside the circles represent the total number of species found in freshwater (left), brackish water (top) and saltwater (right). (A) Gonochorism; (B) Protogyny; (C) Protandry; (D) Simultaneous hermaphroditism.

Hermaphroditism was considered to be absent (Wootton and Smith, 2014) or rare (Pavlov et al., 2009) in freshwater. Here, we found 19 species of hermaphroditic species in freshwater, all of them are diadromous, except *Monopterus boueti* and *Crenicara punctulatum*, that exclusively live in fresh water and do not migrate. It has been argued that freshwater fish may present physical differences in their anatomy that limit the evolution hermaphroditism in this habitat (Sadovy de Mitcheson and Liu, 2008) but the protogynous nature of the two species just mentioned above has been confirmed by gonadal studies (Liem, 1968, Oldfield, 2005, Carruth, 2000). In contrast, the simultaneous hermaphrodite *Squalius alburnoides* and the potamodromous protogynous *Henicorhynchus lobatus* (F. Cyprinidae) are found in rivers (Matos et al., 2010, Collares-Pereira et al., 2013; Fukushima et al., 2014). If their sexual system is confirmed, it will be interesting to know what mechanism have allowed them to adapt to freshwater and why there are no more freshwater hermaphrodites. Some protogynous gobies and epinephelids and protandrous sparids and centropomids are adapted to all salinity habitats. Therefore, all hermaphroditic species are present in saltwater but they have been adapted in freshwater and brackish waters. To the best of our knowledge, there are no records of protandrous hermaphrodites living exclusively in freshwater.

The results of the multinomial linear model showed that all hermaphroditic sexual types tend to be underrepresented in freshwater, but only protogynous hermaphrodites are significantly less frequent in this habitat than in salt water or brackish waters ($P < 0.0001$). Conversely, protandric hermaphrodites are relatively more frequent in brackish waters ($P < 0.0001$), but not as frequent as protogyny (see section 3.5. below).

3.4 The association of sequential hermaphroditism, particularly protogyny, with different marine habitats

Regarding the distribution of marine teleost fish for which we had information on their sexual system, we found that of all species present in reefs (n = 2,073), 355 species of sequential hermaphrodites (17.1%), inhabit the photic layer of the ocean above the 200 m depth, but also are present in demersal (n= 92; 4.9%) and benthopelagic (n=17; 5.2%) areas. It could be said that, overall, sequential hermaphroditism decreases with depth. In contrast, simultaneous hermaphroditism is the predominant form in bathypelagic habitat (n= 21; 9%) (P < 0.001, based on the multinomial linear model results), although, as we will see below, it is also present in shallow waters (Fig. 5A).

In sequential hermaphroditism, protogyny is significantly relatively more frequent in reefs (P <0.001) than in other habitats, but it is significantly less frequent in the bathydemersal (P = 0.033) and in the benthopelagic (P < 0.0001). Protandry is relatively more important in reefs (P < 0.0001), but also in the bathypelagic (P = 0.008). Simultaneous hermaphrodites are overrepresented in reefs (P = 0.001) and bathydemersal (P = 0.005) and bathypelagic (P < 0.0001) marine waters (Fig. 5A).

Focusing exclusively on perciform sequential hermaphrodites, we found an unequal distribution of habitats (Chi-squared = 239.17, df = 3; P < 0.001), with species found in the reefs (n= 286; 74.1%), followed by demersal zones (n= 80; 20.7%) (Fig. 5B). The other two habitats where they are found, benthopelagic zone and bathydemersal, accounted only ~5%, the latter habitat with three species of the family Epinephelidae such as Epinephelus suborbitalis. Our study numerically confirms the preponderance of sequential hermaphroditism, mainly protogynous hermaphrodites, in shallower habitats, corresponding to reefs and demersal areas (0-200 m) as exemplified by families such as Labridae, Scaridae and Serranidae (Warner and Robertson, 1978, Warner, 1984).

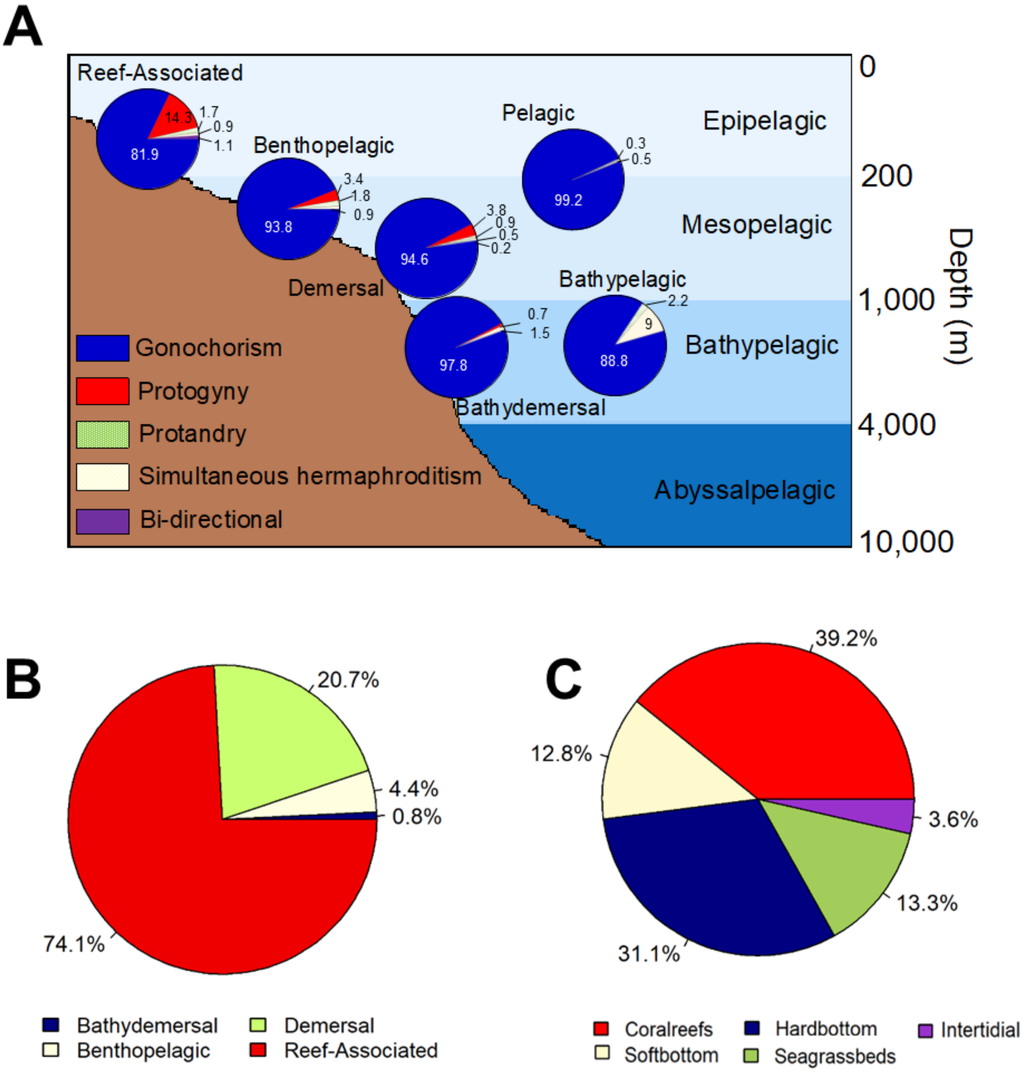

Figure 5. Habitat distribution of teleost hermaphroditic species (**A**) Distribution of 5,287 marine species, Gonochorism, n= 4,751; Protogyny n = 381; Protandry, n = 66; Bi-directional sequential hermaphroditism n = 26; Simultaneous hermaphroditism n = 63, according to type of habitat depth. Numbers refer to percent and in the pie chart sexual system types follow the same order as in the figure legend, i.e., first gonochorism, then protogyny, etc. (**B**) Distribution of 386 species of sequential hermaphrodites in Perciforms, of which 86% are protogynous, according to type of habitat. (**C**) Distribution of 421 perciform protogynous hermaphrodites according to the substrate where they are preferentially found. The FishBase seven original substrate categories were here grouped in the following five categories: "coral reefs" → coral reefs (n= 165); "soft" + "soft bottom" → soft bottom (n= 54); "hard bottom" + "rocky" → hard bottom (n= 131); "seagrass beds" + "macrophytes" →seagrass beds (n= 56); "intertidal" → intertidal (n= 15).

Focusing exclusively on perciform protogynous hermaphrodites, since most of them were found in the reefs type of habitat (Fig. 5B), we could expect that their most common substrate would be coral reefs. We collected information of the preferred substrate of 421 Perciforms protogynous species. We found an unequal distribution (Chi-squared = 97.07, df = 4; P < 0.001) but the coral reefs and hard bottom substrates had similar preference (39.2% vs. 31.1%) (Fig. 5C). In fact, only 13.3% and 12.8% of the species live in seagrass beds and soft bottoms substrates, respectively. These results indicate that not only coral reefs but also other types of substrates, particularly hard bottom substrates, offer environmental conditions capable of favoring the evolution of protogyny. It is easy to conceive that the social haremic structure characteristic of most protogynous species can adequately take place in hard bottom and seagrass beds substrates that offer dominant males a stable environment where to control their harems of females and protect them from other males.

3.5 The association of sequential hermaphroditism, particularly protandry, with rare habitats for hermaphroditism

It has been claimed that hermaphroditism is especially absent or unusual in certain groups such as epipelagic fishes and soft-bottom ground fishes of the continental shelf and estuaries and, if it does occur in these habitats, it tends to be protandry (Sadovy de Mitcheson and Liu, 2008). First, we tested whether protandry was indeed the prevailing sexual system among hermaphrodites in estuaries (Fig. 6A). We did not find support for this since more than half of the hermaphroditic species were protogynous (n= 36; 53.7%) vs (n= 25; 37.3%) protandrous (Chi-squared = 28.34; df = 3; P < 0.001). In addition, a few simultaneous hermaphrodites (n= 3; 4.4%) and bi-directional sequential hermaphrodites (n= 3; 4.4%) species were also present in brackish waters (Fig. 6A). Similarly, protogyny was also the most abundant sexual system in soft bottom (n= 57; 75%) while protandry only reached 22.4% (Fig. 6B). Again, these observations did not support the claim that protandry is the prevailing system in these habitats (Chi-squared = 34.06; df = 3; P < 0.001). Thus, protogyny, not protandry, is the sexual system also most prominent in habitats where hermaphroditism is rare. These results do not depend on the fact that protogyny is the most abundant type of hermaphroditism since for these analyses we took only species present in the estuaries and soft bottom habitats of the continental shelf, regardless of the actual abundance of each sexual system in other habitats.

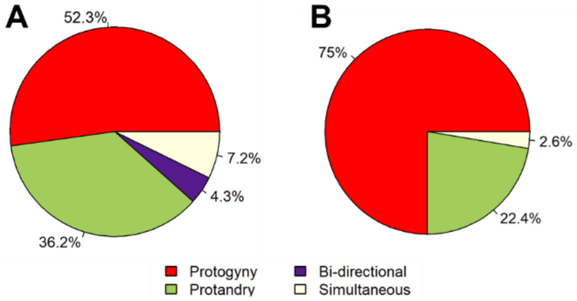

Figure 6. Presence of hermaphroditic teleost fishes in specific habitats. (**A**) Sexual system of 67 hermaphroditic species (Protogyny, n = 36; Protandry, n = 25; Bi-directional, n = 3 and Simultaneous hermaphroditism, n = 3) present in estuarine brackish waters. (**B**) Sexual system of 76 hermaphroditic species (Protogyny, n = 57; Protandry, n = 17; Simultaneous hermaphroditism, n = 2) present in marine soft bottom substrates.

3.6 The distribution of simultaneous hermaphroditism as a function of depth.

The evolution of simultaneous hermaphroditism in animals has been traditionally explained by the low probability of finding mates in populations with low density of individuals, i.e., the low-density model (Ghiselin, 1969). Such conditions are found in the ocean depths and thus in fish the association of simultaneous hermaphroditism with species from the deep-sea, notably of the order Aulopiformes, has been made numerous times (Davis and Fielitz, 2010). However, Sadovy de Mitcheson and Liu (2008) stated that “a few [simultaneous hermaphrodites] are found in shallow waters”. In fact, the family Serranidae contains also several species of simultaneous hermaphrodites and they do not live in the deep sea. Thus, here, we quantitatively analyzed the distribution of simultaneous hermaphroditism as a function of depth to determine their relative abundance.

Of the 65 species with verified simultaneous hermaphroditism, only members of the Aulopiformes inhabit the deep-sea. In contrast, we found that the maximum depth (called depth deep in FishBase) attained by simultaneous hermaphrodite species from other orders, i.e., Perciformes (21 species of the genus Serranus, Hypoplectrus and Diplectrum), Gobiiformes (two Priolepis sp.) and Anguilliformes (three Gymnothorax sp.) are not found beyond the first 500 m (Fig. 7A). A generalized linear model of the probability of being simultaneous hermaphrodite with depth deep shows that this type of hermaphroditism is relatively more frequent with depth ($P < 0.0001$). However, grouping the depth - deep values in in ranges of 200 meters, we observed that 25 species live within the first 200 m and 32 species (i.e., ~50% of the total) within the first 400 m of the water column (Fig. 7B). The mean deep depth values of these groups of species were 69.4 m for the 0–200 m range (n = 25 species); 345.1 m for the 201–400 m range (n = 7 species) and 2,430.3 m for > 400 m (n = 28 species) (Fig. 7C). Thus, our analysis clearly shows that the number of simultaneous hermaphrodite species whose maximum depth (i.e., depth deep) is restricted to shallow waters (≤ 200m) is similar to those whose maximum depth is > 400 m. In any case, and even assuming that all of the 124 species of Aulopiformes of Baldwin and Johnson (1996) for which we could not confirm their sexual system lived exclusively in deep waters, it cannot be stated that the distribution of simultaneous hermaphroditism in fish is associated with the deep sea. Thus, while it is true that simultaneous hermaphroditism is the only type of hermaphroditism present in the deep sea, the opposite is not true, i.e., simultaneous hermaphrodites live both in the deep sea and in shallow waters in roughly similar proportions.

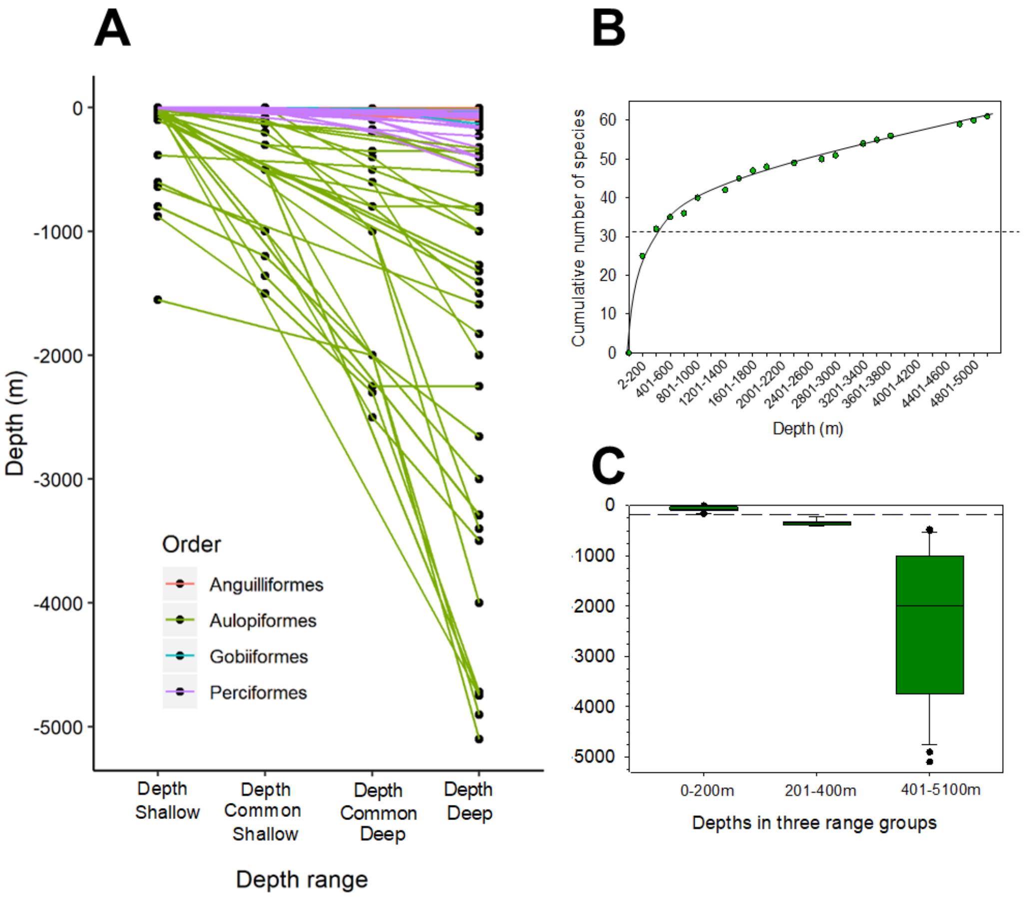

Figure 7. Distribution of fish species exhibiting simultaneous hermaphroditism according to depth. (**A**) Distribution at four depth points: *depth-shallow, depth-common shallow; depth-common deep*, and *depth-deep* (see Methods for definitions) of species belonging to four different orders. Number of species in each one of the four depths are: Aulopiformes, n= 35; 19; 18 and 35, Perciformes, n= 21; 13; 13 and 21, Gobiiformes, n= 2; 2; 2 and 2, and Anguilliformes, n= 3; 0; 0 and 3. (**B**) Cumulative number of species as a function of maximum depth (*depth-deep*). (**C**) Depth deep distribution of simultaneous hermaphrodites in three 200-m depth ranges: 0-200 m (n= 25); 201-400 m (n= 7); 401-5100 m (n= 29). The edges of the box closed and farthest from zero indicate the 25th and 75th percentiles, respectively. Within boxes, the thin line indicates the median. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles respective.

4. Conclusions

1. Hermaphroditism is confirmed in 13 orders, 42 families and 164 genera. The incidence of hermaphroditism has increased in additional 6 orders, 15 families and 70 genera with respect to the 7 orders, 27 families and 94 genera in (2008). In Perciformes, the incidence of hermaphroditism is 14% of the families vs. 10% in the previous study (Sadovy de Mitcheson and Liu, 2008), followed by the Aulopiformes with 10 (vs. 5 families before) and then the rest of orders with presence of one family at the most.

2. Based on the 10,875 teleost species for which there is information about their sexual system, hermaphroditism is present only in 552 species (5.1%). The estimated maximum number of hermaphrodite species in fish is 1500-2000. Sequential hermaphrodites predominate (88.2%) among hermaphrodites in contrast to other major taxa where simultaneous hermaphroditism is most common. Among the sequential hermaphrodites the majority are protogynous hermaphrodites (female first, 80.7%), followed by protandrous (14%) and bi-directional sequential hermaphrodites (5.3%).

3. The majority of hermaphrodites are present in lower-latitudes, likely because this is also the range with the highest abundance of teleost species. Only 3.8% of hermaphrodites, mainly simultaneous hermaphrodites, are found in high-latitudes. This could also be due to a possible physiological limitation of sex change at high latitudes.

4. Protogynous species are abundant mainly in shallower habitats below 200 meters (reefs, 75%) and warmer waters belonging to tropical regions. They show a specific habitat, significantly different from the protandrous and simultaneous hermaphrodites. Protogynous species are present in coral reefs or hard bottom substrates that may favor the establishment of stable social structures typical of this sexual system. Therefore, there is a significant association with specific abiotic factors that may favor sex change.

5. Protandry does not predominate in environments where hermaphroditism is rare such as soft-bottoms of the continental shelf and in estuarine brackish waters as previously suggested. Therefore, it does not seem habitat-specific.

6. Simultaneous hermaphroditism is present in both deep and shallow waters in similar proportions, rather than being mostly associated with deep water. The stability of the simultaneous system at the genus level and the opposite environmental factors according to taxonomic orders suggest that its distribution is phylogeny-dependent.

In summary, following William (1975) assertion, our results show that the distribution of simultaneous hermaphroditism in teleost fish is mostly dependent on phylogeny while sequential hermaphroditism, especially protogyny, is mostly influenced by abiotic factors, as required by its social and mating system.

Finally, we need to remember that the factors best explaining hermaphroditism in fish, particularly the most abundant types, protogyny and protandry, are biotic factors related to the social structure of populations and the mating systems. However, the information on the influence of different abiotic factors provided in this study fill a gap and can be of interest not only for ecology and evolutionary biology, but also for applied biology. Thus, we show some environmental correlates in groups of species important for both fisheries and aquaculture. Further understanding of these environmental correlates can help to forecast changes in distribution or in phenology of hermaphrodites in a global change scenario.

Acknowledgments

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Supplementary Figures

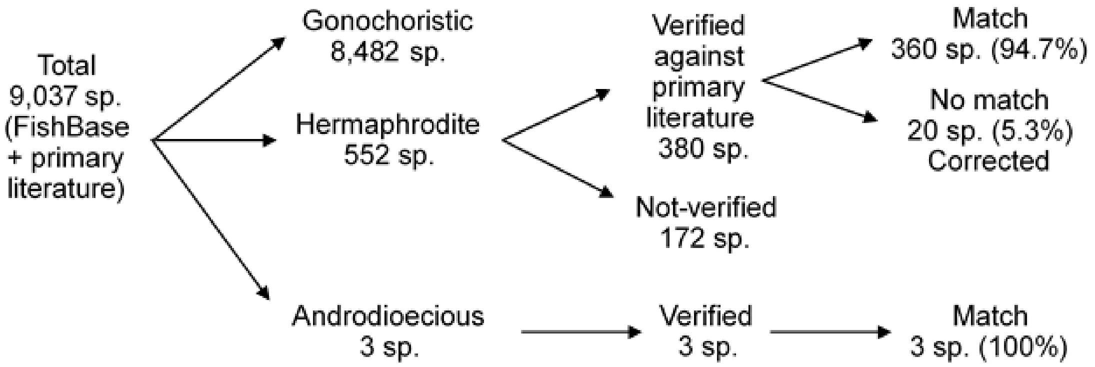

Figure S1. Validation of our database regarding assignment of a particular sexual system to a given species. Of the 9,037 Actinopterygian species in our database, 552 were hermaphrodite, all teleosts. Of these, 380 were validated against the primary literature, of which 360 coincided in the assignment of a particular type of hermaphroditism (~95% match). In case of disagreement, the value of the primary (and usually more recent) literature was taken as valid and corrected in the database. Assuming the same level of error, we can estimate that around 9 species of the 172 that could not be independently verified had a wrong assignment of sexual system. Thus, as a whole the maximum possible error in the hermaphroditic species is 1.63% (9 out of 552).

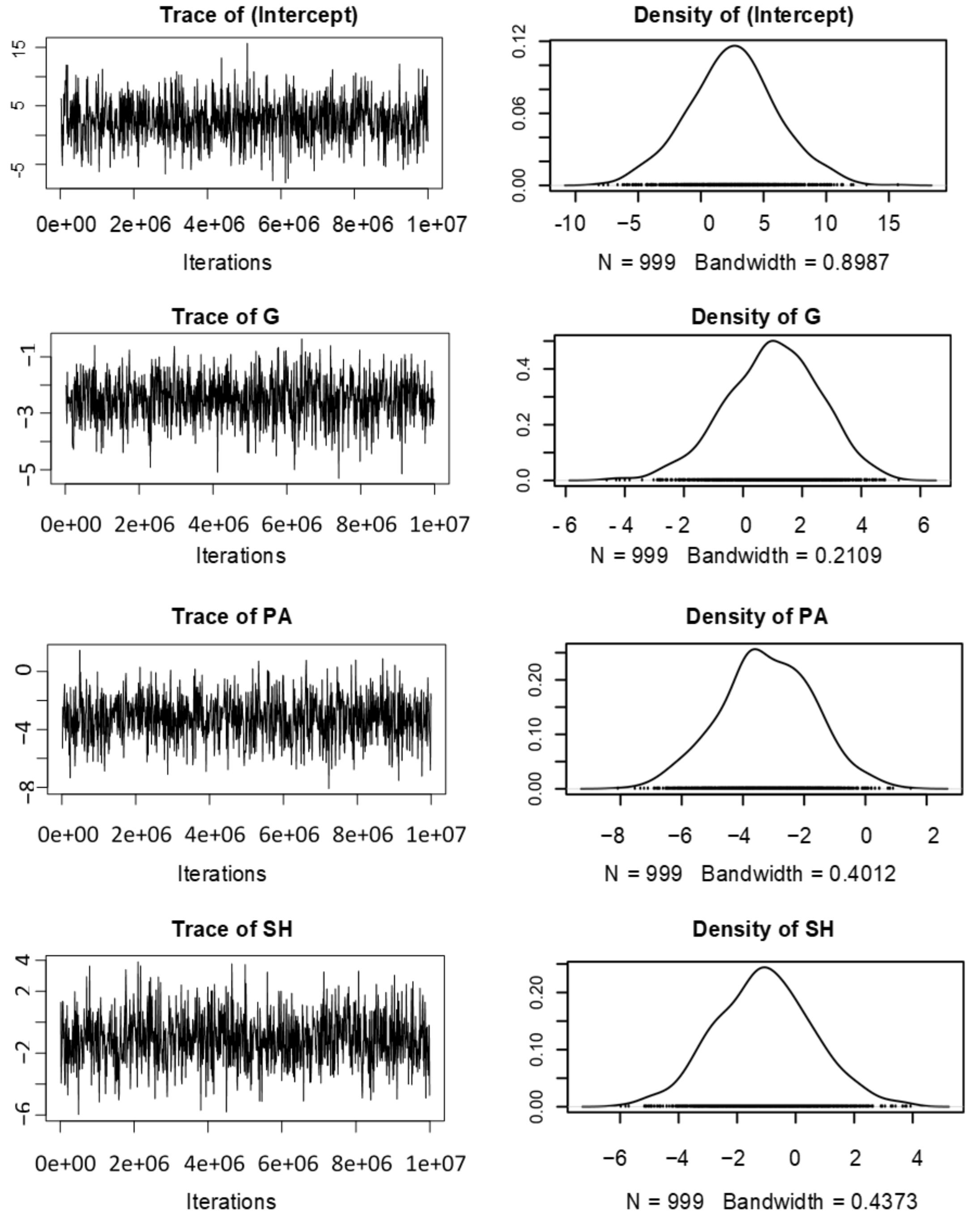

Figure S2. Results of a Bayesian linear mixed model using Markov chain Monte Carlo algorithms (MCMCglmm) evaluating the relationship between sexual systems and tropical or temperate latitude in Actinopterygian fishes. Trace and density plots show good mixing in the MCMC chain. Code: Intercept, protogynous; G, gonochorism, PA, protandrous and SH, simultaneous hermaphroditism.

Supplementary Tables

Table S1. Summary of all specific questions tested in this study, description of variables used and statistical tests applied

Results section	Fig./ Table	Specific question	Indep. variable	Type of variable	Possible values	Dep. variable	Type of variable	Possible values	Test applied
3.2. Association of the different sexual systems between tropical and temperate marine environments	Fig. 3A	We identified the latitudinal distribution of hermaphrodite species with respect to gonochoristic species in Actinopterygian fishes	*Latitude + Mean sea surface temperature*	Continuous numerical (range)	90°N–80ºS -0.33°C–28.68°C	*Sexual system*	Nominal categorical	G, H	-
	Fig. 3B Table S2	We test the probability of finding each sexual system in selected teleost families in tropical vs. temperate latitudes	*Latitude class*	Nominal categorical	Temperate, tropical	*Sexual system*	Nominal categorical	G, PG, PA, SH	MCMCglmm
3.3. The distribution of different sexual systems according to water salinity	Fig. 4	We tested the frequency of the different sexual systems of teleosts in freshwater, brackish water and saltwater	*Salinity*	Binary: 0, absence 1, presence	Freshwater, brackish water, saltwater	*Sexual system*	Nominal categorical	PG, PA, BD, SH	Multinomial glm
3.4. The association of different sexual systems with different marine habitats	Fig. 5A	We test the association of different types of sequential hermaphrodites to specific marine habitats	*Habitat*	Nominal categorical	Bathydemersal, bathypelagic, benthopelagic, demersal, pelagic, reef-associated	*Sexual system*	Nominal categorical	PG, PA, SH	Multinomial glm

	Fig. 5B	In Perciforms, we identify the frequency of sequential hermaphrodites in different habitats	*Habitat*	Nominal categorical	Bathydemersal, bathypelagic, benthopelagic, demersal, pelagic, reef-associated	*Sexual system*	Nominal categorical	SQ	Chi-squared
	Fig. 5C	In Perciforms, we test the association of types of sequential hermaphrodites to specific habitat and whether protogyny is indeed associated with coralreefs	*Substrate type*	Binary: 0, absence 1, presence	Softbottom, hard bottom, seagrassbeds, intertidal, coral reefs	*Sexual system*	Nominal categorical	PG	Chi-squared
3.5. The association of sequential hermaphroditism, particularly protandry, with rare habitats for hermaphroditism	Fig. 6A	We tested whether protandry was the prevailing sexual system among hermaphrodites in estuaries	*Salinity*	Binary: 0, absence 1, presence	Brackish water	*Sexual system*	Nominal categorical	PA	Chi-squared
	Fig. 6B	We tested whether protandry was the prevailing sexual system among hermaphrodites in soft bottom grounds of the continental shelf	*Substrate type*	Binary: 0, absence 1, presence	Soft bottom	*Sexual system*	Nominal categorical	PA	Chi-squared
3.6. The distribution of simultaneous hermaphroditism as a function of depth	Fig. 7	We analyzed the distribution of simultaneous hermaphroditism as a function of depth to determine their relative frequency	*Depth (Depth-deep)*	Continuous numerical (range)	0-5100 m	*Sexual system*	Nominal categorical	SH, SQ	Logit glm

Abbreviations: Fig, Figures; Dep, Dependent; Indep, Independent, G, Gonochorism; SQ, sequential hermaphroditism; PG, Protogyny; PA, Protandry; BD, Bi-Directional; SH, Simultaneous hermaphroditism

Table S2. List of the 42 teleost families where hermaphroditism is present, indicating the number of species in each sexual system

Order[1]	**Family[1]**	**No. of species[1]**	**G**	**Hermaphroditism**				**Total Hermaphr. (PG+PA+BD +SH)**	**Total Reprod. (G+PG+PA+ BD+SH)**	**Total Hermaphr. / Total Reprod. (%)**	**Total Hermaphr. / no. sp. per family (%)**
				PG	**PA**	**BD**	**SH**				
Acropomatiformes	Lateolabracidae	2	-	-	1	-	-	1	1	100	50
Anguilliformes	Muraenidae	213	8	9	1	-	3	13	21	61.9	6.1
Aulopiformes	Alepisauridae	2	-	-	-	-	2	2	2	100	100
	Anotopteridae	3	-	-	-	-	1	1	1	100	33.33
	Bathysauridae	2	-	-	-	-	2	2	2	100	100
	Chlorophthalmidae	21	-	-	-	-	5	5	5	100	23.8
	Evermannellidae	8	-	-	-	-	2	2	2	100	25
	Ipnopidae	33	-	-	-	-	2	2	2	100	6
	Notosudidae	17	-	-	-	-	3	3	3	100	1.6
	Omosudidae	1	-	-	-	-	1	1	1	100	100
	Paralepididae	14	2	-	-	-	9	9	11	81.8	64.2
	Scopelarchidae	18	-	-	-	-	7	7	7	100	38.8
Blenniiformes	Blenniidae	405	368	1	-	-	-	1	369	0.27	0.2
Centrarchiformes	Cirrhitidae	35	3	9	-	-	-	9	12	75	25.7
Cichliformes	Cichlidae	1,724	867	1	-	-	-	1	868	0.11	0.05
Clupleiformes	Clupeidae	197	77	-	2	-	-	2	79	2.53	1.01
Cypriniformes	Cyprinidae	1,718	508	1	-	-	1	2	510	0.4	0.11
Gobiiformes	Gobiidae	1,907	198	27	-	26	2	55	253	21.7	2.88
Perciformes	Callanthiidae	17	-	2	-	-	-	2	2	100	11.7
	Centropomidae	12	11	-	1	-	-	1	12	8.3	8.3
	Eleginopsidae	1	-	-	1	-	-	1	1	100	100
	Epinephelidae	220	12	159	-	-	1	160	172	93	72.7
	Grammatidae	18	-	1	-	-	-	1	1	100	5.5
	Labridae	556	111	63	-	-	-	63	174	36.2	11.3
	Latidae	14	2	-	1	-	-	1	3	33.3	7.1
	Lethrinidae	44	-	8	-	-	-	8	8	100	18.2
	Nemipteridae	73	8	4	-	-	-	4	12	33.3	5.5

	Pinguipedidae	98	1	2	-	-	-	2	3	66.6	2
	Plesiopidae	50	48	1	-	-	-	1	49	2	2
	Polynemidae	42	-	-	5	-	-	5	5	100	11.9
	Pomacanthidae	90	8	16	-	-	-	16	24	66.6	17.8
	Pomacentridae	420	351	5	27	-	-	32	383	8.3	7.6
	Pseudochromidae	154	-	6	-	-	-	6	6	100	3.9
	Scaridae	100	3	29	-	-	-	29	32	90.6	29
	Serranidae	326	36	19	-	-	24	43	79	54.4	13.2
	Siganidae	31	6	1	-	-	-	1	7	14.2	3.2
	Sparidae	166	27	22	19	-	-	41	68	60.3	24.7
	Trichonotidae	10	-	1	-	-	-	1	1	100	10
Scorpaeniformes	Scorpaenidae	231	5	2	-	-	-	2	7	28.6	0.8
	Platycephalidae	84	69	-	5	-	-	5	74	6.7	5.9
Stomiiformes	Gonostomatidae	33	15	-	5	-	-	5	20	25	15.2
Synbranchiformes	Synbranchidae	26	19	4	-	-	-	4	23	17.4	15.4
Other orders	**Other families**	24,651	7,560	-	-	-	-	-	7,560		
TOTAL		**33,787**	**10,323**	**393**	**68**	**26**	**65**	**552**	**10,875**	**5.07**	**1.63**

Abbreviations: G, Gonochorism; PG, Protogyny; PA, Protandry; BD, Bi-Directional; SH, Simultaneous hermaphroditism.

Note: The total number of species with known information on sexual system in this table, 10,875, is lower than the number 10,912 that appears in Table 1 because here we only consider teleosts, while in Table 1 we include Actinopterygians.

[1] Taxonomic classification and recounts from Fricke et al. (2019).

Table S3. List of all 552 teleost species where hermaphroditism is present, indicating order, family and sexual system (SS)

Order	Family	Genus	Species	SS
Acropomatiformes	Lateolabracidae	Lateolabrax	*Lateolabrax japonicus*	PA
Anguilliformes	Muraenidae	Echidna	*Echidna nebulosa*	PG
		Gymnomuraena	*Gymnomuraena zebra*	PG
		Gymnothorax	*Gymnothorax fimbriatus*	PG
			Gymnothorax flavimarginatus	PG
			Gymnothorax gracilicauda	PG
			Gymnothorax griseus	SH
			Gymnothorax margaritophorus	PG
			Gymnothorax pictus	SH
			Gymnothorax thyrsoideus	SH
		Muraena	*Muraena pavonina*	PG
		Rhinomuraena	*Rhinomuraena quaesita*	PA
		Uropterygius	*Uropterygius fasciolatus*	PG
			Uropterygius polyspilus	PG
Aulopiformes	Alepisauridae	Alepisaurus	*Alepisaurus brevirostris*	SH
			Alepisaurus ferox	SH
	Anotopteridae	Anotopterus	*Anotopterus pharao*	SH
	Bathysauridae	Bathysaurus	*Bathysaurus ferox*	SH
			Bathysaurus mollis	SH
	Chlorophthalmidae	Chlorophthalmus	*Chlorophthalmus agassizi*	SH
			Chlorophthalmus albatrossis	SH
			Chlorophthalmus brasilensis	SH
			Chlorophthalmus mento	SH
		Parasudis	*Parasudis truculenta*	SH
	Evermannellidae	Coccorella	*Coccorella_atlantica*	SH
		Evermannella	*Evermannella indica*	SH
	Ipnopidae	Bathypterois	*Bathypterois grallator*	SH
			Bathypterois phenax	SH
	Notosudidae	Ahliesaurus	*Ahliesaurus brevis*	SH
		Scopelosaurus	*Scopelosaurus harryi*	SH
			Scopelosaurus lepidus	SH
	Omosudidae	Omosudis	*Omosudis lowii*	SH
	Paralepididae	Lestidiops	*Lestidiops jayakari*	SH
			Lestidiops ringens	SH
		Lestidium	*Lestidium atlanticum*	SH
		Lestrolepis	*Lestrolepis intermedia*	SH
			Lestrolepis japonica	SH
		Macroparalepis	*Macroparalepis johnfitchi*	SH
		Magnisudis	*Magnisudis atlanticum*	SH
		Stemonosudis	*Stemonosudis macrura*	SH
		Sudis	*Sudis atrox*	SH
	Scopelarchidae	Benthalbella	*Benthalbella dentata*	SH
			Benthalbella infans	SH
			Benthalbella macropinna	SH
		Rosenblattichthys	*Rosenblattichthys volucris*	SH
		Scopelarchoides	*Scopelarchoides nicholsi*	SH
		Scopelarchus	*Scopelarchus analis*	SH
			Scopelarchus guentheri	SH
Blenniformes	Blenniidae	Salaria	*Salaria basilisca*	PG
Centrarchiformes	Cirrhitidae	Amblycirrhitus	*Amblycirrhitus pinos*	PG
		Cirrhitichthys	*Cirrhitichthys aprinus*	PG
			Cirrhitichthys aureus	(BD)
			Cirrhitichthys falco	PG

			Cirrhitichthys oxycephalus	PG
		Cirrhitops	*Cirrhitops hubbardi*	PG
		Cirrhitus	*Cirrhitus pinnulatus*	PG
		Cyprinocirrhites	*Cyprinocirrhites polyactis*	PG
		Neocirrhites	*Neocirrhites armatus*	PG
Cichliformes	Cichlidae	Crenicara	*Crenicara punctulatum*	PG
Clupeiformes	Clupeidae	Tenualosa	*Tenualosa macrura*	PA
			Tenualosa toli	PA
Cypriniformes	Cyprinidae	Henicorhynchus	*Henicorhynchus lobatus*	PG
		Squalius	*Squalius alburnoides*	SH
Gobiiformes	Gobiidae	Bryaninops	*Bryaninops amplus*	PG
			Bryaninops natans	PG
			Bryaninops yongei	PG
		Coryphopterus	*Coryphopterus alloides*	PG
			Coryphopterus dicrus	PG
			Coryphopterus eidolon	PG
			Coryphopterus glaucofraenum	PG
			Coryphopterus hyalinus	PG
			Coryphopterus lipernes	PG
			Coryphopterus personatus	PG
			Coryphopterus thrix	PG
			Coryphopterus urospilus	PG
		Eviota	*Eviota afelei*	PG
			Eviota albolineata	PG
			Eviota disrupta	PG
			Eviota distigma	PG
			Eviota epiphanes	PG
			Eviota fasciola	PG
			Eviola nebulosa	PG
			Eviota saipanensis	PG
			Eviota sparsa	PG
		Fusigobius	*Fusigobius neophytus*	PG
		Gobiodon	*Gobiodon citrinus*	BD
			Gobiodon fulvus	BD
			Gobiodon histrio	BD
			Gobiodon micropus	BD
			Gobiodon oculolineatus	BD
			Gobiodon okinawae	BD
			Gobiodon quinquestrigatus	BD
			Gobiodon rivulatus	BD
		Lophogobius	*Lophogobius cyprinoides*	PG
		Luposicya	*Luposicya lupus*	PG
		Lythrypnus	*Lythrypnus dalli*	BD
			Lythrypnus nesiotes	BD
			Lythrypnus phorellus	BD
			Lythrypnus spilus	BD
			Lythrypnus zebra	BD
		Paragobiodon	*Paragobiodon echinocephalus*	BD
		Pleurosicya	*Pleurosicya mossambica*	PG
		Priolepis	*Priolepis akihitoi*	BD
			Priolepis cinctus	BD
			Priolepis eugenius	SH
			Priolepis hipoliti	SH
			Priolepis semidoliata	BD
		Rhinogobiops	*Rhinogobiops nicholsii*	PG
		Tigrigobius	*Tigrigobius multifasciatus*	PG
		Trimma	*Trimma caesiura*	BD
			Trimma grammistes	BD

			Trimma kudoi	BD
			Trimma maiandros	BD
			Trimma naudei	BD
			Trimma okinawae	BD
			Trimma taylori	BD
			Trimma unisquamis	BD
			Trimma yanagitai	BD
Perciformes	Callanthiidae	Callanthias	*Callanthias parini*	PG
			Callanthias ruber	PG
	Centropomidae	Centropomus	*Centropomus undecimalis*	PA
	Eleginopsidae	Eleginops	*Eleginops maclovinus*	PA
	Epinephelidae	Aethaloperca	*Aethaloperca rogaa*	PG
		Alphestes	*Alphestes afer*	PG
			Alphestes immaculatus	PG
			Alphestes multiguttatus	PG
		Anyperodon	*Anyperodon leucogrammicus*	PG
		Aporops	*Aporops bilinearis*	PG
		Cephalopholis	*Cephalopholis aitha*	PG
			Cephalopholis argus	PG
			Cephalopholis aurantia	PG
			Cephalopholis boenak	(BD)
			Cephalopholis cruentata	PG
			Cephalopholis cyanostigma	PG
			Cephalopholis formosa	PG
			Cephalopholis fulva	PG
			Cephalopholis hemistiktos	PG
			Cephalopholis igarashiensis	PG
			Cephalopholis leopardus	PG
			Cephalopholis microprion	PG
			Cephalopholis miniata	PG
			Cephalopholis nigri	PG
			Cephalopholis oligosticta	PG
			Cephalopholis panamensis	PG
			Cephalopholis polleni	PG
			Cephalopholis sexmaculata	PG
			Cephalopholis sonnerati	PG
			Cephalopholis spiloparaea	PG
			Cephalopholis taeniops	PG
			Cephalopholis urodeta	PG
		Cromileptes	*Cromileptes altivelis*	PG
		Dermatolepis	*Dermatolepis dermatolepis*	PG
			Dermatolepis inermis	PG
			Dermatolepis striolata	PG
		Epinephelus	*Epinephelus adscensionis*	PG
			Epinephelus aeneus	PG
			Epinephelus akaara	(BD)
			Epinephelus albomarginatus	(BD)
			Epinephelus amblycephalus	PG
			Epinephelus analogus	PG
			Epinephelus andersoni	PG
			Epinephelus areolatus	PG
			Epinephelus awoara	PG
			Epinephelus bilobatus	PG
			Epinephelus bleekeri	PG
			Epinephelus bontoides	PG
			Epinephelus bruneus	PG
			Epinephelus caninus	PG
			Epinephelus chabaudi	PG

Epinephelus chlorocephalus	PG
Epinephelus chlorostigma	PG
Epinephelus cifuentesi	PG
Epinephelus coeruleopunctatus	PG
Epinephelus coioides	PG
Epinephelus corallicola	PG
Epinephelus costae	PG
Epinephelus cyanopodus	PG
Epinephelus daemelii	PG
Epinephelus darwinensis	PG
Epinephelus diacanthus	PG
Epinephelus drummondhayi	PG
Epinephelus epistictus	PG
Epinephelus erythrurus	PG
Epinephelus fasciatomaculosus	PG
Epinephelus fasciatus	PG
Epinephelus faveatus	PG
Epinephelus flavocaeruleus	PG
Epinephelus fuscoguttatus	PG
Epinephelus gabriellae	PG
Epinephelus goreensis	PG
Epinephelus guttatus	PG
Epinephelus heniochus	PG
Epinephelus hexagonatus	PG
Epinephelus howlandi	PG
Epinephelus indistinctus	PG
Epinephelus irroratus	PG
Epinephelus itajara	PG
Epinephelus labriformis	PG
Epinephelus lanceolatus	PG
Epinephelus latifasciatus	PG
Epinephelus lebretonianus	PG
Epinephelus longispinis	PG
Epinephelus macrospilos	PG
Epinephelus maculatus	PG
Epinephelus magniscuttis	PG
Epinephelus malabaricus	PG
Epinephelus marginatus	PG
Epinephelus melanostigma	PG
Epinephelus merra	PG
Epinephelus miliaris	PG
Epinephelus morio	PG
Epinephelus morrhua	PG
Epinephelus multinotatus	PG
Epinephelus ongus	PG
Epinephelus poecilonotus	PG
Epinephelus polylepis	PG
Epinephelus polyphekadion	PG
Epinephelus polystigma	PG
Epinephelus posteli	PG
Epinephelus quoyanus	PG
Epinephelus radiatus	PG
Epinephelus retouti	PG
Epinephelus rivulatus	PG
Epinephelus sexfasciatus	PG
Epinephelus sociales	PG
Epinephelus spilotoceps	PG
Epinephelus stictus	PG

		Epinephelus stoliczkae	PG
		Epinephelus suborbitalis	PG
		Epinephelus summana	PG
		Epinephelus tauvina	PG
		Epinephelus timorensis	PG
		Epinephelus trimaculatus	PG
		Epinephelus trophis	PG
		Epinephelus tuamotuensis	PG
		Epinephelus tukula	PG
		Epinephelus undulatostriatus	PG
		Epinephelus undulosus	PG
	Gonioplectrus	*Gonioplectrus hispanus*	PG
	Gracila	*Gracila albomarginata*	PG
	Hyporthodus	*Hyporthodus acanthistius*	PG
		Hyporthodus ergastularius	PG
		Hyporthodus exsul	PG
		Hyporthodus flavolimbatus	PG
		Hyporthodus haifensis	PG
		Hyporthodus mystacinus	PG
		Hyporthodus nigritus	PG
		Hyporthodus niphobles	PG
		Hyporthodus niveatus	PG
		Hyporthodus octofasciatus	PG
		Hyporthodus perplexus	PG
		Hyporthodus quernus	PG
		Hyporthodus septemfasciatus	PG
	Mycteroperca	*Mycteroperca acutirostris*	PG
		Mycteroperca bonaci	PG
		Mycteroperca cidi	PG
		Mycteroperca fusca	PG
		Mycteroperca interstitialis	PG
		Mycteroperca jordani	PG
		Mycteroperca microlepis	PG
		Mycteroperca olfax	PG
		Mycteroperca phenax	PG
		Mycteroperca prionura	PG
		Mycteroperca rubra	PG
		Mycteroperca tigris	PG
		Mycteroperca venenosa	PG
		Mycteroperca xenarcha	PG
	Plectropomus	*Plectropomus areolatus*	PG
		Plectropomus laevis	PG
		Plectropomus leopardus	PG
		Plectropomus maculatus	PG
		Plectropomus oligacanthus	PG
		Plectropomus pessuliferus	PG
		Plectropomus punctatus	PG
	Pseudogramma	*Pseudogramma gregoryi*	SH
		Pseudogramma xantha	PG
	Rypticus	*Rypticus bistrispinus*	PG
		Rypticus saponaceus	PG
	Saloptia	*Saloptia powelli*	PG
	Triso	*Triso dermopterus*	PG
	Variola	*Variola albimarginata*	PG
		Variola louti	PG
Grammatidae	Gramma	*Gramma loreto*	PG
Labridae	Achoerodus	*Achoerodus gouldii*	PG
		Achoerodus viridis	PG

Anampses	*Anampses cuvier*	PG
	Anampses geographicus	PG
Bodianus	*Bodianus bilunulatus*	PG
	Bodianus diplotaenia	PG
	Bodianus eclancheri	PG
	Bodianus rufus	PG
	Bodianus speciosus	PG
Cheilinus	*Cheilinus undulatus*	PG
Choerodon	*Choerodon azurio*	PG
	Choerodon schoenleinii	PG
Cirrhilabrus	*Cirrhilabrus temminckii*	PG
Clepticus	*Clepticus parrae*	PG
Coris	*Coris flavovittata*	PG
	Coris julis	PG
Epibulus	*Epibulus insidiator*	PG
Halichoeres	*Halichoeres bivittatus*	PG
	Halichoeres garnoti	PG
	Halichoeres maculipinna	PG
	Halichoeres marginatus	PG
	Halichoeres melanochir	PG
	Halichoeres pictus	PG
	Halichoeres poeyi	PG
	Halichoeres radiatus	PG
	Halichoeres scapularis	PG
	Halichoeres semicinctus	PG
	Halichoeres trimaculatus	(BD)
Hologymnosus	*Hologymnosus annulatus*	PG
Iniistius	*Iniistius dea*	PG
	Iniistius geisha	PG
	Iniistius melanopus	PG
	Iniistius pavo	PG
	Iniistius pentadactylus	PG
Labroides	*Labroides dimidiatus*	(BD)
Labrus	*Labrus bergylta*	PG
	Labrus mixtus	PG
Lachnolaimus	*Lachnolaimus maximus*	PG
Notolabrus	*Notolabrus celidotus*	PG
Parajulis	*Parajulis poecilepterus*	PG
Pseudolabrus	*Pseudolabrus japonicus*	PG
	Pseudolabrus sieboldi	(BD)
Pteragogus	*Pteragogus flagellifer*	PG
Semicossyphus	*Semicossyphus pulcher*	PG
Stethojulis	*Stethojulis trilineata*	PG
Suezichthys	*Suezichthys ornatus*	PG
Symphodus	*Symphodus melanocercus*	PG
	Symphodus melops	PG
	Symphodus ocellatus	PG
	Symphodus roissali	PG
	Symphodus tinca	PG
Thalassoma	*Thalassoma bifasciatum*	PG
	Thalassoma cupido	PG
	Thalassoma duperrey	PG
	Thalassoma lucasanum	PG
	Thalassoma lunare	PG
	Thalassoma lutescens	PG
	Thalassoma pavo	PG
	Thalassoma purpureum	PG
	Thalassoma quinquevittatum	PG

	Xyrichtys	*Xyrichtys novacula*	PG
		Xyrichtys martinicensis	PG
		Xyrichtys splendens	PG
Latidae	Lates	*Lates calcarifer*	PA
Lethrinidae	Lethrinus	*Lethrinus atkinsoni*	PG
		Lethrinus rubrioperculatus	PG
		Lethrinus genivittatus	PG
		Lethrinus lentjan	PG
		Lethrinus mahsena	PG
		Lethrinus miniatus	PG
		Lethrinus nebulosus	PG
		Lethrinus variegatus	PG
Nemipteridae	Nemipterus	*Nemipterus furcosus*	PG
	Scolopsis	*Scolopsis bilineatus*	PG
		Scolopsis monograma	PG
		Scolopsis taeniopterus	PG
Pinguipedidae	Parapercis	*Parapercis lineopunctata*	PG
		Parapercis snyderi	PG
Plesiopidae	Plesiops	*Plesiops nigricans*	PG
Polynemidae	Eleutheronema	*Eleutheronema tetradactylum*	PA
	Galeoides	*Galeoides decadactylus*	PA
	Polydactylus	*Polydactylus macrochir*	PA
		Polydactylus multiradiatus	PA
		Polydactylus sexfilis	PA
Pomacanthidae	Centropyge	*Centropyge acanthops*	(BD)
		Centropyge bicolor	PG
		Centropyge ferrugata	(BD)
		Centropyge flavicauda	(BD)
		Centropyge flavissima	(BD)
		Centropyge interrupta	PG
		Centropyge multispinis	PG
		Centropyge potteri	PG
		Centropyge resplendens	PG
		Centropyge tibicen	PG
	Genicanthus	*Genicanthus bellus*	PG
		Genicanthus caudovittatus	PG
		Genicanthus lamarck	PG
		Genicanthus semifasciatus	PG
		Genicanthus watanabei	PG
	Holacanthus	*Holacanthus tricolor*	PG
Pomacentridae	Amphiprion	*Amphiprion akallopisos*	PA
		Amphiprion akindynos	PA
		Amphiprion allardi	PA
		Amphiprion bicinctus	PA
		Amphiprion chagosensis	PA
		Amphiprion chrysogaster	PA
		Amphiprion chrysopterus	PA
		Amphiprion clarkii	PA
		Amphiprion ephippium	PA
		Amphiprion frenatus	PA
		Amphiprion fuscocaudatus	PA
		Amphiprion latezonatus	PA
		Amphiprion latifasciatus	PA
		Amphiprion leucokranos	PA
		Amphiprion mccullochi	PA
		Amphiprion melanopus	PA
		Amphiprion nigripes	PA
		Amphiprion ocellaris	PA

		Amphiprion omanensis	PA
		Amphiprion percula	PA
		Amphiprion perideraion	PA
		Amphiprion polymnus	PA
		Amphiprion rubrocinctus	PA
		Amphiprion sandaracinos	PA
		Amphiprion sebae	PA
		Amphiprion thiellei	PA
		Amphiprion tricinctus	PA
	Dascyllus	*Dascyllus aruanus*	PG
		Dascyllus carneus	PG
		Dascyllus flavicaudus	PG
		Dascyllus marginatus	PG
		Dascyllus reticulatus	PG
Pseudochromidae	Anisochromis	*Anisochromis straussi*	PG
	Pseudochromis	*Pseudochromis aldabraensis*	(BD)
		Pseudochromis cyanotaenia	(BD)
		Pseudochromis flavivertex	(BD)
		Pseudochromis fridmani	PG
		Pseudochromis olivaceus	PG
Scaridae	Calotomus	*Calotomus carolinus*	PG
		Calotomus japonicus	PG
		Calotomus spinidens	PG
		Calotomus zonarchus	PG
	Chlorurus	*Chlorurus perspicillatus*	PG
		Chlorurus gibbus	PG
		Chlorurus sordidus	PG
	Cryptotomus	*Cryptotomus roseus*	PG
	Scarus	*Scarus coelestinus*	PG
		Scarus coeruleus	PG
		Scarus dubius	PG
		Scarus festivus	PG
		Scarus frenatus	PG
		Scarus ghobban	PG
		Scarus globiceps	PG
		Scarus guacamaia	PG
		Scarus iserti	PG
		Scarus niger	PG
		Scarus psittacus	PG
		Scarus rivulatus	PG
		Scarus rubroviolaceus	PG
		Scarus schlegeli	PG
		Scarus taeniopterus	PG
		Scarus vetula	PG
	Sparisoma	*Sparisoma atomarium*	PG
		Sparisoma aurofrenatum	PG
		Sparisoma chrysopterum	PG
		Sparisoma radians	PG
		Sparisoma rubripinne	PG
		Sparisoma viride	PG
Serranidae	Anthias	*Anthias anthias*	PG
		Anthias nicholsi	PG
		Anthias noeli	PG
	Baldwinella	*Baldwinella vivanus*	PG
	Centropristis	*Centropristis ocyurus*	PG
		Centropristis philadelphica	PG
		Centropristis striata	PG
	Chelidoperca	*Chelidoperca hirundinacea*	PG

	Diplectrum	*Diplectrum bivittatum*	SH
		Diplectrum formosum	SH
		Diplectrum macropoma	SH
		Diplectrum pacificum	SH
		Diplectrum radiale	SH
		Diplectrum rostrum	SH
		Hemanthias leptus	PG
		Hemanthias peruanus	PG
	Hypoplectrodes	*Hypoplectrodes huntii*	PG
		Hypoplectrodes maccullochi	PG
		Hypoplectrus chlorurus	SH
		Hypoplectrus nigricans	SH
		Hypoplectrus puella	SH
		Hypoplectrus unicolor	SH
	Pronotogrammus	*Pronotogrammus martinicensis*	PG
	Pseudanthias	*Pseudanthias bicolor*	PG
		Pseudanthias conspicuus	PG
		Pseudanthias elongatus	PG
		Pseudanthias squamipinnis	PG
		Pseudanthias taeniatus	PG
	Sacura	*Sacura margaritacea*	PG
	Serraniculus	*Serraniculus pumilio*	SH
	Serranus	*Serranus annularis*	SH
		Serranus atricauda	SH
		Serranus baldwini	SH
		Serranus cabrilla	SH
		Serranus hepatus	SH
		Serranus notospilus	SH
		Serranus phoebe	SH
		Serranus psittacinus	SH
		Serranus scriba	SH
		Serranus subligarius	SH
		Serranus tabacarius	SH
		Serranus tigrinus	SH
		Serranus tortugarum	SH
Sparidae	Acanthopagrus	*Acanthopagrus australis*	PA
		Acanthopagrus berda	PA
		Acanthopagrus bifasciatus	PA
		Acanthopagrus latus	PA
		Acanthopagrus schlegelii	PA
	Argyrops	*Argyrops spinifer*	PG
	Boops	*Boops boops*	PG
	Calamus	*Calamus bajonado*	PG
		Calamus leucosteus	PG
		Calamus nodosus	PG
		Calamus pennatula	PG
		Calamus proridens	PG
	Chrysoblephus	*Chrysoblephus cristiceps*	PG
		Chrysoblephus laticeps	PG
		Chrysoblephus puniceus	PG
	Dentex	*Dentex gibbosus*	PG
	Diplodus	*Diplodus annularis*	PA
		Diplodus argenteus argenteus	PA
		Diplodus capensis	PA
		Diplodus sargus cadenati	PA
		Diplodus sargus kotschyi	PA
		Diplodus sargus sargus	PA
	Evynnis	*Evynnis tumifrons*	PG

		Lithognathus	*Lithognathus aureti*	PA
			Lithognathus mormyrus	PA
		Pachymetopon	*Pachymetopon aeneum*	PG
		Pagellus	*Pagellus acarne*	PA
			Pagellus belottii	PG
			Pagellus bogaraveo	PA
			Pagellus erythrinus	PG
		Pagrus	*Pagrus auriga*	PG
			Pagrus caeruleostictus	PG
			Pagrus pagrus	PG
		Rhabdosargus	*Rhabdosargus globiceps*	PG
			Rhabdosargus sarba	PA
		Sarpa	*Sarpa salpa*	PA
		Sparidentex	*Sparidentex hasta*	PA
		Sparus	*Sparus aurata*	PA
		Spicara	*Spicara maena*	PG
			Spicara smaris	PG
		Spondyliosoma	*Spondyliosoma cantharus*	PG
	Trichonotidae	Trichonotus	*Trichonotus filamentosus*	PG
Scorpaeniformes	Platycephalidae	Cociella	*Cociella crocodilus*	PA
		Inegocia	*Inegocia japónica*	PA
		Kumococius	*Kumococius rodericensis*	PA
		Platycephalus	*Platycephalus fuscus*	PA
		Suggrundus	*Suggrundus meerdervoortii*	PA
	Scorpanidae	Caracanthus	*Caracanthus typicus*	(BD)
			Caracanthus unipinna	PG
Stomiiformes	Gonostomatidae	Cyclothone	*Cyclothone atraria*	PA
			Cyclothone microdon	PA
		Gonostoma	*Gonostoma elongatum*	PA
		Sigmops	*Sigmops bathyphilum*	PA
			Sigmops gracilis	PA
Synbranchiformes	Synbranchidae	Monopterus	*Monopterus albus*	PG
			Monopterus boueti	PG
		Ophisternon	*Ophisternon bengalense*	PG
		Synbranchus	*Synbranchus marmoratus*	PG

Abbreviations: PG, Protogyny; PA, Protandry; BD, Bi-Directional; SH, Simultaneous hermaphroditism; PG and BD are related. (BD) indicates that evidence for bi-directional hermaphroditism has been obtained only under laboratory conditions, not recorded in the field and thus here, as precaution, were treated like PG.

Chapter 2

Switches, stability and reversals: the evolutionary history of sexual systems in fish

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Abstract

Theoretical models, initially developed for plants, suggest that gonochorism (G) and simultaneous hermaphroditism (SH) are evolutionary stable conditions. However, if this is the case in animals is unclear, especially in sexually plastic taxa as teleosts, where in addition to G and SH there is sequential hermaphroditism (SEQ). Understanding the different sexual systems and their transitions is a major challenge in evolutionary biology. Here, we collected information on the sexual system of 4,740 species of fish to reconstruct the ancestral sexual state and the evolutionary transition rates. Life-history traits such as longevity, length at first maturity, and investment on male gonad tissue using the gonadosomatic index (GSI) as a proxy of sperm competition were also collected for ~800 species with different sexual systems. Results showed that gonochorism is the most likely ancestral condition in fish from which protogyny, protandry and, to a lesser extent, simultaneous hermaphroditism evolved at a low rate. Unlike protandry that once evolved quickly changes to a different sexual system, gonochorism, simultaneous hermaphroditism and protogyny are evolutionary stable conditions. Regarding the association between life-history traits and sexual systems, we found that longevity was significantly higher in protogynous species when compared to their gonochoristic relatives. This is not due to the complex social systems typical of many protogynous species living in coral reefs that, theoretically, could favor from higher longevity, as initially surmised, since higher longevity was observed irrespective of habitat. The higher longevity of protogynous species was explained by the age-related increase in fecundity of females to satisfy the fertilization capacity of large males, thereby increasing their lifetime reproductive fitness. However, age at first maturity was similar between protogynous and gonochoristic species, thus indicating that gonochoristic species comparatively reproduce at an older age than protogynous species. Taken together, our results suggest that lifetime female function success is achieved in different ways: by increasing longevity in protogynous species and by delaying age at first maturity in gonochoristic species. Finally, we show that GSI values can serve as good indicators of the level of sperm competition in gonochoristic and protogynous, but not protandrous, species. This study can generate hypothesis regarding the association between important life-history traits and sexual systems that can be tested in future studies focusing on particular families and perhaps also in other taxa where protogyny and gonochorism coexist.

Keywords: Actinopterygian, ancestral sexual reconstruction, haremic, longevity, gonadosomatic index, protogyny, Reversible Jump MCMC, sexual systems

1. Introduction

Sexual reproduction is a predominant feature of Eukaryotes (Speijer et al., 2015), yet it is achieved in a great diversity of forms (Bachtrog et al., 2014). Broadly, sexual systems vary from dioecy/gonochorism (separate fixed sexes) to simultaneous hermaphroditism (one individual can produce both male and female gametes at the same time) in a sexually plastic gradient of intermediate/mixed systems (Ah-King and Nylin, 2010, Leonard, 2018b). Such diversity and plasticity imply multiple transformations from an ancestral state and promote (and respond to) different selective pressure. Thus, a good understanding of the evolution of sexual systems is relevant for evolutionary studies of adaptations and speciation as well as conservation and management of endangered and exploited species. However, despite multiple attempts, understanding evolutionary transitions among different sexual systems, particularly in Metazoa, has been impaired by the limited data available. As a result, just a few studies have thoroughly addressed the adaptive significance of the different sexual systems and, in particular, of hermaphroditism, in large taxonomic groups (e.g., (Eppley and Jesson, 2008, Weeks, 2012, Sasson and Ryan, 2017).

Hermaphroditism prevails in flowering plants (angiosperms) (Käfer et al., 2017), where 94% of the species have male and female sex organs in the same individual/flower, and it is widespread in invertebrates (e.g., crustaceans, mollusks, annelids, cnidarians, echinoderms, nematodes, flat worms, but not insects), totaling 5% of animal species (Jarne and Auld, 2006). Theoretical models, initially developed for plants, suggest that gonochorism and simultaneous hermaphroditism are evolutionary stable conditions. In angiosperms, simultaneous hermaphroditism is the ancestral state, from which dioecy evolved independently several times (Bawa, 1980). The situation is less clear for animals in general where a gonochoristic ancestor is often assumed (e.g. (Ghiselin, 1969, Avise and Mank, 2009, Erisman et al., 2013) but no definite conclusion has been reached (Sasson and Ryan, 2017). Among vertebrates, only fish exhibit hermaphroditism. Fishes represent, with more than 35,000 species (Fricke et al., 2019), approximately half of all known extant vertebrates and are characterized by the broadest variety of sex determination systems (Penman and Piferrer, 2008, Bachtrog et al., 2014). This plasticity results in gonochorism (G), simultaneous hermaphroditism (SH; outcrossing between two partners, which in turn fertilize each other eggs) and sequential hermaphroditism (SQ) or sex change. In the latter, individuals reproduce initially as one sex and later in life switch to the other, with female being the first sex in protogyny (PG) and male the first sex in protandry (PA), or individuals can shift between the two sexes (bidirectional sex change; BD). Few species are androdioecious (they belong to a mixed mating system where self-compatible hermaphrodites coexist with males) (Weeks et al., 2006) and some cases of unisexuality (all-female populations) are also present (Vrijenhoek, 1994). These sexual systems are widespread across the phylogenetic tree of fishes (Devlin and Nagahama, 2002, Sadovy de Mitcheson and Liu, 2008, Avise and Mank, 2009), suggesting multiple evolutionary origins and transitions between sexual systems.

Given the sexual/gonadal plasticity of fish, it is not surprising that various studies have addressed the evolution of sexual systems in individual fish families. For example, a quantitative rate of transitions between sex change and dioecy using the Reversible Jump Markov Chain Monte Carlo (RJ MCMC) method was estimated for the first time in the Labridae (Kazancioglu and Alonzo, 2010). Subsequently, Sunobe et al. (2017), using the maximum-likelihood (ML) optimality criterion with Mesquite software application, investigated the evolutionary transitions of sexuality and mating systems of species in three gobiid genera. They found that gonochorism is the ancestral state in the sex changers *Trimma, Priolepis* and *Trimmaton*. However, a comprehensive analysis of all sexual systems in fish in a phylogenetic framework, has not been attempted yet.

It has been argued that to interpret the distribution and adaptive value of different sexual systems. a better understanding of how life-history parameters interact with the sexual system is needed (Leonard, 2013). Longevity and length at first maturity are two of the most relevant life-history traits connected with sexual systems. Life-history theory predicts how species optimize their survival and reproduction success taking into account the relationship between longevity and maturity (Stearns, 1992, Roff, 1992). Higher longevity facilitates a larger period of reproduction during the lifespan (Beverton, 1987, Das, 1994). Thus, species with great longevity have overlapping generations and this can favor sociality (Arnold and Owens, 1998). Therefore, a possible prediction is that social species are long-lived species than non-social species (Taborsky and Wong, 2017). Protogyny is the most common sexual system among hermaphroditic fishes (Sadovy de Mitcheson and Liu, 2008): in this system, the transition from female to male is socially controlled. Indeed these species present complex social systems. It has been suggested that species with complex social interactions live longer (Leonard, 2013). However, to our knowledge, whether PG species live longer than gonochorism relatives has not been formally tested.

According to the relationship between life-history traits, in reef-associated labrids, the smaller species mature at larger size relative to their maximum adult size than larger species do (Hubble, 2003), depending on its longevity (e.g., Charnov (1993). Individuals can obtain greater reproductive success if they mature later because by being bigger they can produce a larger amount of gametes (Stearns and Crandall, 1981). Therefore, larger prolonged growth is a trade-off with early reproduction (Roff, 1984) but the advantage of delaying maturity is the multiple reproductive events or / and a longer lifespan. Another explanation to delay maturity considers the possible physiological restriction due to body space to store eggs where small species may have a development size-threshold (Roff, 1992, Day and Rowe, 2002) which is not found in larger species. Based on the above, it has been suggested that protogynous hermaphrodites, in which the initial sex is female and small individuals might need to reach a certain size to produce eggs, overall mature later than gonochoristic relatives (Leonard, 2013), an aspect that until now, to the best of our knowledge, has never been formally tested.

Many protogynous species have territorial males that monopolize harems of females (Warner, 1984, Warner, 1988a, Walker and McCormick, 2009). The size-advantage model (Ghiselin, 1969) explains the adaptive advantage of protogyny based on the high reproductive success of large males. Group mating leads to high sperm competition (Warner, 1984, Munoz and Warner, 2003), but in haremic systems competition among males is drastically reduced (e.g., (Sadovy et al., 1994, Donaldson, 1995, Mackie, 2007) and thus a reduced testis size of protogynous species compared to gonochoristic species, has been predicted (Warner, 1975, Stockley et al., 1997, Taborsky, 1998, Molloy et al., 2007). The gonadosomatic index (GSI), the relative weight of the gonad to the total weight of the individual, has been used as a reliable proxy for sperm competition (Stockley et al., 1997). Several comparative studies (encompassing phylogenetic analyses of different taxa and independent contrast methodology; Molloy et al., (2007)) have determined that the GSI of protogynous species are, as predicted, significantly smaller than their gonochoristic congeners. However, testing whether GSI is a good indicator of low sperm competition suggestive of social structures and encompassing gonochoristic, protogynous and protandrous species has not been done phylogenetically on a large scale.

Here, we compiled the largest dataset of sexual system and life-history traits in Actinopterygian fish and first reconstructed the ancestral state and investigated the evolutionary transitions using a large-scale phylogenetic comparative approach. Next, we tested predictions relating key life-history traits within protogyny, the most common form of hermaphroditism Specifically, we tested whether protogynous species live longer, mature later and have lower investment in the male gonads (lower GSI) when compared with protandrous and gonochoristic relatives.

2. Material and methods

2.1. Definition of sexual systems

In this study we define the *sexual system* of a given species as follows: gonochorism, also called dioecy (G), where individuals reproduce only in one sexual role during the lifetime, either as males or as females; protogyny (PG), where individuals first reproduce as females, change sex once with increasing size/age and then reproduce as males; protandry (PA), where individuals first reproduce as males, change sex once with increasing size/age and then reproduce as females; bi-directional sequential hermaphroditism (BD), where individuals change sex several times in both directions throughout their lifespan; simultaneous hermaphroditism (SH), where individuals produce gametes of both sexes at the same time or in a short period of time.

2.2. Data collection and verification

We collected information from FishBase (www.fishbase.com; (Froese and Pauly, 2018) on the sexual system for a total of 10,914 Actinopterygian species, of which 10,875 were teleosts. We also collected information on life-history traits from FishBase and from the literature. We ended up with a database containing information for both sexual system and life-history traits for 9,039 Actinopterygian species (9,005 teleosts). This database contained 552 hermaphrodite species, all of them teleosts.

Of the 552 hermaphroditic species 403 teleost species were present in the phylogenetic tree used for this study (see details in section 2.4 below). Of these 403 teleost hermaphroditic species, we could verify, on a one-by-one basis, the sexual system for 278 of them against the primary literature. The main overall references used were: Sadovy de Mitchelson (2008), Devlin and Nagahama (2002), Allsop and West (2003) but reviews on specific families were given priority, e.g., Sparidae (Buxton and Garratt, 1990), Labridae (Kazancioglu and Alonzo, 2010) and Serranidae (Erisman and Hastings, 2011). Of the 278 species checked, 264 (95%) matched, i.e., the sexual system assigned to each one of them in FishBase coincided with the one assigned in the primary literature, while 14 species (5%) did not match. In case of discrepancy between FishBase and the primary literature in terms of sexual system, we took as valid that of the primary literature. Assuming a similar level of discrepancy in the remaining 125 species for which we could not verify their sexual system, we should have at most six species with wrong assignment of the sexual system, which represent 1.48% of the total of 403 species. We consider this level of error low and acceptable

2.3. Definition of each variable for PGLS, sources, searches, sample sizes

Longevity was defined as the maximum age (in years) recorded in the wild. We collected data from FishBase and additional primary sources (e.g., Carey and Judge (2000), De Magalhaes and Costa (2009)) for the species in the phylogenetic tree and our dataset (n = 803 sp.). Data on *length at maturity*, defined as the mean of total length (cm) at which the fish of a given population develop ripe male/female gonads for the first time, was extracted from FishBase and the primary literature (n = 362 spp.). *GSI* was defined as the mean of gonadosomatic index, i.e. the gonad weight expressed as a percentage of whole body weight. We collected information on male GSI exclusively from the primary literature (n = 103 spp.). Of these 103 species, we could determine that the male GSI values corresponded to monandrous (n = 8 species; all males are secondary males that only come from sex change) or diandrous species (n = 3 species; in addition to secondary males, there are also primary males that mature directly as males). We could not find this information for the remaining 92 protogynous species. Thus, we first used only monandrous protogynous species against gonochorism and protandry, and then we used all protogynous species regardless we had information on monandry or diandry. Finally, to confirm that GSI is a good indicator of sperm competition, we expected lower GSI values to be found in species with mating system where males do not compete for access to females. Therefore, we classified species in two categories, low or high sperm competition, combining mating system and spawning behavior data taken from the primary literature on gonochoristic (n=6), protandrous (n=4) and protogynous (n=24) species. Specifically, we considered species that engage in pair spawning as having low sperm competition while those engaging in group spawning as having high sperm competition (Walker and McCormick, 2009, Allsop and West, 2003). Likewise, we considered species exhibiting monogamous and haremic mating as having low sperm competition, while those exhibiting promiscuous mating and mating in aggregations as having high sperm competition (Stockley et al., 1997, Petersen and Warner, 1998). Note that, in the latter case, we considered high sperm competition if this was confirmed in the primary literature because some species can have pair mating within aggregations (Fish Aggregation Database: https://www.scrfa.org/database/). Therefore, we selected 34 species with known male GSI and levels of sperm competition.

2.4. Phylogenetic comparative analysis

For all phylogenetic comparative analyses, we combined our dataset with the most comprehensive molecular, dated, fully bifurcating phylogeny of teleosts to date (Rabosky et al 2018) that includes 11,638 extant species. Of the 9,039 species in the dataset, 4,740 species were present in this phylogeny of which 4,337 species are gonochoristic; 285 species protogynous; 57 species protandrous, 14 bi-directional and 47 simultaneous hermaphrodites (**Table S1**).

We investigated the evolutionary history of sexual systems using Multistate models in *BayesTraits* V3 (Pagel et al., 2004, Pagel and Meade, 2006) in a Bayesian framework. Multistate estimates instantaneous transition rates between alternative character states of a single categorical variable (i.e., the rate of change between states along individual branches of the phylogeny), based on a continuous time Markov model of evolution (Pagel, 1994, Currie and Meade, 2014). A higher transition rate from one state to another indicates that that the first state changes rapidly to the second state over evolutionary time. Therefore, a character state is evolutionary stable when transition rates ‘into’ it are greater than those ‘out’ of it, i.e. when it is lost more slowly than it is gained. Conversely, a state that is lost more quickly than it is gained suggests that it is evolutionarily unstable (Furness and Capellini, 2019). Multistate also produces posterior distributions of the ancestral character state at the root of the phylogeny.

We scaled the tree by a default constant (mean of 0.1) in all analyses. Scaling the tree allows the algorithm to better explore parameter space as the transition rates are typically very small and so difficult to estimate and search for (Furness and Capellini, 2019). Scaling the tree also scales the estimates of the transition rates by the same constant and therefore does not alter conclusions. We ran all analyses with Markov Chain Monte Carlo (MCMC) and an exponential prior whose mean was seeded from a uniform hyperprior ranging from 0 to 10 to reduce inherent uncertainty and biases of prior choice (Pagel and Meade, 2006). MCMC analysis samples models in direct proportion to their fit to the data and generates a posterior distribution of parameter estimates for each transition rate. All analyses were run in Reversible Jump MCMC (rjMCMC) which sets some parameters equal to zero or equal to one another thereby reducing model complexity and overparametrization (Pagel et al., 2004, Pagel and Meade, 2006, Currie and Meade, 2014). Therefore, not only do we consider the mean and 95% high posterior density (95-HPD) interval for each posterior distribution of parameter estimates, but also the mode and percentage of models with parameters set to 0, as the posterior distributions generated by rjMCMC may not be normal.

We ran all MCMC chains for 400 million iterations with an additional burn-in of half a million iterations and sampled every 200,000 iterations, thus resulting into a 2,000 posterior sample per chain. All chains converged and showed good mixing as indicated by their effective sample sizes (ESS), a measure of possible autocorrelation, well over 1000 for all parameters and visual inspections of their traces in *Tracer* v1.6 (Rambaut et al., 2018). All analyses were run in triplicate and the three independent chains converged on very similar solutions, leading to qualitatively similar results. Here we present the results from the first chain. We ran rjMCMC Multistate analysis on sexual system as a binary state (1= gonochorism; or 2 = hermaphrodite); as a three state categorical variable (1= gonochorism; 2= sequential hermaphrodite including protogyny, protandry, and bidirectional or 3 =simultaneous hermaphroditism); and as a four state categorical variable (1= gonochorism; 2= protogyny, 3= protandry, or 4 =simultaneous hermaphrodite). Bi-directional sequential hermaphrodites was excluded from the latter analysis as the sample size of extant species was too low and the chained failed to converge and mix properly when sexual system was studies as a 5 states categorical variable **(Table S1).**

Because *BayesTraits* does not provide graphic tools, we visually represented the evolutionary history of sexual system on the phylogeny using maximum likelihood (ML) and the R package *ape* v.5.3 (Paradis, 2012), when the latter provided a reasonably close approximation of the rjMCMC Multistate analysis. This could be done only for the analysis at 2 states as for analysis at 3 states (gonochorism, sequential or simultaneous hermaphroditism) and at 4 states (gonochorism, protogyny, protandry or simultaneous hermaphroditism), ML estimation was a poor representation of the rjMCMC results.

We used phylogenetic generalized least square (PGLS) models (Freckleton et al., 2002, Pagel, 1997, Pagel, 1999) to test for the association between longevity, length at first maturity and male GSI, and sexual systems, and the relationship between male GSI and sperm competition, in the R package *caper* (Orme, 2013) with ML estimation. In PGLS the parameter λ quantifies the strength of the phylogenetic signal in the data and ranges between zero, i.e. the similarity between species in trait values is independent of phylogeny, and one, i.e., similarity between species in trait values is directly proportional to their common evolutionary time under Brownian motion model of evolution (Pagel, 1999, Freckleton et al., 2002). We tested PGLS models with longevity, length at first maturity and GSI as the dependent variable, and sexual system as the independent discrete variable with three possible states (G, PG, PA). All continuous variables were $\log_{10}$-transformed to meet assumptions of normality. All model residuals were normally distributed in all analyses. For PGLS analyses differences were considered statistically significant when *P*- value < 0.05.

3. Results

3.1. Evolutionary history

We used 4,740 extant species in the phylogeny (of which 403 were hermaphroditic) and rjMCMC Multistate analysis (gonochorism vs. hermaphroditism) to reconstruct the ancestral sexual system of fishes. This means that our analysis involved 73% of the 552 hermaphroditic species known to date (**Chapter 1**). We found that gonochorism is the ancestral state in teleosts and hermaphroditism is gained slowly but reverts very quickly to gonochorism, indicating that hermaphroditism is an evolutionary unstable condition (**Table S2, Fig. S1).** Visual inspection of the ancestral state reconstruction in ML shows that hermaphroditism evolves and is lost multiple times along fish phylogeny (**Fig. 1**).

When the sexual system was analyzed as a 3-state variable (gonochorism, sequential hermaphroditism and simultaneous hermaphroditism), Multistate indicates that sequential hermaphroditism evolves from the ancestral condition of gonochorism at a very low rate and it can either revert quickly to gonochorism or it can switch to simultaneous hermaphroditism at a relatively low rate. Conversely, simultaneous hermaphroditism is very unlikely to evolve directly from gonochorism as 99% of models estimates this transition rate to be equal to zero. Once evolved from sequential hermaphroditism, simultaneous hermaphroditism evolves slowly to gonochorism or to a lesser extent back to sequential hermaphroditism since nearly 60% of models estimate that the latter transition rate is equal to zero (**Fig. S2, Table S3**).

Finally, we repeated the analysis by considering the four main sexual systems – gonochorism, protogyny, protandry and simultaneous hermaphroditism–. The rjMCMC Multistate analysis revealed that all 3 types of hermaphroditism evolve at a very low rate from the ancestral condition of gonochorism, but protogyny and protandry revert very rapidly back to gonochorism while the rate of loss for simultaneous hermaphroditism is low and similar to its rate of gain (**Fig. 2, Table S4**). Protogyny may also switch to either protandry or simultaneous hermaphroditism at a very low rate. Conversely, protandry is lost very quickly to protogyny or to a lesser extent to simultaneous hermaphroditism as 38 % of the models estimate this latter transition to be zero. Finally, while simultaneous hermaphroditism may evolve at a very low rate from gonochorism, protogyny, and possibly protandry, it is lost at a very low rate to gonochorism, and possibly to protogyny or protandry, even though 45% and 48% of models, respectively, estimate these latter two transitions to be 0 (**Fig. 2, Table S4**).

Altogether, these results indicate that gonochorism is the ancestral state of fishes and that is an evolutionarily stable state; protogyny is evolutionarily more stable than protandry, while simultaneous hermaphroditism evolves rarely but is evolutionarily stable, being lost slowly to gonochorism, and possibly to protogyny and protandry (**Fig. 2, Table S4**).

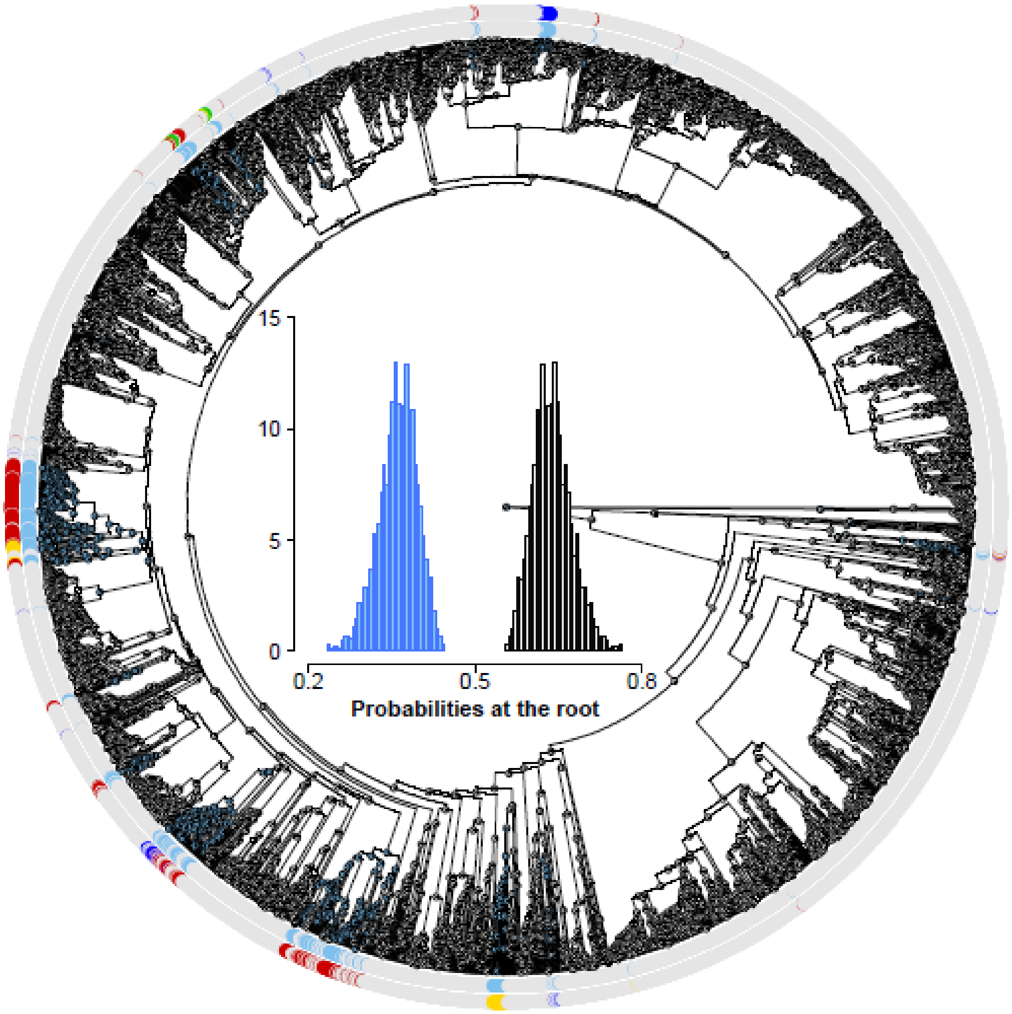

Figure 1. Visual representation of the evolutionary history of sexual system in Actinopterygian fishes (gonochorism or hermaphroditism) in maximum likelihood using the R package Ape v4. In the inner circle and in the internal nodes of the phylogeny the sexual system of extant species and their ancestors are color coded as gonochorism in grey and hermaphroditism in light blue. In the external circle, the sexual system of extant species is color coded as gonochorism (grey), protogyny (red), protandry (blue), bidirectional (green) and simultaneous hermaphroditism (yellow). The density plots in the inset show the estimated probability of character state at the root of the phylogeny derived from the rjMCMC Multistate analysis in BayesTraits and color coded as gonochorism in grey and hermaphroditism in light blue.

3.2. Life history traits

We did not find overall significant differences in longevity among gonochorism, protogyny and protandry, although protogynous species had a maximum lifespan significantly longer that gonochoristic species (**Fig. 3A; Table 1**). There was no significant difference in total length at first maturity between teleost species with different sexual systems (**Fig. 3B; Table S5**). When the 8 monandric species were included in the analysis, the average GSI values of the protogynous species were lower than those of the gonochoristic and protandrous species ($t= -4.53$; $df= 63$; $\lambda= 0.757$; $P < 0.001$), but there was no difference between gonochorism and protandry. The same results were obtained if we considered all 103 teleost species in the tree where male GSI values were available, i.e., all protogynous species, regardless whether they are monandry or diandry (**Fig. 3C; Table 2**). Teleost fish that have low sperm competition because of their mating system showed a significantly smaller GSI value than individuals that exhibit high sperm competition ($t= 4.27$, $df= 32$; *P*-value= 0.0001; $\lambda= 0.244$, $R^2= 0.363$; **Fig. S3**).

Table 1. Results of phylogenetic generalized least square model of longevity across sexual systems. For each independent variable we report the parameter estimate (Beta), t-statistics (T), and P-value, and the model statistics including the degrees of freedom (df), the ML lambda value and R^2. This analysis is based on sample size of 803 extant species of which 696 are gonochoristic, 88 are protogynous, and 19 are protandrous

Variables					**Model stats**		
Dependent	**Independent**	**Beta**	**T**	**P**	**df**	**λ**	**R^2**
Longevity	Sexual system - PA[1]	0.027	0.274	0.783	2,800	0.903	0.004
($\log_{10}$	Sexual system - PG[1]	0.118	1.967	**0.049**			
transformed)	Sexual system - PG[2]	-0.090	-0.881	0.378			

Abbreviations: λ = phylogenetic signal; G = Gonochorism; PA = Protandry; PG = Protogyny. Significant differences are indicated in bold.

[1] G as reference level.

[2] PA as reference level.

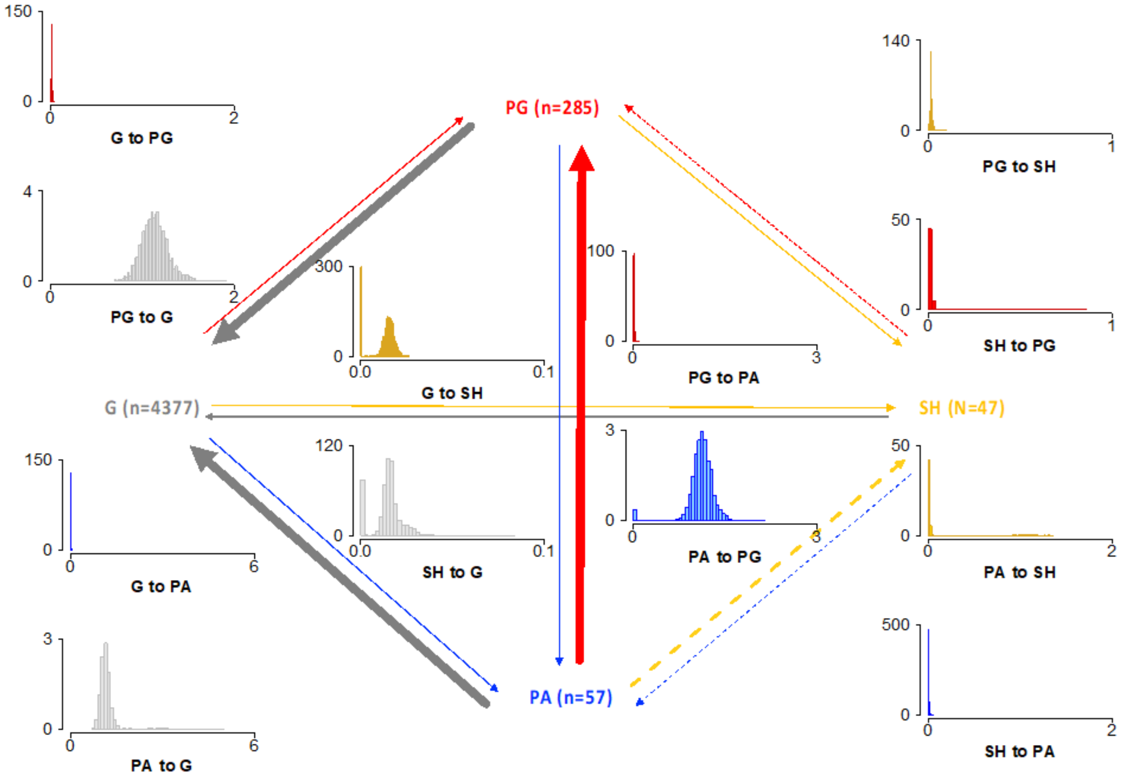

Figure 2. Summary of rjMCMC Multistate analysis with density plots of the posterior distribution of the transition rates between character states of sexual systems. Transition rates are color coded as follows: grey into gonochorism, red into protogyny, blue into protandry, yellow into simultaneous hermaphroditism. Note, only x-axis, but not y-axis, are the same for each pair of transition rates. The thickness of the arrows in the diagram is proportional to the mean magnitude of the transition rates from the posterior distribution. Dotted lines indicate transition rates estimated to be equal to 0 in 30% of more of the models in the posterior distributions.

Table 2. Results of phylogenetic generalized least square model of gonadosomatic index (GSI) across sexual systems. For each independent variable we report the parameter estimate (Beta), t-statistics (T), and P-value, and the model statistics including the degrees of freedom (df), the ML lambda value and R^2. This analysis is based on sample size of 103 extant species of which 45 are gonochoristic 45 are protogynous, and 13 are protandrous

Variables						**Model stats**	
Dependent	**Independent**	**Beta**	**T**	**P**	**df**	**λ**	**R^2**
GSI ($\log_{10}$ transformed)	Sexual system - PA[1]	0.071	0.553	0.581	2,100	0.788	0.142
	Sexual system - PG[1]	-0.366	-3.721	**0.000**			
	Sexual system - PG[2]	-0.438	-3.154	**0.002**			

Abbreviations: G = Gonochorism; PA = Protandry; PG = Protogyny. Significant differences are indicated in bold.

[1] G as reference level.

[2] PA as reference level.

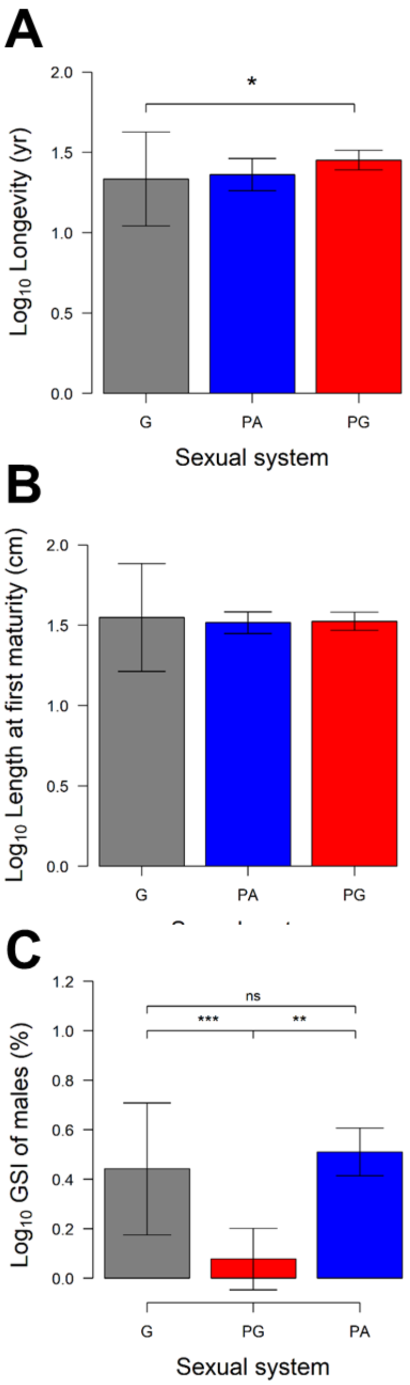

Figure 3. Phylogenetic generalized least square results of life-history traits in $\log_{10}$ transformed according to the sexual systems of extant species is color coded as gonochorism (G, grey), protogyny (PG, red), protandry (PA, blue). (**A**) Longevity (sample sizes: G= 696, PA= 19, PG= 88). (**B**) Length at first maturity (sample sizes: G= 302, PA= 17, PG= 43). (**C**) Male gonadosomatic index (GSI) (sample sizes: G= 45, PA= 13, PG= 45).

4. Discussion

4.1) Ancestral reconstruction and evolutionary transitions

Our results of the evolutionary history of sexual system show that the ancestral sexual system of teleosts is more likely to be gonochorism than hermaphroditism as a whole (64 vs 36%), as previously suggested (Avise and Mank, 2009) without such a rigorous testing as the one presented here. This contrasts with the situation in plants, where hermaphroditism is thought to be the ancestral state (Bawa, 1980). Our results also show that hermaphroditism is gained slowly but reverts very quickly to gonochorism, suggesting that hermaphroditism as a whole is not a stable evolutionary condition. Fish are the only vertebrates exhibiting hermaphroditism. They are unique in that both male and female gonads derive from the cortical part of the mesonephros whereas in the rest of vertebrates gonads have a dual origin: ovaries are derived from the cortical part and testes from the stromal part. This means that, from an anatomical or morphological point of view, all fish species could be, in principle, hermaphrodites (Adolfi et al., 2018).

Our results support Leonard (2018b) view of sequential hermaphroditism as an intermediate state between gonochorism and simultaneous hermaphroditism. Fish can be used as an ideal study system to assess the role of sequential hermaphroditism in the evolution of sexual systems since the low transition rates to simultaneous hermaphroditism from both protogyny and protandry explain why in fish, in contrast to plants or invertebrates, sequential hermaphroditism is more common that simultaneous hermaphroditism (Sadovy de Mitcheson and Liu, 2008, Smith and Wootton, 2016)Pla et al 2019a; *submitted*). However, gonochorism is very unlikely to evolve to simultaneous hermaphroditism, although such transition may be possible in Aulopiformes (Pla and Piferrer, 2019; *submitted*).

Multistate analysis with the 4 states confirmed previous results and added new information. Comparing among all four sexual systems, gonochorism, protogyny and simultaneous hermaphroditism are stable, while protandry is unstable. Protogyny may also switch to either protandry, as shown in a study focusing on Sparidae (Pla et al., 2019b; *submitted*), or simultaneous hermaphroditism, but in both cases at a very low rate. Conversely, protandry is lost very quickly to protogyny or to simultaneous hermaphroditism, although this last transition is very unlikely to occur. Indeed, for protandry to switch to simultaneous hermaphroditism would require for some individual to retain male functions when gaining female functions (the normal path from male to female). In fact, this occurs in some bi-directional fish when the environment is inconsistent but retaining the male gonad is more expensive than the female gonad and this is thought to be due to structural differences in male and female fitness payoffs (Yamaguchi and Iwasa, 2017). Thus, in fish this transition is difficult to occur. However, in some invertebrates such the shrimp of the genus *Lysmata*, a protandric simultaneous hermaphroditism, individuals first mature as males and then become females retaining male tissues (Baeza, 2006). Also, the low transition from protogyny to simultaneous hermaphroditism means maintaining female function in addition to gaining male function, possibly made feasible by this trend of fish towards early differentiation of females. These transitions from protogyny to simultaneous hermaphroditism have been observed in the families Serranidae and Muraenidae (Pla and Piferrer, 2019; *submitted*).

In contrast, the transition from protandry to protogyny may be favored by the peculiarities of gonadal development in protandrous species. Thus, many protandrous species develop as a female during the initial stage of gonadal formation, with ovaries containing cysts of oocytes and an ovarian cavity. Later, testicular development is superimposed on this arrangement, causing the animal to mature first as a male. This has been observed in protandrous species of different families such as the gilthead sea bream, *Sparus aurata* (Zohar et al., 1978), the black porgy, *Acanthopagrus schlegelii* (Chang and Yueh, 1990) and the anemonefish *Amphiprion clarkii* (Miura et al., 2008). Thus, the transition to protogyny is easier because the first sex to develop, not the one that first matures, is female.

Finally, simultaneous hermaphroditism is one of the less common (in terms of number of species) types of hermaphroditism, probably because the cost of maintaining both types of gonads in the same individual is complex (Bull and Charnov, 1985). However, it showed to be evolutionarily stable as occur in plants (Charnov, 1979). Their stability in the phylogeny may be due to the fact that they evolve in low rates because of these difficulties, but once they do they have a great reproductive strategy, i.e., the egg-trade in Serranidae (Petersen, 2006).

4.2) Life-history traits: longevity and size at first maturity

Many protogynous species live in complex and diverse habitats such as coral reefs. This sort of environmental complexity combined with the stable and complex social structures they form has led some authors to suggest that protogynous species may be long-lived (Munday et al., 2006, Sadovy de Mitcheson and Liu, 2008).

Our results show that longevity was significantly higher in protogynous, but not protandrous hermaphrodites, when compared to gonochoristic relatives, which, in principle, would support the view that the combination of complex habitat and social systems typical of protogynous species would select for higher longevity. However, it is worth mentioning that in our analysis we included many protogynous species that live in habitats such as soft bottoms that are less complex than coral reefs. Although the longevity of the coral-reef associated species was slightly higher than that of non-coral reef-associated species, differences were not statistically different (data not shown; $P > 0.05$). This suggests that increased longevity is an inherent property of protogyny per se regardless of habitat. A possible explanation for the higher longevity of protogynous species would be that since protogyny females are, by definition, smaller than males and that in females fecundity increases with age, by increasing longevity protogynous species would also increase the fecundity of females. This could produce a higher amount of eggs that would satisfy the fertilization capacity of large males, thereby increasing their lifetime reproductive fitness. Thus, although in protogyny the largest fish are males, natural selection would favor higher longevity to take advantage of the higher fecundity of older and thus larger females.

The trade-off between life-history traits such as longevity, maturity, growth and mortality, shows how species adapt a particular reproductive strategy throughout life (Beverton, 1987). Thus, we expected that protogynous species would mature later than gonochoristic relatives, especially if the former had higher longevity, as we found, since maturity delay has also been positively related to longevity (Charnov, 1993). Surprisingly, our results comparing size at first maturity (females in protogynous species, males in protandrous species and both sexes in gonochoristic species) showed no differences in size at maturity, as had been once surmised (Leonard, 2013). The fact that gonochoristic species, who are shorter lived than protogynous species, have a similar size at first maturity than protogynous hermaphrodites indicates that gonochoristic species comparatively delay maturation and suggests that this can represent a benefit in the long term. Larger females can get more eggs fertilized, investing more energy in reproduction (Barneche et al., 2018). Taken together, our results suggest that lifetime female function success is achieved in different ways: by increasing longevity in protogynous species and by delaying size (age) at first maturity in gonochoristic species.

4.3) Sperm competition

Sex allocation theory predicts that in protogynous species, where mating occurs between dominant males that monopolize a harem of females, sperm competition is low and therefore this should lead to low GSI values. In a previous study, the GSI was compared between protogyny and gonochorism across different families, only data on the GSI of 8 sex-changing diandric species was used (Molloy et al., 2007), but information on the actual species considered was not made available. The results showed that the GSI of protogynous species was lower than those of gonochoristic species Subsequent research confirmed prediction in specific families such as Epinephelidae (Erisman et al., 2009) and Sparidae (Erisman et al., 2013). However, in all cases the number of species was limited. Here we used a larger dataset, considering the three families with higher number of protogynous species, i.e., Labridae, Sparidae and Epinephelidae, using a recent Actinopterygian phylogenetic tree (Rabosky et al., 2018).

For the comparison of 103 values of male GSI between protogyny, protandry and gonochorism, we did not differentiate between primary and secondary males and thus it could be argued that GSI values of protogynous species had been overestimated. This is because primary males, defined as those that develop directly as males, which are small and non-dominant, typically have higher GSI values than secondary males, which come from a sex change, defend the harem and spawn in pairs (Robertson and Warner, 1978, Warner and Robertson, 1978, Warner, 1982, Warner and Lejeune, 1985). We found indeed that the GSI values of diandric species were higher than those of monandric species. Two of them concerned species such as *Boops boops* and *Pagellus erythrinu*s, which are diandric, and reported GSI values >2 and sex ratios that did not deviate from 1:1 (Smida et al., 2014, Dobroslavić et al., 2017), suggesting that the analyzed males were primary males. However, in a third study, carried out with *Epinephelus tauvina*, the GSI was 0.8 and only 7% of the animals were males (Abu-Hakima, 1987), strongly suggesting that all of the were secondary males. Based on this, it is likely that we are probably considering 10 species (23% of all protogynous) with GSI values > 2 that correspond then to primary males. Even in this worst case scenario, our results show a large and significant difference between the GSI values of protogynous and gonochoristic species.

Therefore, these values indicate that not only secondary males but also primary males of protogynous species must experience low levels of sperm competition as assessed by their GSI values compared to gonochoristic and protandrous relatives. This indicates that protogynous males must never experience elevated male-male competition environments. Therefore, if the harem-polygyny structure is taken as the main social structure of protogynous fish, which allows them to form their complex social structures in environments without sperm competition.

Further evidence that the GSI is a reliable indicator of sperm competition (Petersen and Warner, 1998) was provided integrating information about mating and spawning systems (Stockley et al., 1997). Our results matched the predictions that species where low sperm competition is expected, regardless of sexual system, will have lower GSI values than species that experience elevated sperm competition. However, this indicator is not reliable in protandrous species, whose variability of GSI values depends on sexual selection of small males to be successful with large females rather than due to sperm competition environments (Pla et al 2019b; *submitted*). Therefore, except for protandrous species, the GSI values in protogynous or gonochorism can suggest the social structure in fish, i.e., low sperm competition as in haremic or high sperm competition in others systems.

In summary, protogyny is a stable condition in fish and, through life-history traits, it is possible to understand that its evolutionary success may be due in part to its greater longevity that is not associated with coral reefs as supposed. The social systems of low sperm competition allow them to interact in complex systems could help provide their stability and live-longer. Simultaneous hermaphroditism is the most complex system because individuals must keep both sexual tissues in the same gonad at all times. Thus, once evolved it is stable with few losses. In contrast, the protandrous system is unstable, probably because the first sex is the opposite of the development of the gonad in fish and is less easy to evolve. These suggestions of stability in sexual systems with data on life-history traits may help to understand how individuals optimize their strategies. Studies focusing in partiuclar groups are required to confirm thesethese interpretations

Acknowledgments

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Supplementary Figures

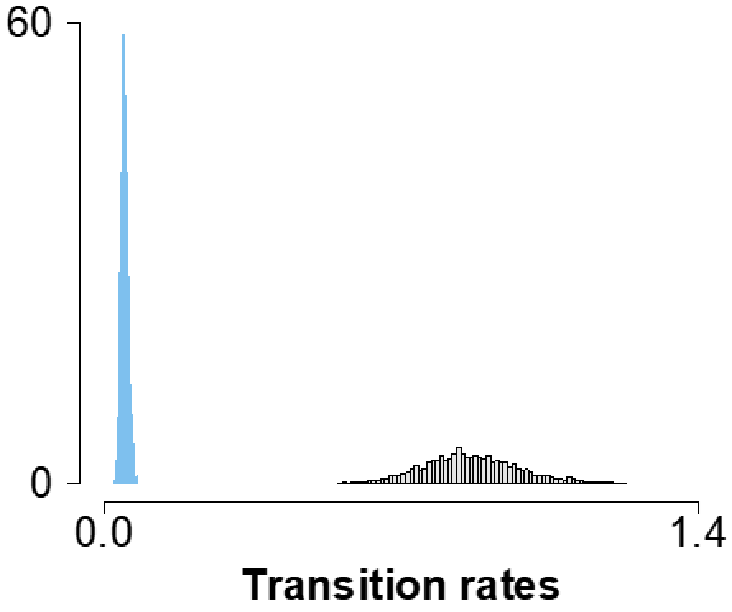

Figure S1. Posterior distributions of the transition rates (2 states) from gonochorism (n = 4337 species) to hermaphroditism (light blue) and from hermaphroditism (n = 403 species) to gonochorism (grey) from the rjMCMC Multistate analysis.

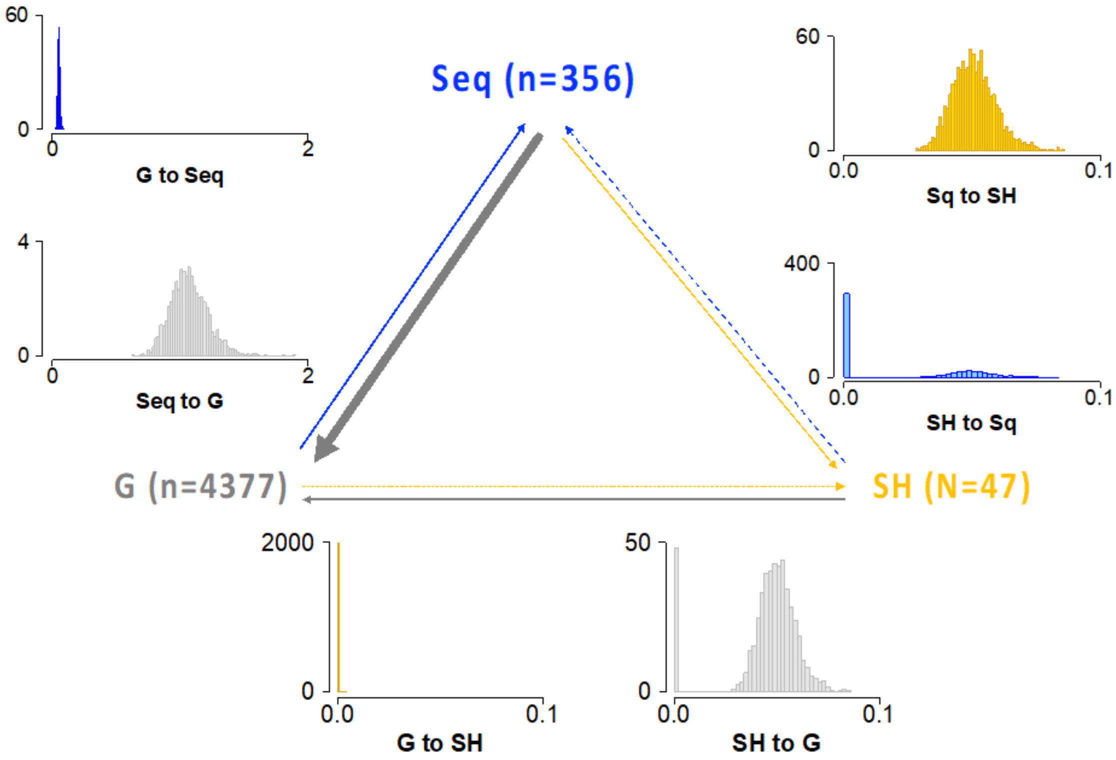

Figure S2. Summary with posterior distributions of transition rates between 3 sexual systems (gonochorism, G, in grey; sequential hermaphroditism, Seq, in blue; and simultaneous hermaphroditism, SH, in yellow) from the Multistate analysis. Transitions to gonochorism in grey, to sequential hermaphroditism in blue and to simultaneous hermaphroditism in yellow. Sample sizes of extant species for each sexual system category are indicated between parentheses. Note, only the x-axis, but not y-axis, is the same for each pair of transition rates. The thickness of the arrows is proportional to the mean magnitude of the transition rates from the posterior distribution. Dotted lines indicate transition rates estimated to be equal to 0 in 30% of more of the models in the posterior distributions.

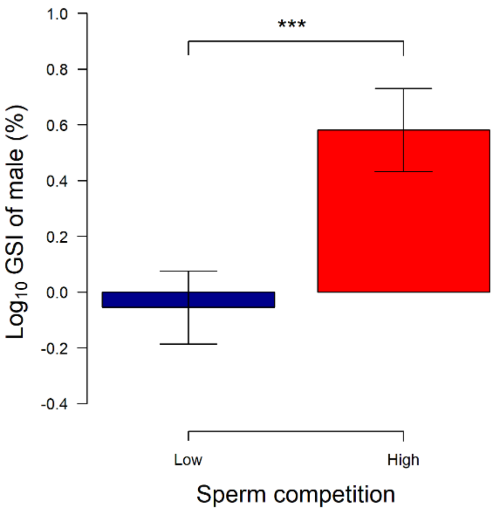

Figure S3. Male gonadosomatic index (GSI) as a function of sperm competition in 34 perciform species (Gonochoristic, n= 6; Protandry, n= 4; Protogyny, n= 24).

Supplementary Tables

Table S1. Number of species used for the study of evolutionary transitions among different sexual systems taken from a total sample of 4,740 Actinopterygian species. Note that 3 androdioecius species were not included

Sexual system	**2 states** (#|1, #2)	**3 states** (#1, #3, #6)	**4 states** (#1, #4, #5, #6)
#1. Gonochorism	4337	4337	4337
#2. Hermaphroditism (#3 + #6)	403*		
#3. Sequential hermaphroditism (#4 + #5)		356*	
#4. Protogyny			285
#5. Protandry			57
#6. Simultaneous hermaphroditism		47	47
Total	4740	4740	4726

* = Includes an additional 14 (13) species of bi-directional sequential hermaphrodites, which were not included anywhere in the 4 states for representing a too small sample size on its own. The number between patrenthesis is the number of hermaphrodite species verified againts the primary literature.

Table S2. Results of the rjMCMC Multistate analysis in BayesTraits of sexual pattern as a binary trait (gonochorism, G, or hermaphroditism, H). For each posterior distribution, we report the effective sample size (ESS), the mean and 95% high posterior density (95-HPD) interval, the mode, and the percentage of models in which the parameter is estimated as zero. This analysis is based on sample size of 4740 extant species of which 4337 are gonochoristic and 403 hermaphrodites.

Transition rates	**ESS**	**Mean**	**95-HPD**	**Mode**	**% Zero**
G to H	2000	0.047	0.032 – 0.065	0.045	0
H to G	2000	0.864	0.663 – 1.098	0.831	0
Root probabilities					
G	2000	63.8	57.3 – 70.7	64.5	0
H	2000	36.2	29.3 – 42.7	35.5	0

Table S3. Results of the rjMCMC Multistate analysis in BayesTraits of sexual pattern as a 3 states categorical variable (gonochorism, G; sequential hermaphroditism, Seq, or simultaneous hermaphroditism, SH). For each posterior distribution we report the effective sample size (ESS), the mean and 95% high density interval (95-HPD), the mode and the percentage of models in which the parameter is estimated as zero. This analysis is based on sample size of 4740 extant species of which 4337 are G, 356 Seq, and 47 are SH

Transition rates	**ESS**	**Mean**	**95-HPD**	**Mode**	**% Zero**
G to Seq	2000	0.051	0.033 – 0.066	0.050	0
Seq to G	1796	1.071	0.804 – 1.349	1.020	0
G to SH	2000	0.000	0.0 – 0.0	0.0156	99.8
SH to G	2000	<0.001	0 – 0.065	0.050	9.6
Seq to SH	2000	0.051	0.033 – 0.066	0.050	0
SH to Seq	1791	0.021	0 – 0.060	0	58.7
Root probabilities					
G	1793	60.9	55.4 – 66.7	61.0	0
Seq	1796	38.9	33.0 – 44.4	38.3	0
SH	2000	0.2	0 – 5.4	0.1	3.9

Table S4. Results of the RJMCMC Multistate analysis in BayesTraits of sexual pattern as a 4 state categorical variable (gonochorism, G; protogyny PG; protandry, PA, or simultaneous hermaphroditism, SH). For each posterior distribution we report the effective sample size (ESS), the mean and 95% high density interval (95-HPD), the mode and the percentage of models in which the parameter is estimated as zero. This analysis is based on sample size of 4726 extant species of which 4337 are G, 285 are PG, 57 are PA and 47 are SH

Transition rates	**ESS**	**Mean**	**95-HPD**	**Mode**	**% Zero**
G to PG	2000	0.0172	0.0097 – 0.0291	0.0156	0.2
PG to G	2000	1.1370	0.8381 – 1.4181	1.1096	0
G to PA	2000	0.0172	0.0097 – 0.0291	0.0156	0
PA to G	2000	1.1770	0.8427 – 1.4866	1.1096	0
G to SH	2000	0.0132	0 – 0.0201	0.0156	15
SH to G	2000	0.0147	0 – 0.0275	0.0162	15
PG to PA	2000	0.0142	0 – 0.0277	0.0161	19
PA to PG	2000	1.1160	0 – 1.4893	1.1096	0.3
PG to SH	2000	0.0170	0.0089 – 0.0309	0.0156	2
SH to PG	2000	0.0102	0 – 0.0252	0	45
PA to SH	2000	0.0611	0 – 0.0589	0	38
SH to PA	2000	0.0090	0 – 0.0247	0	48
Root probabilities					
G	2000	40.7	35.7 – 48.2	39.2	0
PG	2000	29.9	25.5 – 32.8	30.3	0
PA	2000	29.3	23.7 – 37.0	30.4	0
SH	2000	<0.1	0 – 0.9	<0.01	2.3

Table S5. Results of the phylogenetic generalized least square model of length at first maturity across sexual systems (gonochorism, G; protogyny PG or protandry, PA). For each independent variable we report the parameter estimate (Beta), t-statistics (T), and P-value (P), and the model statistics including the degrees of freedom (df), the ML lambda value and R^2. This analysis is based on sample size of 362 extant species of which 302 are G, 43 are PG, and 17 are PA

Variables						**Model stats**	
Dependent	**Independent**	**Beta**	**T**	**P**	**df**	**λ**	**R^2**
Length Maturity ($\log_{10}$ transformed)	Sexual system - PA[1]	-0.031	-0.470	0.638	2,359	0.972	0.0009
	Sexual system - PG[1]	-0.023	-0.412	0.680	2,359		
	Sexual system - PG[2]	0.008	-0.099	0.920	2,359		

[1] G as reference level.

[2] PA as reference level.

Chapter 3

A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae)

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Abstract

The Sparids are an ideal group to study the evolution of sexual systems since they exhibit a great diversity of them, from gonochorism (separate sexes) to protandrous (male-first) and protogynous (female-first) sequential hermaphroditism (sex-change). According to the size-advantage model (SAM), selection should favour sex change when the second sex achieves greater reproductive success at a larger body size than the first sex. Using phylogenetic comparative methods on 68 sparid species, we show that protogyny and protandry can evolve from gonochorism but evolutionary transitions between these two forms of sequential hermaphrodites are unlikely to happen. Using male gonadosomatic index (GSI) as a measure of investment in gametes and proxy for sperm competition, we find that, while gonochoristic and protogynous species support the predictions of SAM, protandrous species do not as they exhibit higher GSI values than expected even after considering mating system. We suggest that small males of protandrous species have to invest disproportionally more in sperm production than predicted not only when spawning in aggregations with high levels of sperm competition, but also when spawning in pairs due to the need to fertilize highly fecund females, much larger than themselves. We suggest that this compensatory mechanism to ensure fertilization success of eggs released by large females, together with Bateman's principles in sequential hermaphrodites, should be formally incorporated in the SAM.

Keywords: sex allocation theory, size-advantage model, sperm competition, Bateman's principles, protandry, gonadosomatic index

1. Introduction

Among vertebrates, fishes exhibit the broadest diversity in sexual systems (Beukeboom and Perrin, 2014, Smith and Wootton, 2016), ranging from gonochorism (separate sexes) to hermaphroditism (sequential and simultaneous), unisexuality (all individuals are females) and mixed sexual systems (i.e., co-occurrence of hermaphrodites and males, as in *Kryptolebias* killifish (Costa et al., 2010, Mackiewicz et al., 2006). Sex allocation theory aims to answer questions such as what allocation of resources to males and females is favoured in gonochoristic species, when and in which direction to change sex in sequential hermaphrodites (from male to female in protandry and from female to male in protogyny), how much energy should be devoted to male *vs*. female function in simultaneous hermaphrodites, and under what ecological conditions these different sexual systems are evolutionary stable strategies (Charnov, 1982). Thus, understanding the evolutionary drivers and consequences of the transitions from one type of sexual system to another has been of great interest since the early days of the study of the evolution of sex (Maynard Smith, 1978, Neiman et al., 2017).

In fish, gonochorism is believed to be the ancestral condition from which all other sexual systems evolved (Ghiselin, 1969, Policansky, 1982, Cole, 2010). In contrast to this view, recent phylogenetic reconstructions of the evolutionary history of sexual systems in some fish families have found ancestral protogyny in Labridae (Kazancioglu and Alonzo, 2010) (where species are gonochoristic, protogynous and bi-directional sex changers) and in Serranidae (Chiba et al., 2009) (where species are gonochoristic, protogynous and simultaneous hermaphrodites). However, an earlier study using parsimony could not resolve the ancestral state for the sexual system in the family Sparidae, porgies or seabreams (Erisman et al., 2013). The Sparidae are a diverse, monophyletic group (Orrell and Carpenter, 2004, Orrell et al., 2002) consisting of ~150 coastal fish species (Nelson et al., 2016) with a wide geographical distribution, mainly in tropical and temperate waters (Chiba et al., 2009). This group is an ideal model system to study the evolution of sexual systems because it also exhibits diverse sexual systems (**Table 1**), ranging from gonochoristic to protogynous and protandrous species, with even some genera including species with different sexual systems. In particular, the sparids offer the unique possibility to explore the adaptive significance of sex change for both protogynous and protandrous species, something that cannot be done in most fish families.

The main theoretical framework to explain the evolutionary advantage of sequential hermaphroditism based on sex allocation theory is the size advantage model (SAM) (Ghiselin, 1969, Warner, 1975, Leigh et al., 1976). The original model predicts that individuals should switch sex when the second sex achieves a greater fitness at a larger body size than the first sex, thus increasing lifetime fitness. When large males have higher reproductive success than smaller males and similar sized females, fish should reproduce initially as female and change later to male (protogyny). Conversely, protandry is expected when large females have greater fitness than smaller females and similar sized males (Warner, 1975, Leigh et al., 1976). Empirical studies support this model in several fish families, explaining quantitatively the size or age at which an individual should change sex, as well as the overall population sex ratio (Allsop and West, 2003, Allsop and West, 2004). Typically, male-biased sex ratios are observed in populations of protandrous species, while female-biased sex ratios are observed in populations of protogynous species, especially in haremic groups (Shapiro, 1987, Warner, 1975). Indeed, mating systems (considering the number and social dominance of mates involved) and spawning mode (i.e., group spawning, where several males spawn with one or several females, or pair mating, where a single male spawns with a single female), determine the fitness that a fish of a given size can achieve as a female or as a male (Benvenuto et al., 2017). Thus, in this model, mating system was recognized as important but incorporated when deriving model predictions only in general terms, grouping all protandrous and all protogynous species in broad categories to generate sex-specific differences in reproductive expectations (Warner, 1988a). A consequence of this was the prediction that protandry should be favored when sperm competition is low and when mating occurs between members of monogamous pairs or those of random pairs (Warner, 1988a), and that mate monopolization by large males should occur in protogynous species (Warner, 1984).

The combined effect of sexual and mating systems on reproductive success has been reported not only in many fish families, including Serranidae, Labridae, Platycephalidae, Pomacanthidae, Pomacentridae and Gobidae (Warner, 1975, Warner, 1984, Munday et al., 2006, Erisman et al., 2009, Erisman and Hastings, 2011, Erisman et al., 2013, Sunobe et al., 2017, Kuwamura and Nakashima, 1998), but also in other taxa, e.g., crustaceans (Bauer, 2006, Chiba, 2007) and molluscs (Collin, 2006, Collin, 2013, Broquet et al., 2015). Overall, the SAM is well supported (Kazancioglu and Alonzo, 2010), but in some cases model expectations do not hold when tested in the field (Charnov, 1986). Thus, given the plasticity of sexual systems and the combined interactions of multiple factors that can influence reproductive success (e.g., success of sneakers that alter sperm competition predictions, size-skew effects of larger fish of the first sex) that were not considered in the initial SAM, there are cases that do not conform to the SAM predictions (Munoz and Warner, 2003, Munoz and Warner, 2004). Nevertheless, the SAM provides an elegant and simple evolutionary framework to understand sequential hermaphroditism, but there is still more to add to the picture, given the complexity and variety of reproductive strategies in animals. Thus, a new version of SAM, that incorporates explicitly mating systems, fecundity skew and sperm competition to derive the model's predictions, has been proposed focusing on protogynous sex change (Munoz and Warner, 2003).

Fish mating systems range from monogamy (a single male mating with a single female), to harem polygyny (one dominant male controls access to multiple females (Rios-Cardenas and Morris, 2009), lek-type polygyny (temporary harems (Allsop and West, 2003)), and large polygamous or promiscuous aggregations (where group spawning occurs (Fischer and Petersen, 1987)). Sperm competition is particularly intense in large aggregations with multiple males. The intensity of sperm competition that a male fish faces can be reliably estimated by the gonadosomatic index (GSI), a proxy of gamete production (Rowley et al., 2018). The GSI is defined as the proportion of gonad weight relative to total body weight (Birkhead and Møller, 1998). Thus, high GSI values (>3%) in males are typically observed in species that spawn in large aggregations (Buxton and Garratt, 1990, Tucker Jr et al., 1993), where males compete at the post-copulatory stage for fertilizing the eggs, as it occurs in many pelagic gonochoristic spawners. According to the SAM, protogyny occurs when males can dominate a social group, with stable or temporary territorial males (e.g., in lek) that monopolize mating harems (Warner, 1984, Warner, 1988a). These social systems diminish sperm competition due to absence of group spawning (as found in many members of the family Serranidae (Taborsky, 1998, Mackie, 2007, Sadovy et al., 1994, Donaldson, 1995). Thus, the SAM predicts protogynous species to have low sperm competition and so low GSI values. Because gonochoristic species can have either high or low sperm competition levels as they exhibit a variety of matingsystems and spawning modes, their GSI should be on average higher than that of protogynous species (Warner, 1975, Taborsky, 1998, Molloy et al., 2007).

While direct male dominance should select for protogyny, the combined effects of the increase of female fitness with size (Klinkhamer et al., 1997) and the interaction of just few males resulting in low sperm competition (Cadet et al., 2004) (also known as 'budget effects') should select for protandry. Although we know little about mating systems in protandrous species, at least a few sparids appear to conform to these predictions (e.g., *Sparus aurata* (Ibarra-Zatarain and Duncan, 2015); *Rhabdosargus sarba* (Leu, 1994)) as they have been mainly reported to mate in pairs or small groups, thus in the absence of multiple males competing among themselves (Ibarra-Zatarain and Duncan, 2015, Leu, 1994). Therefore, we can expect low levels of sperm competition in protandrous sparids (Warner, 1988b) and consequently low GSI values. However, a handful of protandrous species engage in random matings in large spawning aggregations (e.g., *Acanthopagrus berda (Garratt, 1993)*) (Warner, 1975) where sperm competition should be intense, and thus higher GSI values than pair-mating species should be expected. Due to this diversity of mating systems, a wide range of GSI values has accordingly been observed in protandrous Sparidae (Erisman et al., 2013). Altogether, the SAM incorporating sperm competition and mating systems appears to be well supported in the Sparidae, with protogynous species exhibiting low levels of sperm competition as predicted; however, protandrous species exhibit much higher GSI values on average than predicted by SAM, suggesting that they face more intense sperm competition than previously thought (Erisman et al., 2013).

A previous study (Erisman et al., 2013) based on recorded GSI values suggested high level of sperm competition in some protandric sparids but did not consider any potential effect of mating system. In addition, the high GSI values reported in that study were derived from only 8 protandrous species (Erisman et al., 2013), three of which were in fact GSI values corresponding to individuals when female rather than male, or do not correspond to the maximum recorded value. That study also ignored the potential confounding effects of allometry. Although GSI, the percentage of gonad tissues on body mass, may provide an estimate of investment in the gonads irrespective of size, it is possible that at least some of the observed differences in male GSI across species are in part determined by differences in male size, if investment in the gonads is easier at larger or smaller sizes. Finally, recent phylogenetic trees, which are essential for all comparative studies across species, are better resolved and more comprehensive than those employed by earlier studies (e.g. Chiba et al., (Chiba et al., 2009)), while modern phylogenetic comparative methods allow overcoming the limitations of approaches used in previous studies, such as parsimony reconstruction and phylogenetic independent contrasts, that can lead to incorrect conclusions (Schluter et al., 1997, Freckleton et al., 2002).

Here we have built the largest dataset to date of sexual systems, spawning modes and body size in the family Sparidae, doubling the number of species with data on sexual system relative to previous studies. Using modern phylogenetic comparative approaches, we have investigated the evolutionary history of sexual systems and tested the predictions of the SAM that protogynous and protandrous should exhibit lower GSI values due to expected lower sperm competition, while accounting for body size. Our study therefore combines sexual systems, mating systems, sperm competition and the principles of the SAM, while accounting for allometric effects, a potential confounding factor ignored in previous studies, and phylogeny.

2. Material and Methods

2.1 Data collection

We used FishBase (www.fishbase.org (Froese and Pauly, 2018)) to gather information on the sexual systems of Sparidae, ranging from gonochorism (G), protandrous hermaphroditism (PA) and protogynous hermaphroditism (PG), and verified the sexual system reported for each species against the primary literature (Devlin and Nagahama, 2002, Buxton and Garratt, 1990, Allsop and West, 2003, Sadovy de Mitcheson and Liu, 2008, Erisman et al., 2013). We carefully revised previous assignments of sexual systems in 4 species, which were mainly based on the gonadal morphology of individuals collected at single or different ages (Sadovy and Shapiro, 1987). Sometimes this approach cannot distinguish functional (active) hermaphrodites from non-functional hermaphrodites (i.e., individuals that despite presenting both male and female tissues reproduce only as one sex throughout their life). The assignment of the correct sex is further complicated in non-reproducing juveniles, which can present a bisexual gonadal stage. Altogether, these peculiarities can make diagnosis of sexual system extremely challenging (Buxton and Garratt, 1990, Erisman et al., 2013, Sadovy de Mitcheson and Liu, 2008). We resolved any discrepancies from previous studies using newly published data in which care was taken not to incur in the above problems (Table S1).

Out of a total of 148 recognized sparid species, we could assign the sexual system in 68 species (Table S1). For these species, we extracted data from FishBase on male maximum body weight (in g; n= 37 spp.), male maximum total body length (in cm; n= 47 spp.) and male total body length at maturity (in cm; n= 36 spp.), to account for possible allometric effects on GSI. Finally, we extracted data from the primary literature on male GSI (n=49) and spawning mode (pair or group spawning, based on the presence of one or more males, respectively; n=10 spp.). When several GSI values were reported for a given species (e.g., monthly means along the year), we consistently used the highest value.

2.2 Ancestral state reconstruction

We used two molecular phylogenetic trees with time-calibrated branch lengths, an essential step for robust analyses in a phylogenetic comparative framework (Harvey and Pagel, 1991). Specifically, we used a phylogeny of Actinopterygians (Rabosky et al., 2018) based on a 27-genes (6 mitochondrial and 21 nuclear genes) and a phylogeny specific for the family Sparidae (Santini et al., 2014), based on three mitochondrial and two nuclear genes. These trees included 58 and 55 species, respectively, out of the 68 species with sexual pattern information in our dataset.

The ancestral state reconstruction infers the evolutionary history of a trait along a phylogeny given the character states of species in the tree and provides estimates of the probable character state of each node in the phylogeny. This approach is based on a Markov model of evolution for discrete traits (Pagel, 1994). We reconstructed the ancestral character states of sexual pattern using maximum likelihood (ML), setting all transition rates between G, PA and PG free to vary. We ran these analyses on both phylogenetic trees using the R package *ape* (Paradis et al., 2004). However, here we can only report the results of the ancestral state reconstruction using the phylogenetic tree of Rabosky *et al.*(Rabosky et al., 2018) since the analysis using the Santini *et al.*(Santini et al., 2014) tree did not converge to a maximum likelihood solution.

2.3 Testing new version of SAM predictions

We used phylogenetic generalized least square models (PGLS,(Pagel, 1997, Pagel, 1999, Freckleton et al., 2002)) to test the predictions of the SAM using the R package *caper*(Orme, 2013) and ML estimation, with both phylogenetic trees(Rabosky et al., 2018, Santini et al., 2014). By incorporating the phylogeny, PGLS models can quantify the strength of phylogenetic signal in the data through the parameter lambda (λ), which can vary between zero (no phylogenetic signal) and one (high phylogenetic signal, whereby the species exhibit phenotypic similarity in direct proportion to their common evolutionary time)(Pagel, 1999, Freckleton et al., 2002). GSI was entered as the dependent variable, while sexual pattern was the independent discrete variable with three possible states (0 = G, 1 = PA, 2 = PG), and body size (as maximum length, weight or length at maturity) the independent continuous covariate. Possible allometric effects on the GSI were thus accounted for using either body length or weight as additional independent variable. Continuous variables were $\log_{10}$-transformed to meet assumptions of normality, with the exception of GSI values. Results for GSI were qualitatively similar whether this variable was log10-transformed, transformed with logit function or left untransformed, thus we report the results of GSI in percentage, not transformed. All model residuals were normally distributed in all analyses.

We also tested the prediction of the SAM within the genus Diplodus. This was the only genus that provided limited but sufficient data to consider at least two different sexual systems (G *vs*. PA) for statistical analysis of the relationship between sexual pattern and male GSI values in very closely related species. Importantly, *Diplodus* species have a narrow range of body sizes and thus there is less variability and potential confounding effects of allometry. However, the analysis could not be carried out in a phylogenetic context in this genus because too many species were missing from both phylogenies. Therefore, we used Student's *t*-test for independent samples to test differences in total male body length and GSI between the two sexual systems, and general linear model to test for the relationship between maximum male length at reproduction and GSI. In all analyses, performed in R(Team, 2016), differences were considered statistically significant when $p < 0.05$.

3. Results

Our dataset on the sexual system of 68 sparid species across 28 genera (out of 37) includes 27 gonochoristic, 22 protogynous and 19 protandrous species (**Table 1**, **Table S1 and S2**), with the three sexual systems roughly present in similar proportions (range~28–40%; Table 1). This represents a substantial increase in the number of species previously investigated(Erisman et al., 2013).

Reconstruction of the ancestral character state in a phylogenetic context showed that gonochorism was only slightly more likely to be the ancestral sexual pattern in the Sparidae family (likelihood at the root 37.4%) than protandry (33.5%) and protogyny (29.1%; **Fig. 1**). While both forms of sequential hermaphroditism, especially protogyny, evolved rapidly to gonochorism (PA to G: 0.035±0.013; PG to G: 0.064±0.033), gonochorism reverted as quickly back to protogyny (G to PG: 0.054±0.039) and much less so to protandry (G to PA: 0.010± 0.024). Finally, the transitions between the two forms of sequential hermaphroditism were both estimated to be zero (PA to PG: 0.000± 0.012; PG to PA: 0.000± 0.027**; Fig. 2**).

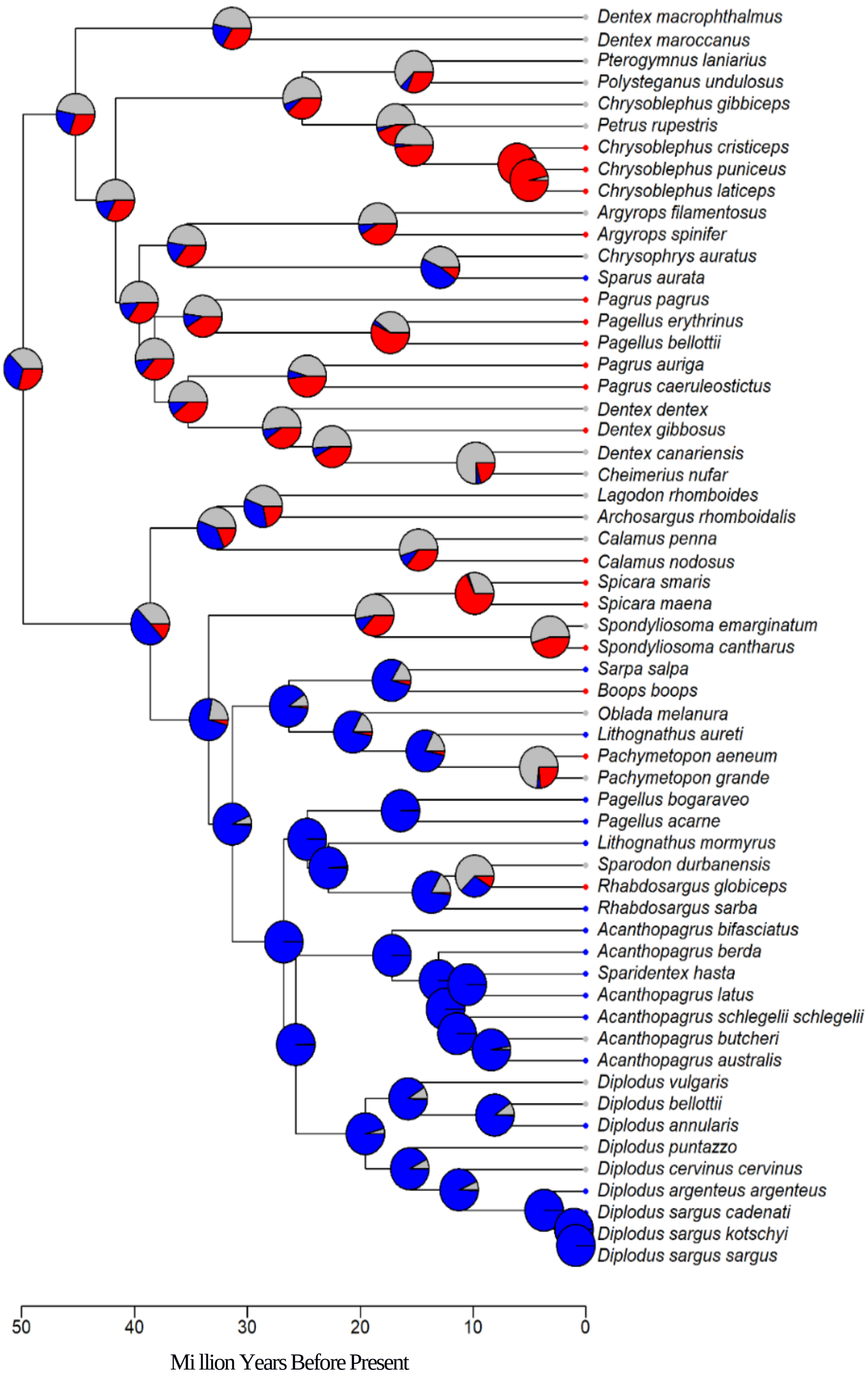

Figure 1. Ancestral state reconstruction in the Sparidae using the phylogenetic tree by Rabosky *et al*.(Rabosky et al., 2018). Sexual system is coded as gonochorism (grey), protandry (blue) and protogyny (red). The pie area indicates the likelihood of character state at each node for the three states.

Table 1. Distribution of the major types of sexual systems among sparids, indicating absolute numbers and percentages with respect to all fishes in the family

	Genera	**Species**
Total number	37	148
With known sexual system	28 (73.68%)[1]	68 (45.94%)[1]
Gonochorism	20 (68.96%)[2]	27 (39.70%)[2]
Protogyny	12 (41.37%)[2]	22 (32.35%)[2]
Protandry	8 (27.58%)[2]	19 (27.95%)[2]

[1] Percentage with respect to total number.

[2] Percentage with respect to number with known sexual system. Percentages add to 100 only for species with known sexual system since in some genera different sexual systems can be present.

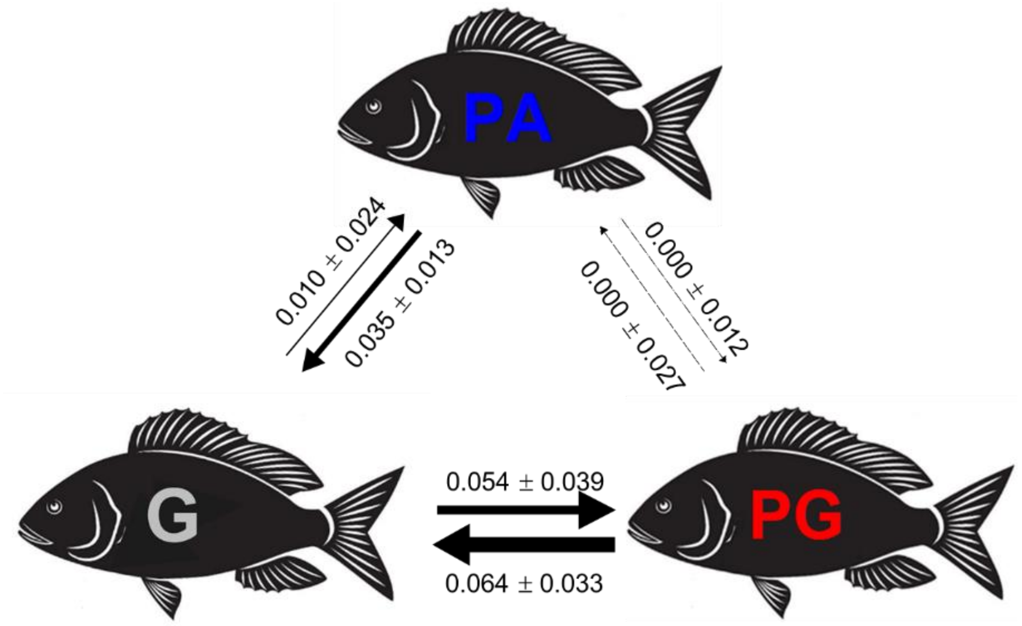

Figure 2. Transitions rates between sexual systems in the Sparidae derived from the ancestral state reconstruction in maximum likelihood. G: gonochorism; PA: protandry; PG: protogyny.

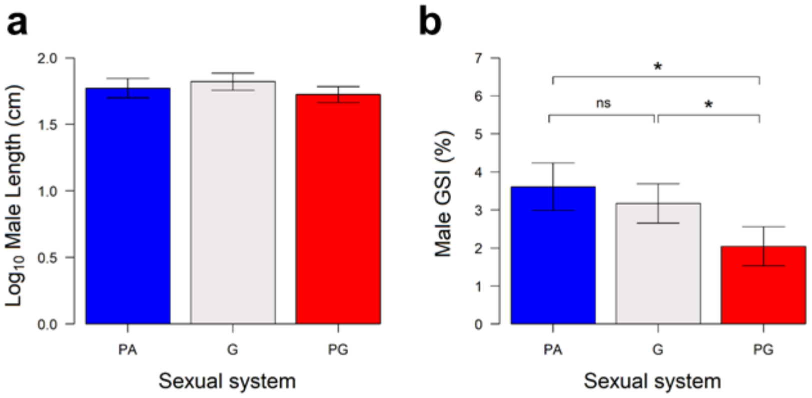

Figure 3. Phylogenetic means and standard errors of **A**) Log_{10} Male total length (G: n= 19; PA: n= 12; PG: n= 10), **B**) GSI (G: n= 15; PA: n= 15; PG: n= 14) across sexual systems (G: gonochorism; PA: protandry; PG: protogyny). Asterisks indicate statistically significant differences with the following equivalence: *= $p < 0.05$.

We did not find any significant difference in total male body length (Fig. 3A), male length at maturity or maximum male body weight between sparid species with different sexual systems (**Table 2**). For the 46 sparid species in the tree where male GSI values were available (Table S3), the average GSI values of protandrous and protogynous species were higher and lower respectively than that of gonochoristic species, with statistically significant differences between protogyny and the other two sexual systems (**Fig. 3B; Table 2**). Results were qualitatively similar regardless the phylogenetic tree used (Table S4) and were not influenced by allometric effects, tested using either length or weight as a covariate in the model (Table S5). Unfortunately, limited data were available on spawning mode, for both species that spawn in groups (G, n=2; PA, n= 4; PG, n= 0) or in pairs (G: n=1; PA: n=2; PG: n=1). These low numbers did not allow testing predictions for mating systems formally. However, the data appear to suggest that protandrous sparids have higher GSI values than gonochoristic species regardless of whether they spawn in groups or pairs (Fig. 4).

Analyses were also carried out focusing on the genus *Diplodus*, which contains only gonochoristic and protandrous species with a narrower range of lengths (~25–60 cm) when compared to that of sparids as a whole (~20–200 cm). Accordingly, we detected no significant differences in weight ($t_{2.98}$=-0.54; p=0.62) between gonochoristic and protandric species. However, the latter had a significantly shorter length than the former ($t_{4.61}$=2.71; p=0.04; Fig. 5A). Importantly, despite being smaller in size, protandric *Diplodus* species had a significantly higher GSI ($t_{4.82}$=-3.19; p=0.02) than the gonochoristic species of the same genus (Fig. 5B), confirming the results found across all sparid species. Moreover, GSI was unrelated to male total body length (F=11.65; df=2,6; R^2=0.79; p=0.246), suggesting that GSI differences between sexual systems were not determined by allometric effects.

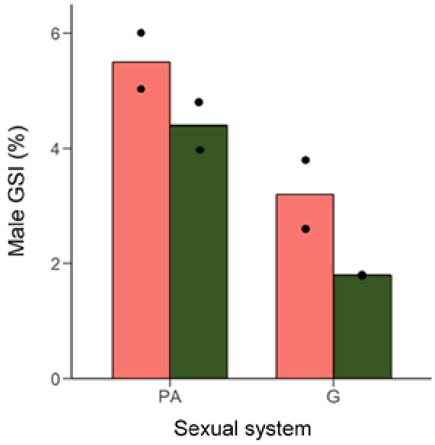

Figure 4. Mean of male gonadosomatic index (GSI) in protandrous (PA) *vs*. gonochoristic (G) species of Sparidae as a function of their spawning mode (Red, group spawning; Green, pair mating). The black dots indicate individual values in species with information for the three variables: sexual system, spawning mode and male GSI.

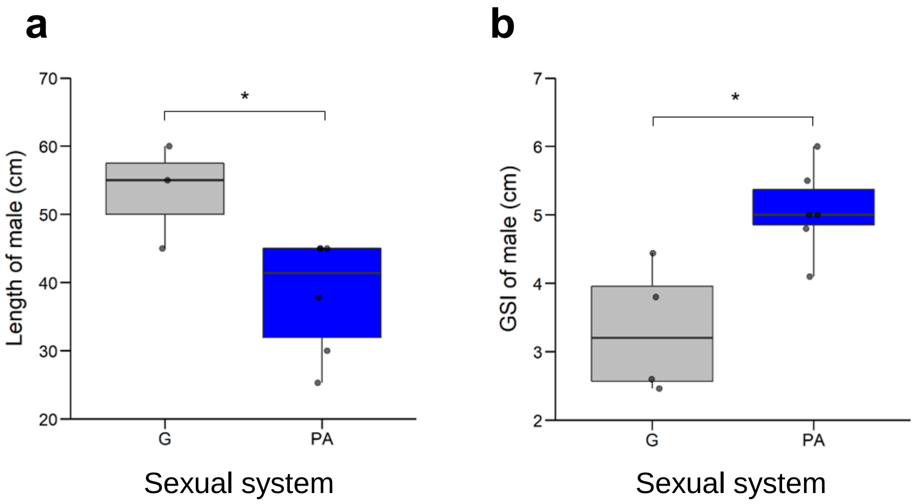

Figure 5. Maximum length and gonadosomatic index (GSI) of males in the genus *Diplodus* as a function of their sexual system (G = Gonochorism; PA = Protandry). **a)** Maximum length (G: n = 3; PA: n = 6). **b)** GSI (G: n = 4; PA: n = 6). The lower and upper edges of the boxes indicate the lower and upper quartiles, respectively; upper whisker = min (max(x), Q3+1.5 * IQR); lower whisker = max (min(x), Q1-1.5* IQR, where IQR= inter-quartile range, defined as the third quartile (Q3) – first quartile (Q1). The median is indicated by solid black horizontal line. The black dots indicate individual values. Asterisks indicate statistical significant differences: * = $p < 0.05$.

4. Discussion

With a larger dataset of sexual systems in the family Sparidae than previously used, this study reveals that protandry and protogyny can evolve from gonochorism, although the ancestral state remains still uncertain in this family. Importantly, we find that transitions between the two forms of sequential hermaphroditism are unlikely, if ever, to occur. We find strong support for the SAM predictions that protogynous species should exhibit lower levels of sperm competition relative to gonochoristic species, as quantified by their low GSI values, consistent with the fact that their mating systems allow large males to monopolize access to fertile females. However, we find no evidence in support of similar predictions in protandrous sparids, i.e., low levels of sperm competition because they are expected to spawn in pairs or small groups. Unexpectedly, protandrous species have similar GSI values to those of gonochoristic species and higher GSI than protogynous species, regardless of their mating system. Here we propose how an underappreciated compensatory mechanism, together with mating system and spawning mode, may explain these unexpected outcomes.

Using twice as many species relative to an earlier study, recent molecular, time-calibrated phylogenies and modern phylogenetic comparative approaches, our study shows that gonochorism is only marginally more likely to be the ancestral state in this family. We find that gonochorism can evolve into both protogyny and protandry. However, sequential hermaphroditism is an evolutionary unstable state as it reverts quickly back to gonochorism, suggesting that both types of sequential hermaphroditism are costlier to sustain than gonochorism. These results may explain why, despite hermaphroditism being anatomically and physiologically possible in fish in contrast to other vertebrates(Adolfi et al., 2018), gonochorism predominates among fishes(Sadovy de Mitcheson and Liu, 2008). Female has been often considered the 'default sex' in fishes because, even in some gonochoristic species, all individuals, exhibit ovarian differentiation at the early stages of development, a process that is subsequently halted in the individuals that become males(Devlin and Nagahama, 2002, DeFalco and Capel, 2009). Thus, it is perhaps not surprising that our analysis reveals that the evolutionary transition rate from gonochorism to protandry is very low and that transitions from protogyny to protandry and *vice versa* are unlikely to occur; once canalized towards initial development as a female, it may be too costly to switch the developmental pathway to male-first sex changer (and *vice versa*). We tested whether the sparids conform to the SAM predictions when incorporating sperm competition. Specifically, we tested whether gonochoristic species, which often spawn in large groups or aggregations and are typically characterized by intense sperm competition, have a higher GSI than species with either types of sequential hermaphroditism. Sperm competition indeed is less intense in haremic species (often protogynous), where the presence of few dominant large males drastically reduce the interaction between sperm of different males(Molloy et al., 2007). Also, it is generally accepted that protandrous hermaphrodites normally reproduce in small, random mating groups (no size-assortative) or in strictly monogamous pairs(Sunobe et al., 2016, Warner, 1988b) as, for example, in most clownfish (family Pomacentridae) of the genus

Amphiprion, such as *A. melanopus* and *A. percula* (Choi et al., 2016, Madhu and Madhu, 2007, Iwata et al., 2010). In both mating systems, this would imply a low degree of sperm competition and thus low values of GSI, as predicted by SAM(Warner, 1988a). As expected, we found that the average male GSI in gonochoristic sparids was > 3%, in agreement with previous reports (Molloy et al., 2007, Erisman et al., 2009, Erisman et al., 2013), and significantly higher than the mean GSI of protogynous sparids (≤ 2%). These results, therefore, support the SAM prediction, incorporating sperm competition, in protogynous species. However, our study also reveals that protandrous sparids have, on average, the highest male average GSI value (3.6%), regardless of their mating system. Thus, even when mating in pairs or small groups, protandrous males invest heavily in the gonads, indeed even more than gonochoristic species that mate in large aggregations with intense sperm competition. Further, we demonstrate that these results are not determined by body size differences as we find no allometric influences on GSI values, neither across all species, nor within one genus (*Diplodus*) containing closely related species of similar size. Altogether, our study unambiguously demonstrates that, while gonochoristic and protogynous sparids conform to the SAM predictions, protandrous sparids do not. We suggest that these results may be explained by both spawning mode and a compensatory mechanism determined by high sexual size dimorphism in protandrous species.

Some protandrous sparid species like *Acanthopagrus berda*, *Sarpa salpa*(Van der Walt and Mann, 1998), *Diplodus capensis*(Mann and Buxton, 1998) and D. *annularis*(Pajuelo and Lorenzo, 2002), spawn in aggregations(Garratt, 1993), similarly to many gonochoristic species(Erisman et al., 2013) and should therefore experience intense sperm competition, leading to a high GSI value. Indeed, we often tend to oversimplify the complexity of sequential hermaphroditism: not all protogynous species are haremic, as not all protandrous species mate in pairs. A recent study has revealed a broader variation in effective population size in protogynous species, different from the more limited expectations obtained when, in a simplistic way, all protogynous species were considered haremic by default(Benvenuto et al., 2017, Waples et al., 2018). Instead, some protogynous species were found to be group spawners, altering the expectations of low effective population size (as a result of an haremic system) for all protogymous species. Similarly, not all protandrous species are monogamous or mate in pairs and small groups. But, surprisingly, many of the protandrous sparids that engage mostly or exclusively in pair mating, including *Sparus aurata*(Hadj-Taieb et al., 2013) and *Rhabdosargus sarba*(Leu, 1994), exhibit an average GSI of 4.4% which, albeit lower than values of group spawning sparids (5.5%), is still higher than most gonochoristic species (3%). Therefore, while spawning in aggregations can explain the high GSI of many gonochoristic and protogynous species, mating system and spawning mode alone cannot explain the high GSI consistently found in protandrous sparids. This is not unique to the sparids. For example, the majority of damselfishes (family Pomacentridae) reproduce in pairs and, as predicted by SAM, exhibit lower GSI values (max. GSI < 1%(Fishelson, 1998)); however, other protandrous damselfishes, such as the yellowtail clownfish *Amphiprion clarkii*, reproduce in pairs(Moyer and Nakazono, 1978) but exhibit unexpectedly high GSI (4.14%)(Nakamura et al., 2015). This corroborates that protandrous species can exhibit high GSI values, regardless of mating system.

Table 2. Phylogenetic analysis of male life-history traits (see text for full definition) according to sexual systems (G = Gonochorism; PA = Protandry; PG = Protogyny) using the Rabosky *et al.*(Rabosky et al., 2018)'s phylogenetic tree

Variables						**Model stats**	
Dependent	**Independent**	**Beta**	**T**	**P**	**df**	**λ**	**R^2**
Weight (log$_{10}$ transformed)	Sexual system - PA^1	0.11	0.77	0.44	2,29	1.00	0.06
	Sexual system - PG^1	-0.19	-1.07	0.29	2,29		
	Sexual system - PG^2	-0.30	-1.41	0.16	2,29		
Length (log$_{10}$ transformed)	Sexual system - PA^1	-0.04	-0.77	0.44	2,44	0.71	0.06
	Sexual system - PG^1	-0.09	-1.64	0.10	2,44		
	Sexual system - PG^2	-0.04	-0.61	0.54	2,44		
Length Maturity (log$_{10}$ transformed)	Sexual system - PA^1	-0.06	-0.85	0.39	2,24	1.00	0.03
	Sexual system - PG^1	-0.05	-0.65	0.51	2,24		
	Sexual system - PG^2	0.01	0.13	0.89	2,24		
GSI (%)	Sexual system - PA^1	0.43	0.84	0.40	2,43	0.70	0.14
	Sexual system - PG^1	-1.12	-2.19	**0.03**	2,43		
	Sexual system - PG^2	-1.56	-2.55	**0.01**	2,43		

Notes: Gonochorism in ^{1} and protandry in ^{2} were set as the reference level, respectively. Abbreviations: T: t-value; df: degrees of freedom; P: *p*-value; λ: phylogenetic signal. Significant *p*-values are in bold.

Here we propose that it is precisely the nature of protandry what explains the high GSI in protandrous sparids with pair mating. Specifically, protandrous males (first sex) are always smaller than females. Thus, given that fecundity increases with size in females(Jennings et al., 2009), small protandrous males need to produce large amounts of sperm to effectively fertilize highly fecund females much larger than themselves, even when mating in pairs and under conditions of low levels of sperm competition. To do so successfully, they need to invest disproportionally in the gonads. For small-sized protandrous sparids, there might be a body size threshold below which the GSI has to increase to ensure enough sperm production to fertilize the eggs produced by the larger females. Consistently, evidence of sperm production adjustment in relation to the amount of eggs to be fertilized has been reported in a coral reef fish(Shapiro et al., 1994). Further, in many species with alterative male mating strategies smaller male "sneakers" invest more in gonads than larger territorial males(Taborsky, 1998) due to the competitive environment as well as to compensate for their reduced size. Taken together, these evidences support the idea that protandrous males have higher GSI than expected because they need to ensure the fertilization of eggs produced by much larger females than themselves.

The potential limitation in the fertilization capacity of small males is in agreement with the current debate questioning Bateman's principles(Tang-Martínez, 2012, Leonard, 2005, Tang-Martínez, 2016). Briefly, Bateman principles state that, due to the smaller cost of producing sperm when compared to eggs: (i) male reproductive success (RS) increases with mate number whereas female RS does not; (ii) males have greater variance in RS than females, and (iii) the sex with the greater variance in RS undergoes stronger sexual selection(Tang-Martínez, 2012). Bateman's principles have been used to test the strength of sexual selection across sexes in gonochoristic(Janicke et al., 2016) and simultaneous hermaphroditic species, although whether they really apply to the latter is currently highly debated(Leonard, 2005, Tang-Martínez, 2012, Tang-Martínez, 2016). Surprisingly, although the presence of sexual selection in sequential hermaphrodites has been recognized with the assumption that it is stronger on the most abundant sex(Leonard, 2006), Bateman's principles have not been formally tested. We argue that it is the combination of the existence of male-skewed sex ratios and male-male competition, on the one hand, and the problem for small males of producing enough sperm to fertilize large females, on the other hand, that together explain the high GSI in males of protandrous sparids, depending on the social/mating system. In fact, there is evidence that sperm depletion is a problem for many males across several taxa (reviewed in(Tang-Martínez, 2016)). For example, in the simultaneous hermaphrodite polychaete *Ophryotrocha diadema*, small protandrous males can have difficulties fertilizing a full clutch of eggs(Sella, 1988). Sperm depletion has been also documented in fish(Nakatsuru and Kramer, 1982, Weir and Grant, 2010). Moreover, in external spawners as most fishes are, mating rates (how many females can be fertilized) and ejaculation rates (how many sperm should be released in the water) should also be considered potential causes of higher GSI(Parker, 2016). Thus, a higher than expected GSI may be related not only to sperm competition, which would relate to strong sexual selection in males in protandrous species spawning in groups, but also to a physiological compensatory mechanism that allows males to fertilize the many eggs that large females produce.

To test these ideas further, field studies are needed to corroborate whether the protandrous sparids with the highest GSI values are those that spawn in large groups or aggregations rather than in random matings or pairs. Furthermore, laboratory experiments aimed at determining the actual fertilization capacity of small-sized protandrous sparids, specifically fertilization rates with different amounts of sperm, will be key to determine whether there is indeed a size threshold below which the GSI needs to increase in order to ensure fertilization of the eggs released by the larger females. This evidence would advance substantially our understanding of the relationship between sexual systems and mating systems in this diverse family in particular and in teleosts in general.

To conclude, this study provides the most updated analysis of the distribution and incidence of different sexual systems in the family Sparidae, an ideal model taxon in which to study the evolution of sexual systems and SAM predictions. We have found that both protandry and protogyny can evolve from gonochorism and back, but evolutionary transitions between the two types of sequential hermaphroditism are unlikely if ever to occur. We show that the predictions of the new SAM incorporating the role played by sperm competition, hold well for protogynous and gonochoristic species. In contrast, protandrous species do not conform to theoretical expectations. The high GSI values of some protandrous sparids suggests that males compete to fertilize the eggs of females; yet other protandrous sparids mate in pairs in absence of male-male competition but still invest greatly in gonad tissue. We suggest that this is due to a compensatory mechanism that is intrinsic to protandry; boosting male GSI to ensure successful fertilization of the considerable number of eggs released by highly fecund females that are much larger than protandrous males.

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Supplementary Tables

Table S1. List of the 68 sparid species used in this study, with information on their sexual system retrieved from FishBase and checked against the primary literature

Species	First description	SS	Reference (**)
Acanthopagrus australis	Günther, 1859	PA	Kailola et al., 1993
Acanthopagrus berda	Forsskål, 1775	PA	Tobin et al., 1997
Acanthopagrus bifasciatus	Forsskål, 1775	PA	Buxton and Garratt, 1990
Acanthopagrus butcheri	Munro, 1949	G	Kailola et al., 1993
Acanthopagrus latus	Houttuyn, 1782	PA	Buxton and Garratt, 1990
Acanthopagrus schlegelii	Bleeker, 1854	PA	Breder and Rosen, 1966
Archosargus rhomboidalis	Linnaeus, 1758	G	Chavance et al., 1986
Argyrops filamentosus	Valenciennes, 1830	G	Jayabalan et al., 2011
Argyrops spinifer	Forsskål, 1775	PG	El-Sayed and Abdel-Bary, 1993
Boops boops	Linnaeus, 1758	PG	Bauchot and Hureau, 1986
Boopsoidea inornata	Castelnau, 1861	G*	Buxton and Garratt, 1990
Calamus arctifrons	Goode & Bean, 1882	G	Darcy, 1986
Calamus bajonado	Bloch & Schneider, 1801	PG	García-Cagide et al., 1994
Calamus leucosteus	Jordan & Gilbert, 1885	PG	Waltz et al., 1982
Calamus nodosus	Randall & Caldwell, 1966	PG	Horvath et al., 1990
Calamus penna	Valenciennes, 1830	G	Darcy et al., 1986
Calamus pennatula	Guichenot, 1868	PG	García-Cagide et al., 1994
Calamus proridens	Jordan & Gilbert, 1884	PG	García-Cagide et al., 1994
Centracanthus cirrus	Rafinesque, 1810	G	Tortonese, 1986
Cheimerius nufar	Valenciennes, 1830	G	Garratt, 1991
Chrysoblephus cristiceps	Valenciennes, 1830	PG	van der Elst and Adkin, 1991
Chrysoblephus gibbiceps	Valenciennes, 1830	G	Garratt, 1986
Chrysoblephus laticeps	Valenciennes, 1830	PG	van der Elst and Adkin, 1991
Chrysoblephus puniceus	Gilchrist & Thompson, 1908	PG	Garratt, 1986
Dentex canariensis	Steindachner, 1881	G	Bauchot and Hureau, 1986
Dentex dentex	Linnaeus, 1758	G	Bauchot and Hureau, 1986
Dentex gibbosus	Rafinesque, 1810	PG	Bauchot et al., 1981
Dentex macrophthalmus	Bloch, 1791	G	Bauchot and Hureau, 1986
Dentex maroccanus	Valenciennes, 1830	G	Bauchot and Hureau, 1986
Dentex tumifrons	Temminck & Schlegel, 1843	PG	Buxton and Garratt, 1990
Diplodus annularis	Linnaeus, 1758	PA	Salekhova, 1961
Diplodus argenteus	Valenciennes, 1830	PA	David et al., 2005
Diplodus bellottii	Steindachner, 1882	G	Bauchot and Hureau, 1986
Diplodus capensis	Smith, 1844	PA	David et al., 2005
Diplodus cervinus	Lowe, 1838	G	Bauchot and Hureau, 1986

Diplodus puntazzo	Walbaum, 1792	G	Garratt, 1986
Diplodus sargus cadenati	de la Paz, Bauchot & Daget, 1974	PA	Pajuelo and Lorenzo, 2004
Diplodus sargus kotschyi	Steindachner, 1876	PA	Abou-Seedo et al., 1990
Diplodus sargus sargus	Linnaeus, 1758	PA	Bauchot and Hureau, 1986
Diplodus vulgaris	Geoffroy Saint-Hilaire, 1817	G	Bauchot and Hureau, 1986
Lagodon rhomboides	Linnaeus, 1766	G	Cody and Bortone 1992
Lithognathus aureti	Smith, 1962	PA	Buxton and Garratt, 1990
Lithognathus mormyrus	Linnaeus, 1758	PA	Bauchot and Hureau, 1986
Oblada melanura	Linnaeus, 1758	G	Buxton and Garratt, 1990
Pachymetopon aeneum	Gilchrist & Thompson, 1908	PG	van der Elst and Adkin, 1991
Pachymetopon grande	Günther, 1859	G*	Buxton and Garratt, 1990
Pagellus acarne	Risso, 1827	PA	Bauchot et al., 1981
Pagellus bellottii	Steindachner, 1882	PG	Bauchot et al., 1981
Pagellus bogaraveo	Brünnich, 1768	PA	de Mitcheson and Liu, 2008
Pagellus erythrinus	Linnaeus, 1758	PG	Buxton and Garratt, 1990
Pagrus auratus	Forster, 1801	G	Kailola et al., 1993
Pagrus aurigaa	Valenciennes, 1843	PG	Alekseev, 1982
Pagrus caeruleostictus	Valenciennes, 1830	PG	Buxton and Garratt, 1990
Pagrus major	Temminck & Schlegel, 1843	G	Buxton and Garratt, 1990
Pagrus pagrus	Linnaeus, 1758	PG	Pajuelo and Lorenzo, 1996
Petrus rupestris	Valenciennes, 1830	G*	Buxton and Garratt, 1990
Polysteganus undulosus	Regan, 1908	G*	Buxton and Garratt, 1990
Pterogymnus laniarius	Valenciennes, 1830	G	Hecht and Baird, 1977
Rhabdosargus globiceps	Valenciennes, 1830	PG	van der Elst and Adkin, 1991
Rhabdosargus sarba	Forsskål, 1775	PA	de Mitcheson and Liu, 2008
Sarpa salpa	Linnaeus, 1758	PA	Bauchot and Hureau, 1986
Sparidentex hasta	Valenciennes, 1830	PA	Lone et al., 2003
Sparodon durbanensis	Castelnau, 1861	G*	Buxton and Garratt, 1990
Sparus aurata	Linnaeus, 1758	PA	Bauchot et al., 1981
Spicara maena	Linnaeus, 1758	PG	Tortonese, 1986
Spicara smaris	Linnaeus, 1758	PG	Tsangridis and Filippousis, 1992
Spondyliosoma cantharus	Linnaeus, 1758	PG	Bauchot and Hureau, 1986
Spondyliosoma emarginatum	Valenciennes, 1830	G	Smith and Smith, 1986

Abbreviations: SS, sexual systems; G, Gonochorism; PA, Protandry; PG, Protogyny.
* Information on the sexual pattern missing in FishBase and obtained from Buxton and Garratt, 1990.
** References on sexual system from FishBase.

Table S2. List of sparid genera with known sexual system

Genus	No. of species	Gonochorism (G)	Hermaphroditism	
			Protandry (PA)	**Protogyny (PG)**
Acanthopagrus	20	1	5	-
Archosargus	3	1	-	-
Argyrops	4	1	-	1
Boops	2	-	-	1
Boopsoidea	1	1	-	-
Calamus	13	2	-	5
Centroacanthus	1	1	-	-
Cheimerius	2	1	-	-
Chrysoblephus	6	1	-	3
Dentex	13	4	-	2
Diplodus	15	4	6	-
Lagodon	1	1	-	-
Lithognathus	4	-	2	-
Oblada	1	1	-	-
Pachymetopon	3	1	-	1
Pagellus	6	-	2	2
Pagrus	6	2	-	3
Petrus	1	1	-	-
Polysteganus	8	1	-	-
Pterogymnus	1	1	-	-
Rhabdosagrus	6	-	1	1
Sarpa	1	-	1	-
Sparidentex	4	-	1	-
Sparodon	1	1	-	-
Sparus	1	-	1	-
Spicara	8	-	-	2
Spondyliosoma	2	1	-	1
(10 other genera)	14			
37	**148**	**27**	**19**	**22**

Table S3. Male gonadosomatic index (GSI) of some sparid species

Species	**Sexual system**	**GSI**	**In Rabosky tree 2018**	**In Santini tree**	**Reference**	**Spawning mode**	**Reference**
Acanthopagrus australis	PA	5.0	Yes	Yes	(Pollock, 1984)		
Acanthopagrus berda	PA		Yes	Yes		GS	(Garratt, 1993, Sheaves et al., 1999)
Acanthopagrus bifasciatus	PA		Yes	Yes			
Acanthopagrus butcheri	G	6.8	Yes	No	(Walker and Neira, 2001)		
Acanthopagrus latus	PA	5.0	Yes	Yes	(Abou-Seedo et al., 2003)		
Acanthopagrus schlegelii	PA	3.4	Yes	Yes	(Law and Sadovy de Mitcheson, 2017)		
Archosargus rhomboidalis	G		Yes	Yes			
Argyrops filamentosus	G	1.3	Yes	Yes	(Jayabalan et al., 2011)		
Argyrops spinifer	PG	1.1	Yes	Yes	(Al Mamry et al., 2009)		
Boops boops	PG	4.0	Yes	Yes	(Dobroslavić et al., 2017)		
Boopsoidea inornata	G		No	Yes			
Calamus arctifrons	G		No	No			
Calamus bajonado	PG		No	No			
Calamus leucosteus	PG		No	No			
Calamus nodosus	PG	0.1	Yes	Yes	(Horvath et al., 1990)		
Calamus penna	G		Yes	Yes			
Calamus pennatula	PG		No	No			
Calamus proridens	PG		No	No			

Cheimerius nufar	G	1.3	Yes	Yes	(Al-Marzouqi, 2013)	GS	(Garratt, 1993, Buxton and Garratt, 1990)
Centracanthus cirrus	G		No	Yes			
Chrysoblephus cristiceps	PG	0.5	Yes	Yes	(Buxton and Garratt, 1990)		
Chrysoblephus gibbiceps	G		Yes	No			
Chrysoblephus laticeps	PG	1.2	Yes	Yes	(Buxton and Garratt, 1990)	PS	(Buxton and Garratt, 1990)
Chrysoblephus puniceus	PG		Yes	Yes			
Dentex canariensis	G		Yes	Yes			
Dentex dentex	G	4.0	Yes	Yes	(Grau et al., 2016)		
Dentex gibbosus	PG	2.6	Yes	Yes	(Alves, 2010)		
Dentex macrophthalmus	G	4.5	Yes	Yes	(Soykan et al., 2015)		
Dentex maroccanus	G	0.8	Yes	Yes	(Mohdeb and Kara, 2015)		
Dentex tumifrons	PG	1.8	Yes	Yes	(Tominaga et al., 2005)		
Diplodus annularis	PA	5.0	Yes	Yes	(Mouine et al., 2012)	GS	(Pajuelo and Lorenzo, 2002)
Diplodus argenteus	PA	6.0	Yes	Yes	(David et al., 2005)		
Diplodus bellottii	G	4.4	Yes	Yes	(Ndiaye, 2015)		
Diplodus capensis	PA	5.5	No	No	(Mann and Buxton, 1998)	GS	(Mann and Buxton, 1998)
Diplodus cervinus	G	2.6	Yes	Yes	(Winkler et al., 2014)	GS	(Mann and Buxton, 1998)
Diplodus puntazzo	G	3.8	Yes	Yes	(Pajuelo et al., 2008)		
Diplodus sargus cadenati	PA	5.0	Yes	No	(Pajuelo and Lorenzo, 2004)		
Diplodus sargus kotschyi	PA	4.1	Yes	No	(Abou-Seedo et al., 1990)		
Diplodus sargus sargus	PA	4.8	Yes	Yes	(Mouine et al., 2012)		
Diplodus vulgaris	G	3.0	Yes	Yes	(Mouine et al., 2012)		
Lagodon rhomboides	G	5.0	Yes	Yes	(Nelson, 2002)		
Lithognathus aureti	PA		Yes	No			

Lithognathus mormyrus	PA	5.8	Yes	Yes	(Eyman Faraj Abd et al., 2016)		
Oblada melanura	G		Yes	Yes			
Pachymetopon aeneum	PG	1.9	Yes	Yes	(Buxton and Clarke, 1986)		
Pachymetopon grande	G		Yes	Yes			
Pagellus acarne	PA	3.5	Yes	Yes	(Arculeo et al., 2000)		
Pagellus bellottii	PG	2.2	Yes	Yes	(Sadovy de Mitcheson and Liu, 2008)		
Pagellus bogaraveo	PA	2.0	Yes	Yes	(Lechekhab et al., 2010)		
Pagellus erythrinus	PG	2.0	Yes	Yes	(Smida et al., 2014)		
Pagrus auratus	G	5.5	Yes	Yes	(Scott and Pankhurst, 1992)		
Pagrus auriga	PG	2.0	Yes	Yes	(Pajuelo et al., 2006)		
Pagrus caeruleostictus	PG	1.5	Yes	Yes	(Ismail et al., 2018)		
Pagrus major	G	7.5	No	Yes	(Gen et al., 2000)		
Pagrus pagrus	PG	4.8	Yes	Yes	(Kokokiris et al., 1999)		
Petrus rupestris	G	1.5	Yes	Yes	(Smale, 1988)		
Polysteganus undulosus	G		Yes	No			
Pterogymnus laniarius	G	1.2	Yes	Yes	(Booth and Buxton, 1997)		
Rhabdosargus globiceps	PG	3.2	Yes	Yes	(Griffiths et al., 2002)		
Rhabdosargus sarba	PA	4.0	Yes	Yes	(Hughes et al., 2009)	PS	(Leu, 1994)
Sarpa salpa	PA	6.0	Yes	Yes	(Paiva et al., 2016)	GS	(Van der Walt and Mann, 1998)
Sparidentex hasta	PA	2.3	Yes	Yes	(Lone et al., 2001)		
Sparodon durbanensis	G	5.0	Yes	Yes	(Buxton and Garratt, 1990)		
Sparus aurata	PA	4.8	Yes	Yes	(Hadj-Taieb et al., 2013)	PS	(Ibarra-Zatarain and Duncan, 2015)
Spicara maena	PG	1.8	Yes	Yes	(Soykan et al., 2010)		

Spicara smaris	PG		Yes	Yes			
Spondyliosoma cantharus	PG	1.1	Yes	Yes	(Pajuelo and Lorenzo, 1999)		
Spondyliosoma emarginatum	G	1.8	Yes	No	(Fairhurst et al., 2007)	PS	(Buxton and Garratt, 1990)

Abbreviations: G, Gonochorism; PA, Protandry; PG, Protogyny; PS, Pair spawning; GS, Group spawning.

Table S4. Phylogenetic analysis of male life-history traits according to sexual systems (G, PA, PG) from the Santini *et al*. (2014) phylogenetic tree

Variables						**Model stats**	
Dependent	**Independent**	**Beta**	**T**	**P**	**df**	**λ**	**R^2**
Weight ($\log_{10}$ transformed)	Sexual system - PA[1]	0.21	0.97	0.33	2,26	0.91	0.07
	Sexual system - PG[1]	-0.16	-0.88	0.38	2,26		
	Sexual system - PG[2]	-0.37	-1.41	0.16	2,26		
Length ($\log_{10}$ transformed)	Sexual system - PA[1]	-0.03	-0.48	0.63	2,40	0.67	0.05
	Sexual system - PG[1]	-0.10	-1.57	0.12	2,40		
	Sexual system - PG[2]	-0.06	-0.71	0.48	2,40		
Length at maturity ($\log_{10}$ transformed)	Sexual system - PA[1]	-0.12	-1.54	0.13	2,23	0.00	0.10
	Sexual system - PG[1]	-0.10	-1.28	0.21	2,23		
	Sexual system - PG[2]	0.02	0.29	0.76	2,23		
GSI (%)	Sexual system - PA[1]	0.99	1.71	0.09	2,41	0.00	0.31
	Sexual system - PG[1]	-1.44	-2.61	**0.01**	2,41		
	Sexual system - PG[2]	-2.44	-4.26	**0.00**	2,41		

Abbreviations: df = degrees of freedom; T, t-value; P = *p*-value; λ = phylogenetic signal; G = Gonochorism; PA = Protandry; PG = Protogyny. Significant differences are indicated in bold.

[1] G as reference level.

[2] PA as reference level.

Chapter 4

Simultaneous hermaphroditism in fish: Incidence, evolutionary transitions and selective pressures constraining body size

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Abstract

The incidence and evolutionary transitions leading to simultaneous hermaphroditism (SH) are well studied in plants, where is considered the ancestral state, and invertebrates. In many invertebrates, species with SH have lower maximum body size (MBS) than related gonochoristic (dioecious) species. This has been explained by the parasitic or interstitial life of many of these SH species, although numerous exceptions exist. However, in fish, the only vertebrate group where SH is present, the number of SH species, the evolutionary transitions and a possible link with small MBS is unknown. Here, we show that, in sharp contrast with the situation in plants and invertebrates, in fish SH is a derived, rare character that has evolved independently from gonochorism, and protogynous hermaphroditism, but not from protandry. We also found that SH species have lower MBS than gonochoristic relatives only when the mating system allows sufficient egg production by other means to compensate the lesser fecundity associated with smaller females, something actually occurring in SH invertebrates with low MBS. Therefore, this study clarifies the incidence and evolution of SH in fish and brings in the mating system, providing a framework to make testable predictions on the selective pressures constraining MBS in some SH animals.

Keywords: Hermaphroditism, ancestral state reconstruction, evolutionary transitions, sexual systems, William’s paradox, growth-reproduction trade-offs.

1. Introduction

Simultaneous hermaphroditism (SH) is the sexual system where both male and female gametes are produced by the same individual, either at the same time or within a very short period of time (Atz, 1964, Yamamoto, 1969). Simultaneous hermaphroditic species can reproduce either by outcrossing, whereby an individual can mate with another individual in both roles in a single reproductive event or season (Leonard, 2018a); or selfing, whereby the eggs of an individual are fertilized by its own sperm. Outcrossing is found in many large taxa as in most angiosperms and in many invertebrates (Wright et al., 2013). Selfing is rarer, particularly in animals, although it occurs in some invertebrates (Jarne and Charlesworth, 1993, Jarne and Auld, 2006, Leonard, 2018a, Leonard, 2018b).

The major benefit of SH is that it doubles the possibility to find mates, since any other individual encountered is a potential mate. In animals, SH is present in 5-6% of species (Leonard, 2013, Ghiselin, 1969, Jarne and Auld, 2006) and it has been argued that selection should favour the evolution of SH to ensure fertilization in species that are sessile, parasitic or with low population densities (Ghiselin, 1969, Leonard, 2013). However, there are many sessile or parasitic species that are gonochoristic (dioecious) rather than SH (Leonard, 2013) and, conversely, mobile species, e.g., some fish, that are SH. Thus, there might be other causes and different selective pressures can be responsible for the evolution of SH.

In plants, there is a well-developed body of theory concerning the evolutionary pathways from SH to gonochorism or the other way around (Delph, 2009), with gonochorism considered to be a derived and a less frequent condition (Barrett, 2002). In contrast, in animals SH is generally considered a condition evolved from gonochorism (Ghiselin, 1969), as it occurs, for example, in molluscs (Collin, 2013), although the opposite has been documented in barnacles (Kelly and Sanford, 2010). Mixed systems such as protandric SH have also been described, where males become simultaneous hermaphrodites. This occurs in some shrimps of the genus *Lysmata*, thought to be derived from protandry (sex-change from male to female) (Bauer, 2006).

In some invertebrate taxa, such as Platyhelminthes and few Nematoda, some Mollusca, Brachiopoda, Phoronida, and some Cirripedia, SH species have a smaller maximum body size (MBS) when compared to their gonochoristic relatives (Clark, 1978b). Why this is the case is far from clear. It may be related to the parasitic nature or interstitial life of some of these species (Clark, 1978b). Another explanation concerns economy of resources: small-bodied outcrossing simultaneous hermaphrodites may achieve higher fecundity by extending the period of egg production, rather than by a larger MBS as in gonochoristic species (Clark, 1978b, Leonard, 2013). Empirical support for the latter has been found in Caribbean brachiopods of the genus *Argyrotheca* (Kaulfuss et al., 2013). Further, a trade-off in energy allocation possibly due to the cost of egg production could lead to lower MBS (van Straalen et al., 2000, Baeza, 2006).

Compared to plants and invertebrates, far less is known about SH in vertebrates, where it is exclusively present in fish (Piferrer, 2018). In fish, SH has been documented in the orders Aulopiformes (several families), Anguilliformes (F. Muraenidae), Cyprinodontiformes (F. Rivulidae) and Perciformes (F. Serranidae) (Sadovy de Mitcheson and Liu, 2008, Avise and Mank, 2009). Most of the SH species in these families outcross (Avise, 2011), with the exception of *Kryptolebias marmoratus* and *K. hermaphroditus* of the family Rivulidae: (Harrington, 1961, Costa et al., 2010, Leonard, 2013), which can self-fertilize. Actually, some species of the genus *Kryptolebias* are androdieocious, i.e., natural populations consisting of males and SH individuals, but never females, a rare condition among vertebrates (Costa, 2016, Weeks et al., 2006). Nevertheless, the incidence of SH in fish is not fully resolved (Sadovy de Mitcheson and Liu, 2008).

The advantage of SH in fish has traditionally been explained by the low-density model, relating SH with species that have isolated populations with a low density of individuals (Ghiselin, 1969). Under these conditions male fitness can saturate for lack of females (Charnov, 1979, Charnov, 1982). Evolutionary transitions leading to SH have been documented in deep-sea species of the order Aulopiformes, contrasting with other species of the same order inhabiting shallower waters, which remain gonochoristic (Baldwin and Johnson, 1996, Davis and Fielitz, 2010). This scenario, therefore, provides support for the low-density model. However, within the family Serranidae —one of the fish families with the highest number of documented SH species— simultaneous hermaphrodites (which constitute in the subfamily Serraninae) inhabit shallow waters and hence do not necessarily constitute low-density populations, nor individuals should have problems in finding mates.

In serranids, protogyny is believed to be the ancestral sexual system (Erisman and Hastings, 2011). In that study, however, the evolutionary transitions leading to SH could not be resolved (Erisman and Hastings, 2011). Further, androdioecy evolved from SH in *Serranus baldwini* and *S. psittacinus*.

Interestingly, the Serraninae in general exhibit the smallest size among serranids and in fact are known as the ‘dwarf seabasses’ (Warner, 1984, Petersen and Fischer, 1986, Fischer and Petersen, 1987, Petersen, 2006). However, the association between SH and MBS documented in some invertebrates has not been formally tested in the Serranids as a whole or, for that matter, in Aulopiformes or in any other groups of fishes where SH is present. Dwarf seabasses have a shorter lifespan than longer-lived relatives and thus it is quite possible that they allocate energy to reproduction rather than grow. SH serranids reproduce by outcrossing and have a mating system called “egg trade”, whereby an individual of a reproducing pair trades eggs in return to have the chance to fertilize the partner’s eggs with his own sperm. It is believed that this direct reciprocity stabilizes SH at high mating rates (Henshaw et al., 2015). This system of sex allocation with egg trading allows to increase reproductive performance without the need to increase fecundity through larger MBS. Therefore, a trade-off between reproduction and growth, be either as a result of egg trading or through extended period of egg production, as in the brachiopods of the genus *Argyrotheca* discussed above, suggests that lower MBS than gonochoristic relatives may be an inherent property of SH species provided they can compensate egg production in order not to jeopardize reproductive performance.

Based on the above, the goal of this study was, first, to clarify as precisely as possible the number and distribution of SH in fishes, a task never specifically undertaken before. Next, we studied the evolutionary transitions leading to SH in the different families where it is present, something also never done before, to determine whether there are common general evolutionary pathways. Next, taking advantage of the different mating systems of the different groups of fishes where SH is present, we tested the hypothesis that lower MBS of SH species when compared to gonochoristic relatives will only occur when the mating system allows sufficient egg production by other means to compensate the lower fecundity associated with smaller females.

2. Material and Methods

2.1 Data collection

Data on SH sexual system across Actinopterygian fishes was obtained from an extensive search of the primary literature, taking as valid the cases where the diagnosis was based on histological analysis or on the verification of the presence of functional testes and ovaries in the same individual (**Table S1**). Notes explaining why some particular species were not included can be found in **Table S2**. The classification of the fish taxa mentioned in this study followed that of Eschemeyer's Catalog of fishes (Van der Laan et al., 2019).

Maximum body length is a useful estimate of body size and associated life history traits in most fish species (Albert and Johnson, 2012, Roxo et al., 2017). Data on maximum total body length (or maximum body size, MBS) were retrieved from FishBase (Froese and Pauly, 2018). We took data concerning MBS of males, which is available for many species, in contrast with data on MBS of females, which is scarce. Taxa with SH species also contained protogynous sequential hermaphrodites (female first), where the MBS is usually attained by males after sex change, although exceptions may occur (Munoz and Warner, 2003, Munoz and Warner, 2004, Munday et al., 2006). Thus, we took MBS of males of protogynous hermaphrodites as well as gonochoristic species to compare it with MBS of SH species. Since in many gonochoristic species females attain larger sizes than males (Parker, 1992), lower mean values of MBS in SH species when compared to gonochoristic species, if found, can be regarded as relevant because with the conservative approach taken we may be underestimating the MBS of some gonochoristic species and thus the possibility of finding differences.

2.2 Reconstruction of the ancestral sexual system

For the reconstruction of the ancestral sexual system of the families where SH is present we used the latest phylogenetic tree available for the Actinopterygian fishes (Rabosky et al., 2018), which includes 11,638 species. Comparison of the favourable performance of this tree with other trees focused in some of the families dealt with in this study because it contains a larger number of species for analysis is shown in **Table S2**. The ancestral state reconstruction of sexual system was carried out using maximum-likelihood (ML) approach (Pagel, 1999) with the R package *ape* (Paradis et al., 2004). Specifically, we compared two different models of evolution and assessed their fit to the data: the equal-rates (ER), in which the rate of gain and loss is forced to be the same, and the all-rates-different (ARD) model, in which the rates can differ. The best fitting model was tested using likelihood ratio (LR) test calculating the *p*-value per the chi-squared distribution. In addition, Akaike Information Criterion (AIC) values are shown, with 2 AIC values as evidence of support for the model with the lowest AIC score and if greater than 4 considered strong evidence. In addition, stochastic character mapping was performed in R, using the *make.simmap* commands in *phytools* v.0.6.99 (Revell, 2012).

2.3 Phylogenetic analysis of MBS as a function of sexual system and reconstruction of the ancestral MBS

To compare the MBS as a function of sexual system, taking into account evolutionary relationships, we utilized the phylogenetic generalized least square (PGLS) analysis (Pagel, 1997, Pagel, 1999, Freckleton et al., 2002) using the R package *caper* (Orme, 2013) and ML estimation. In PGLS analysis, the lambda parameter (0–1) indicates the magnitude of phylogenetic signal in the data. A lambda value of 0 indicates that the character is independent of phylogeny, while value of 1 that the character has evolved following the Brownian motion model of evolution and there is strong phylogenetic signal (Pagel, 1999, Freckleton et al., 2002). In all cases, MBS was $\log_{10}$-transformed prior analysis. We ran the PGLS analysis in clades with a sufficient number of species, i.e., the family Serranidae, order Aulopiformes and subfamily Gobiinae. However, due to small sample sizes, in the families Muraenidae and Rivulidae we just plotted the distribution of MBS or the median according to sexual system, considering SH species and only closely-related species (same or sister genus). Differences were considered as statistically significant when *P*-value < 0.05.

Next, we reconstructed the evolution of MBS in the phylogenetic tree to detect if the size of extant SH species was smaller or larger than the estimated MBS of the ancestor. To estimate MBS evolution, we evaluated the best-fit model for continuous traits among Brownian motion (BM), the Ornstein-Uhlenbeck (OU) or 'early-burst' model (EB), measuring the greatest strength of evidence of AICc (Akaike Information Criterion sample-size corrected) through the fitContinuous function of *geiger*. The best-fit model in all cases was the Brownian motion model of evolution (BM) (Pagel, 1999). In addition, we mapped ancestral size states along the phylogenetic tree with a gradient color using the *contMap* function in *phytools* (Paradis et al., 2004). To represent the evolution of sexual system and of MBS in mirror trees we plotted only the species for which we had information for both characters.

3. Results and Discussion

3.1. Distribution of SH in Actinopterygian fishes

Out of ~35,000 fish species, there is information on their sexual system for ~11,000 of them. Among these, SH could be confirmed only in 62 species. In addition, three species are androdioecious (populations where individuals are SH or male, but never female) and another three where SH would require further confirmation (**Table 1**). Depending on whether the latter are included or not, SH is thus present in 6 or 4 out of the 59 orders of the Class Actinopterygii. The subfamily Serraninae contained the highest number of confirmed SH species, 24. Next, with lower numbers, there were several families of the order Aulopiformes, such as Paralepididae (9 species), Scopelarchidae (7 species) and Chlorophtalmidae (5 species). In the rest of the families of this order, the presence of SH was limited to 1–3 species (but see notes in **Table S2**). The same figures also apply to the rest of families/subfamilies with SH species examined in this study, namely: Muraenidae, Leuciscidae, Epinephelidae, Gobiinae and the three androdieocious species in the family Rivulidae (**Table 1)**. We did not consider two serranids, *Serranus baldwini* and *S. psittacinus*, in which some large hermaphrodites loose the female function and start dominating a harem of small hermaphrodites (Petersen, 1987). This is different from the Rivulidae, where androdioecy is not a derived condition from SH.

3.2. Phylogenetic reconstruction of ancestral sexual system and ancestral MBS

The best-fit ancestral state reconstruction in all groups of interest in this study was the ER model (equal rates), based on the chi-squared of likelihood ratio test (**Table S3)**. The results of PGLS for each sexual system was used in taxa with enough number of species such as Serranidae, Aulopiformes and Gobiinae, as discussed below.

3.2.1 Serranidae family (Perciformes)

The Serranidae was the fish family where we found the highest number of documented SH species. The reconstruction of the ancestral sexual system of the 30 serranid species present in the Rabosky et al. (2018) phylogenetic tree, using ER model (df= 5, *P*-value = 0.81), showed that protogyny is the ancestral character state of this family (likelihood > 99%). PG is also the likely character state at both subfamilies, the Anthiadinae (n= 6 species; likelihood > 99%) and Serraninae (n= 24 species; likelihood >90%) (**Fig. 1;**

Table 1). In the Serraninae only *Centropristis striata*, which is considered the basal species of this subfamily (Erisman and Hastings, 2011), retained protogyny while most of the remaining species evolved to SH from protogyny, i.e., those of the genus *Hypoplectrus*, *Diplectrum* and *Serranus*. Finally, gonochorism evolved only in the genus *Paralabrax* (**Fig. 1**).

In a previous study on the evolutionary transitions in Serranids, Erisman and Hasting (2011) included 46 species but they used a most parsimony approach and the species relationships were different from trees using methodologies currently considered more reliable, such as ML and Bayesian methods (Craig and Hastings, 2007, Ma and Craig, 2018). Furthermore, the immediate ancestor of the *Paralabrax* and *Serranus* clades was not resolved (Erisman and Hastings, 2011), although it was pointed out that it would likely be protogyny based on previous evidence showing the evolution of gonochorism from rotogyny in some species of the family Epinephelidae (Erisman et al., 2009). Our analysis, however, shows that sexual transitions in Serranidae are protogyny → SH → gonochorism, instead of protogyny → (SH and gonochorism), as proposed by Erisman and Hastings (2011). In this regard, the lack of information of the sex determining mechanism of species in the genus *Paralabrax* does not allow to determine whether the transition from SH to G involves genetic (GSD) or environmental (ESD) sex determination, especially taking into account that analysis of adult sex ratio in *P. auroguttatus* showed values of 0.61 female:male adult sex ratio (Sadovy and Domeier, 2005), which would suggest GSD, while sex ratio in *P. clathratus* was 0.95 (Sadovy and Domeier, 2005), suggesting ESD.

M BS differed significantly between sexual systems (F= 10.85, df= 21, *P*-value = 0.0004, lambda= 0; **Fig. 2A**), and in particular between gonochoristic species of the genus *Paralabrax* and SH species (Serraninae) (T= -4.57, *P*-value = 0.0001), and between gonochoristic and protogynous species (Anthiadinae) (T= 2.51, *P*-value = 0.019). Sexual system accounted for ~48% of MBS variance (**Table 2**). Other factors such as the social system of SH serranids (Petersen, 1990, Petersen, 2006, Fischer, 1986, Fischer and Petersen, 1987) likely contributes to observed MBS values. Thus, in the species where the female function is lost in large SH individuals the smaller size of the remaining SH in an haremic social structure may be due to the costs of maintaining female function. On the other hand, the SH that mostly reproduce by egg-trading have a very short life and thus cannot achieve a large size. In this situation, a better strategy is to reproduce frequently. Therefore, the smaller MBS of SH in serranids is influenced by their particular mating systems (loss of female function and egg-trading). Furthermore, the reconstruction of the ancestral MBS showed a further reduction of MBS in some SH serranids, supporting the idea that at least some SH species in this family experience evolutionary pressures that select for reduced MBS. In summary, our analysis showed that serranids with SH had a smaller MBS than gonochoristic relatives, that the ancestral state was protogyny, and in some species there was further reduction of MBS during the evolution to SH.

Table 1. Actinopterygian taxa, following Eschemeyer's Catalog of fishes, where simultaneous hermaphrodite species are present. Numbers represent number of species

Order	**Suborder**	**Family**	**Subfamily**		**Hermaphroditism**			
				G	PG	PA	BD	SH
Anguilliformes		*Muraenidae*	*Muraeninae*	8	6	-	-	3
Aulopiformes	*Alepisauroidei*	*Alepisauridae*		-	-	-	-	2
		Anotopteridae		-	-	-	-	1
		Evermannellidae		-	-	-	-	2
		Omosudidae		-	-	-	-	1
		Paralepididae		2	-	-	-	9
	Chlorophthalmoidei	*Chlorophthalmidae*		-	-	-	-	5
		Ipnopidae		-	-	-	-	2
		Notosudidae		-	-	-	-	3
		Scopelarchidae		-	-	-	-	7
	Giganturoidei	*Bathysauridae*		-	-	-	-	2
Cypriniformes		*Leuciscidae*	*Leuciscinae*	191	-	-	-	1 ‡
Cyprinodontiformes		*Rivulidae*	*Cynolebiinae*	39	-	-	-	3†
Gobiiformes		*Gobiidae*	*Gobiinae*	121	28	-	26	2 ‡
Perciformes		*Epinephelidae*	*Epinephelinae*	11	159	-	-	1
		Serranidae	*Serraninae*	12	4	-	-	24

Abbreviations: G, gonochorism; PG, protogyny; PA, protandry; BD, bi-directional; SH, Simultaneous hermaphroditism. †Androdioecy system; ‡ Uncertain SH classification (see Discussion).

Table 2. Phylogenetic analysis of maximum male body size, MBS ($\log_{10}$-transformed) according to sexual systems in Serranidae, Aulopiformes and Gobiinae

Taxonomic level	**Variable statistics**						**Model stats**	
	Dependent	**Independent**	**Beta**	**T**	***p***	**df**	**λ**	***R*²**
Serranidae	MBS	Sexual system – PG^1	0.33	2.51	**0.02**	2,23	0.00	0.48
		Sexual system – SH^1	-0.52	-4.57	**0.00**	2,23		
		Sexual system – SH^2	-0.19	-1.94	0.06	2,23		
Aulopiformes	MBS	Sexual system – SH	0.05	0.45	0.65	1,19	0.00	0.01
Gobiinae	MBS	Sexual system – PG^1	-0.09	-1.34	0.18	3,71	0.80	0.28
		Sexual system – SH^1	-0.49	-3.14	**0.01**	3,71		
		Sexual system – BD^1	-0.32	-4.26	**0.00**	3,71		
		Sexual system – BD^2	-0.23	-2.44	**0.01**	3,71		
		Sexual system – SH^2	-0.40	-2.37	**0.02**	3,71		
		Sexual system – BD^3	0.17	0.99	0.32	3,71		

Abbreviations: df, degrees of freedom; λ, phylogenetic signal; *p*, p-value; R^2, Multiple R-squared; PG, Protogynous; SH, simultaneous hermaphroditism and BD, bi-directional. Indices of references level: ^{1}Gonochorism; ^{2}Protogynous and ^{3}Simultaneous hermaphroditism.

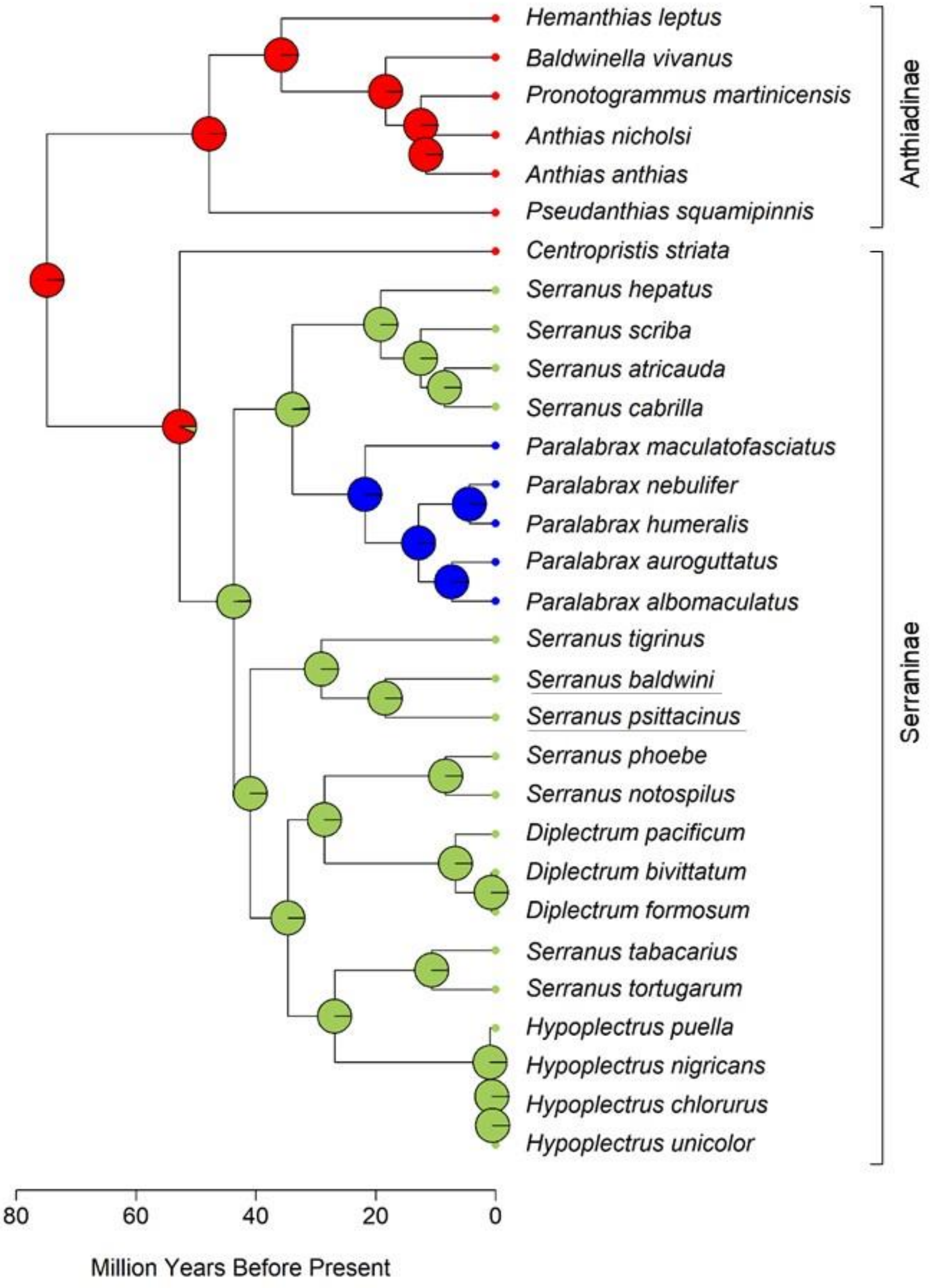

Figure 1. Reconstruction of the ancestral sexual system in the Serranidae (Perciformes). The two subfamilies described by Smith and Craig (2007), Anthiadinae and Serraninae, are noted. Sexual system is indicated by colours: gonochoristic (blue), protogynous (red) and simultaneous hermaphroditism (green). Species underlined are possible androdioecious.

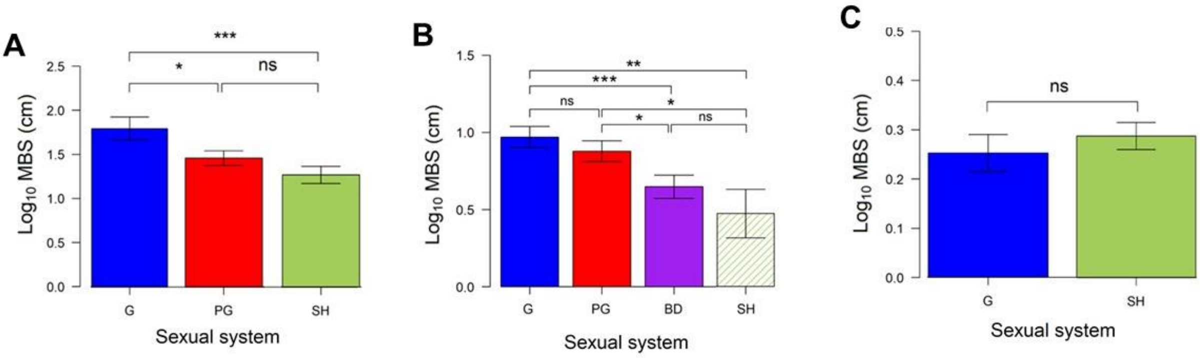

Figure 2. PGLS analysis of maximum body size (MBS) as a function of sexual system: gonochorism (G), protogyny (PG), bi-directional sequential (BD) and simultaneous hermaphroditism (SH). **A**) Phylogenetic mean and standard error of Serranidae: G, n = 4; PG, n = 6; SH, n = 16. **B**) Phylogenetic mean and standard error of Gobiinae; G, n = 49; PG, n = 13; BD, n = 11; SH, n = 2. **C**) Phylogenetic mean and standard error of Aulopiformes families: G, n = 10; SH, n = 11. Sexual system colors are gonochoristic (blue), protogynous (red), bi-directional (purple) and simultaneous hermaphroditism (dashed, indicating not fully confirmed). Abbreviations: ns, not significant. Asterisks indicate statistical significant differences with the following equivalence; * = p < 0.05, ** = p < 0.01 and *** = p < 0.001

3.2.2 Gobiinae subfamily (Gobiiformes)

The Gobiinae is the only fish family where bi-directional sequential hermaphroditism (BD) has been documented in the field. In contrast to protogynous or protandrous species who change sex only once during their lifetime, BD species can change sex several times from one sex to another just in a matter of days and depending on the social context (Munday et al., 2006).

The reconstruction of the ancestral sexual system in 75 species of the Gobiinae subfamily by the ER model (df= 11, P-value = 0.40) showed that gonochorism is the ancestral character state (likelihood >99%). From a gonochoristic ancestor, protogyny evolved three times, BD evolved three times (once from a gonochoristic ancestor, twice from protogynous ancestors) and SH evolved once from BD (**Fig. 3A)**. In the subfamily Gobiinae, all species of a given genus have the same sexual system, except two cases: *Trigrigobius*, which contains both gonochoristic and protogynous species, and *Priolepis*, with one BD species, *P. cincta*, and the two SH species, *Priolepis hipoliti* and *P. eugenius*.

The PGLS analysis showed that MBS differed across sexual systems (F= 9.421, df= 68, P-value = 2.554e^{-05}; lambda = 0.807; **Table 2**), which explained 28.4% of the variance in size. Gonochoristic gobies had the highest MBS, which is not statistically greater than protogynous gobies (T= 1.34; P-value = 0.184). On the other hand, BD gobies were significantly smaller than gonochoristic gobies (T= 4.26, P-value = 5.973e^{-05}) and protogynous ones (T= 2.44; P-value = 0.017). The two SH gobies were the smallest species, although no significant differences were found between BD and SH gobies, most likely because of the small sample size (**Fig. 2B**).

The ancestral state reconstruction showed that many branches leading to non-SH species exhibit a reduction in size. Therefore, MBS reduction is not specific to SH (**Fig. 2B**). The gobies are generally small (< 10 cm) and often short-lived (Helfman et al., 2009, Froese and Pauly, 2018). This suggests that the small-size in this subfamily could derive to specific life-traits as continuous growth or early maturity (Hernaman and Munday, 2005, Herler et al., 2011), which would favour the development of several reproduction systems (BD and/or SH) and thus avoid the loss of mating opportunities (Sunobe and Nakazono, 1990, Manabe et al., 2007).

3.2.3 Families of the order Aulopiformes

SH is present in 10 families of the order Aulopiformes (lizardfishes). The number of SH species with *confirmed* information of sexual system in many of these families did not allow considering families separately. The reconstruction of the ancestral sexual system in 41 species belonging to different families of the order Aulopiformes by the ER model (df= 1, *P*–value = 0.87) showed that gonochorism was the ancestral character state of this order (likelihood > 99%) (**Fig. 3B**) and that, as noticed before (Davis and Fielitz, 2010), the appearance of SH in Aulopiformes ~95 MYBP took place much earlier than in all the other cases examined (≤ 40 MYBP). Gonochorism prevailed in the families Synodontidae (21 species) and Aulopidae (*Aulopus filamentosus*), which form the suborder Synodontoidei. SH evolved once (likelihood at the common ancestor of the family > 99%) and has been maintained until the present in 10 families (Alepisauridae, Anotopteridae, Evermannellidae, Omosudidae, Paralepididae, Chlorophthalmidae, Ipnopidae, Notosudidae, Scopelarchidae, and Bathysauridae) with few verifiednSH species each (range 1–9 species; mean 3.4) distributed in the suborders Giganturoidei, Chlorophthalmoidei and Alepisauroidei (**Fig. 3B**). Here, then, not only the genus but also the entire families with information of sexual systems and their presence in tree to date, seem to maintain the same sexual system.

The evolution to SH in some species of Aulopiformes has been traditionally explained by their deep-sea habitat, in contrast to gonochoristic relatives inhabiting shallower waters (Ghiselin, 1969, Baldwin and Johnson, 1996, Davis and Fielitz, 2010). It has been argued that fishes that inhabit extreme depths could decrease their MBS due to low availability of resources (Moranta et al., 2004). The mating system of deep-sea SH aulopiformes is poor understood. Nevertheless, gonochoristic species of this family are promiscuous and competition of males occur during spawning rush (Donaldson, 1990). PGLS analysis showed that MBS of SH was not significantly different from that of gonochoristic species **(**F= 0.206; df= 18; *P*-value = 0.654**;** lambda = 0; **Fig. 2C; Table 2**). Reconstruction of the ancestral MBS of Aulopiformes (**Fig. 3B**) showed no major change in MBS and thus SH species had similar MBS than ancestral gonochoristic relatives. Then, some SH evolved to smaller sizes except in the Alepisauridae family, where branches leading to

SH species *Alepisaurus ferox* and *A. brevirostris* show an important increase in size (to ~200 cm in length). However, even without considering these two species, PGLS analysis showed that differences were not significant (T= 0.84; *P*-value = 0.41; data not shown). Thus, our analysis showed a lack of reduction in MBS of SH species when compared to shallower gonochoristic Aulopiformes.

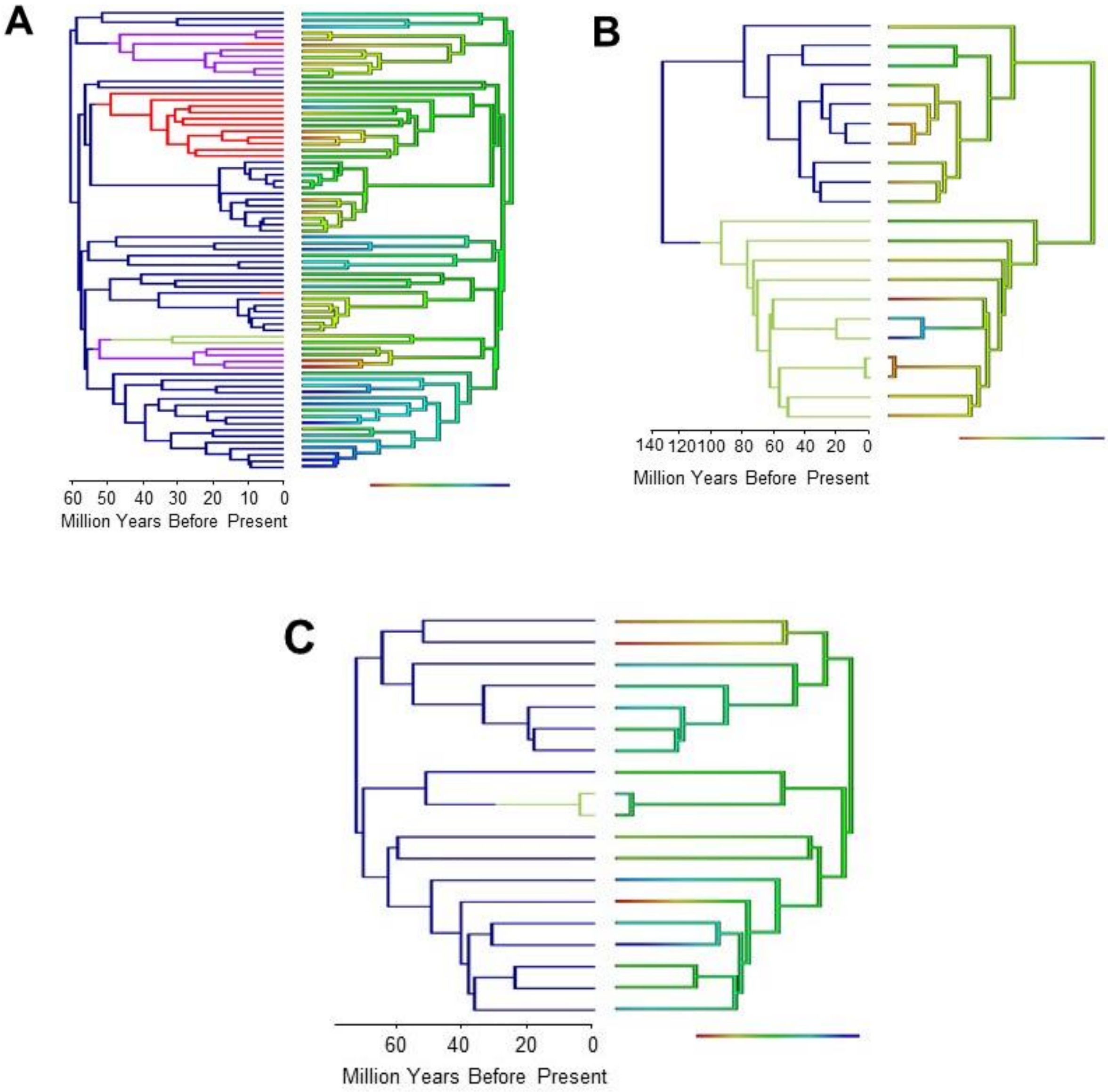

Figure 3. Stochastic mapping of sexual system (left) and MBS (right). **A)** Gobiinae. **B)** Aulopiformes. **C)** Rivulidae. Lower values are shown in redder colors; higher values are shown in blue. Sexual system colors are gonochoristic (blue), simultaneous hermaphroditism (green) and bi-directional (purple).

3.2.4 Muraenidae family (Anguilliformes)

The reconstruction of the ancestral sexual system in the family Muraenidae with the ER model (df= 5, *P*-value = 0.50) showed that protogyny is the ancestral character state (likelihood > 99%) in the family and particularly is retained in the *Gymnothorax* and *Echidna* genus. From protogyny, two sexual systems evolved: SH, present only in *Gymnothorax pictus*, *G. griseus* and *G. thyrsoidea*, and gonochorism, which evolved several times in the *Gymnothorax*, *Muraena* and *Pseudechidna* genus. Thus, the genus *Gymnothorax* exhibits a great diversity, presenting all sexual systems known in the family Muraenidae.

The PGLS analysis of MBS in family Muraenidae was not possible since we had data for only three SH species. Nevertheless, taking into account all species with information of sexual system, it could be determined that gonochoristic species (n = 8; mean = 164 cm) had the highest MBS, followed by protogynous species (n = 7; mean = 103 cm) while SH species had the lowest MBS (n= 3, mean = 90 cm) (**Fig. 4A**). Thus, protogynous species had in general lower MBS values than gonochoristic species, while *G. pictus*, the only SH present in the Rabosky et al (2018) tree, had an intermediate MBS value, similar to that of its direct ancestor, *E. nebulosa.* The mating system of *Gymnothorax pictus*, *G. griseus* and *G. thyrsoidea* is, to the best of our knowledge, unknown.

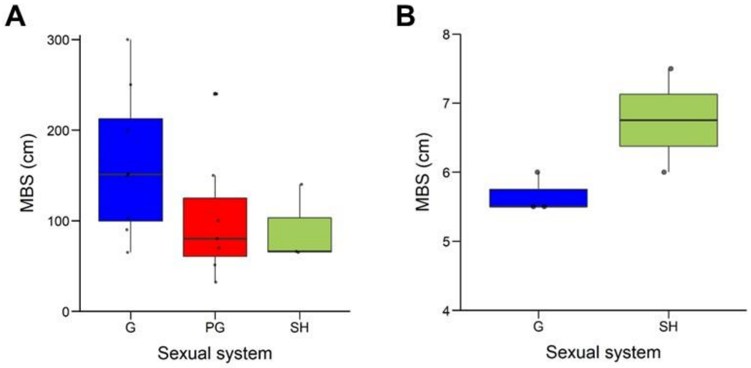

Figure 4. Maximum body size (MBS) as a function of sexual system in specific groups and without phylogenetic analysis. **A)** Muraenidae. Median of MBS: G, n = 8; PG, n = 7; SH, n = 3. **B)** Rivulidae. Median MBS in *Kryptolebias* and their close *Rivulus* genus. G, n= 3; SH, n = 2. The lower and upper edges of the boxes indicate the lower and upper quartiles, respectively; upper whisker = min (max(x), Q3+1.5 * IQR); lower whisker = max (min(x), Q1-1.5* IQR, where IQR= inter-quartile range, defined as the third quartile (Q3) – first quartile (Q1). The median is indicated by a black horizontal line. The black dots indicate individual values. Sexual system colors are gonochoristic (G, blue), protogynous hermaphroditism (PG, red) and simultaneous hermaphroditism (SH, green).

Reconstruction of the ancestral MBS of Muraenidae showed that there are small increases along branches leading to a few gonochoristic species and decreases in others. Similarly, pattern is observed in protogynous species. However, the SH species *G. pictus* did not show any major change in MBS with respect to the ancestral size. These data, albeit scarce, suggest that there was no reduction of MBS in the evolution of SH in Muraenidae.

3.2.5 Rivulidae family (Cyprinodontiformes)

The sexual system of SH species of this family is actually androdioecy, whereby natural populations consist of both SH individuals and males, but never females (Costa et al., 2010, Costa, 2016). Thus, the presence of SH individuals warranted the inclusion of this family in our study.

The reconstruction of the ancestral sexual system in 19 species of the family Rivulidae by the ER model (df= 1, *P*-value = 0.34) showed that gonochorism is the ancestral character sexual state (likelihood >99%) in this monophyletic family (**Fig. 2C)**. Androdioecy evolved once and recently, ~5 MYBP, in three species of the genus *Kryptolebias: K. marmoratus, K. ocellatus* and *K. hermaphroditus*, while *K. brasiliensis* retained gonochorism (**Fig. 2C**). The mating system of SH species is self-fertilization for *K. marmoratus and K. hermaphroditus*.

The PGLS analysis of MBS between species of the family Rivulidae exhibiting different sexual systems was not possible due to the low number of androdioecious species. However, taking into account only the closely related species of the genus *Kryptolebias* and *Rivulus*, constituted by androdioecious and gonochoristic species, respectively, we found similar or even higher MBS in androdioecy (G = 5.5 cm, SH = 6.75 cm) (**Fig. 4B**).

Reconstruction of the ancestral MBS of the family Rivulidae showed no major changes in the branches leading to the SH from a mid-sized gonochoristic ancestor (**Fig. 2C**). The only branches that showed an evolutionary change in MBS were those leading to gonochoristic species, either increase (e.g., *Gnatholebias zonatus*) or a decrease (e.g., *Notholebias minimus*) (**Fig. 2C**). Thus, when compared to the closely related gonochoristic species of the genus *Rivulus*, SH species of the *Kryptolebias* genus did not show a reduction of MBS.

4. Conclusions

4.1 Distribution of simultaneous hermaphroditism in fishes

With a largest dataset assembled so far, this study reveals that SH has evolved independently in at least 15 families belonging to 4 different orders (Anguilliformes, Aulopiformes, Cyprinodontiformes, and Perciformes) comprising ~65 species (but see notes in **Table S2** regarding SH in Aulopiformes). Our dataset identifies SH species in five additional families of Aulopiformes (Anotopteridae, Evermannellidae, Notosudidae, Omosudidae and Paralepididae) relative to what was previously reported. Thus, the number of families in which SH has been reported in at least one species has increased from 10 (Sadovy de Mitcheson and Liu, 2008) to 15 in this study. The ~65 SH species in fish represents a significant increase with respect to the 11 species mentioned in Sadovy de Mitcheson and Liu (2008). Thus, an important result of this study is that SH is a relatively rare condition in fish, even if an additional ~115 species of aulopiformes (Baldwin and Johnson, 1996) for which we could not find confirmation were included. This is certainly a small number when compared to the >35,000 known species of fish (Van der Laan et al., 2019) or even with the ~500 known species of sequential hermaphrodites. The reason why SH is rare in fish, or even when compared to other forms of hermaphroditism, is not known. Possible explanations include antagonism between male and female hormones in the same gonad (Devlin and Nagahama, 2002) and high level of initial inbreeding depression if self-fertilization is possible. Nevertheless, it has been argued that, at least as it is exemplified by the Serranines, once evolved SH appears to be a fairly robust sex-allocation pattern, specially taking into account that there might be potentially destabilizing influences of a strong male size-advantage if the female function is lost in SH individuals of some species (Fischer and Petersen, 1987). In any case, the patchy distribution of SH in fish contributes to the debate about William’s paradox, which contrasts the statement that the best predictor of SH in large taxa would be ecological conditions (Williams, 1975) while in fact in many cases is phylogeny (Leonard, 2013).

4.2 Evolutionary transitions

We identified multiple possible origins of SH in fish from different sexual systems: SH can evolve from gonochorism, as in Aulopiformes and Rivulidae; protogynous hermaphroditism, as in Serranidae and Muraenidae; and from BD sequential hermaphroditism, as in Gobiinae, but it is never an ancestral condition. Unexpectedly, fish families with SH species do not contain protandrous hermaphrodites. This is in contrast to what found in some invertebrates such as Entoprocta that contain both SH and protandrous species (Leonard, 2018b). Thus, with the data available so far it can be said that in fish SH is not derived from protandry. This suggests the existence, in addition of possible ultimate environmental pressures related to the social or mating systems, of some proximate morphological and/or physiological constraints in the evolution of SH. In fish, females has often been considered the ‘default sex’, because ovarian differentiation can naturally start in all individuals but ultimately halted in the individuals that become males even in some gonochoristic species (Devlin and Nagahama, 2002, DeFalco and Capel, 2009). Thus, evolving from protogyny to SH means retaining female function in addition to gaining the male one, something theoretically possible with this tendency of fish towards early female differentiation. In contrast, evolving from protandry to SH means retaining male function, which may be more difficult.

Recently, Leonard (2018) stated that a plausible evolutionary sequence for transitions between gonochorism and SH in animals was lacking, and proposed that selection for decreasing phenotypic plasticity in sexual phenotype could explain the transition between SH and gonochorism through intermediate stages of sequential hermaphroditism. While our results in Serranidae, Gobiidae and Muraenidae are consistent with this suggestion, we found also that SH can evolve straight from gonochorism in the Aulopiformes and the Rivulidae.

It has been argued that gynodioecy and androdioecy are not intermediate states in the transition between SH and gonochorism in animals (Weeks, 2012). In fishes exhibiting SH, androdioecy has been documented in *Kryptolebias marmoratus*, *K. ocellatus* and *K. hermaphroditus* (Harrington, 1961, Costa et al., 2010, Leonard, 2013) but we have no data supporting a further transition to gonochorism. In the Serraninae, there is a transition from SH to gonochorism in the genus *Paralabrax* but without androdioecious intermediaries. Furthermore, in small-sized serranines androdioecy can evolve when — in populations of elevated densities— large SH individuals become males after losing the ovarian tissue and defend harems consisting of SH (Leonard, 1993, Petersen, 2006, Hastings and Petersen, 1986, Petersen and Fischer, 1986), supporting the size-advantage model that predicts which sex is favourable according to their fitness by size (Ghiselin, 1969, Fischer and Petersen, 1987).

4.3 SH and MBS

This study clearly reveals that the mean MBS of extant SH fish species is either significantly smaller (Serranidae, Gobiinae) or similar (Aulopiformes, Rivulidae, Muraenidae) than that of gonochoristic conspecifics but never larger, the only exception being two species of Aulopisauridae. Thus, with the currently available data, it cannot be concluded that SH species in fish as a whole necessarily have a lower MBS than gonochoristic species. Further, taking into account the habitats where the species examined in this study live, we cannot find common aspects between the SH species that reduce MBS when compared to their gonochoristic relatives with those that do not.

However, if we bring in the mating system, we find that SH species have lower MBS than gonochoristic relatives only when the mating system allows sufficient egg production by other means to compensate the lesser fecundity associated with smaller females. Thus, contrasting with the situation in sequential hermaphroditism, where size determine fitness, in some SH species total female reproductive output is more dependent on the mating system and frequency of reproduction than size. This is the case of the Serraninae, where there is a clear reduction of MBS (egg-trading), and the Gobiidae, where SH evolved from already small-sized BD but that reproduce at high rate. The lower MBS of SH invertebrates has been linked to parasitic or interstitial life (Clark, 1978b) but many exceptions do occur, so this does not seem a fully satisfactory explanation. Invertebrates with lower MBS can achieve higher fecundity by extending the period of egg production, rather than by a larger MBS as in gonochoristic species (Clark, 1978b, Leonard, 2013), as found in the brachiopod of the genus *Argyrotheca* (Kaulfuss et al., 2013). This does not conflict with a trade-off in energy allocation due to the cost of egg production, also favouring lower MBS (van Straalen et al., 2000, Baeza, 2006). Based on what we have found in this study examining different families of fish where SH is present, we propose, therefore, that the relationship between SH and lower MBS is dependent on the mating system in each case, not necessarily of the parasitic or interstitial lifestyle. This does not mean that the habitat has no influence. In this study, the smallest-sized SH (serranids and gobies) have been found in shallow marine areas, while larger-sized SH are found in deep water areas (aulopiforms) or in brackish water and saltwater (muraenids) and also in fresh and brackish water (the androdioecy rivulids). It is tempting to conclude that, in animals, the smaller size of some SH is not an inherent feature but, rather, a consequence of their particular mating system. In summary, this study clarifies the incidence and evolution of SH in fish and shows that a reduction of MBS only occurs in association with particular mating systems. This study, therefore, provides a framework where to make predictions on the selective pressures constraining MBS based on mating systems that can be tested in other taxa containing SH species.

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Supplementary Tables

Table S1. List of all the fish species that belong to taxa containing simultaneous hermaphrodites for which we collected information on their sexual system

Order, Family and Species name	**Sexual system**	**References**
Perciformes		
Serranidae		
Anthias	PG	(Reinboth, 1964)
Anthias nicholsi	PG	(Anderson and Baldwin, 2000)
Baldwinella vivanus	PG	(Hastings, 1981)
Centropristis striata	PG	(Lavenda, 1949)
Diplectrum bivittatum	SH	(Touart and Bortone, 1980)
Diplectrum formosum	SH	(Bubley and Pashuk, 2010)
Diplectrum macropoma	SHǂ	(Bortone, 1977)
Diplectrum pacificum	SH	(Bortone, 1977)
Diplectrum radiale	SHǂ	(Bortone, 1977)
Diplectrum rostrum	SHǂ	(Bortone, 1977)
Hemanthias leptus	PG	(Anderson and Baldwin, 2000)
Hypoplectrus chlorurus	SH	(Barlow, 1975)
Hypoplectrus nigricans	SH	(Fischer, 1980)
Hypoplectrus puella	SH	(Barlow, 1975)
Hypoplectrus unicolor	SH	(Smith, 1965, Barlow, 1975)
Pronotogrammus martinicensis	PG	(McBride et al., 2009)
Pseudanthias squamipinnis	PG	(Fishelson, 1970, Shapiro, 1981)
Serraniculus pumilio	SHǂ	Hastings (1973)
Serranus annularis	SHǂ	Longley and Hildebrand (1941)
Serranus atricauda	SH	(García-Díaz et al., 2006)
Serranus baldwini	SH	(Petersen and Fischer, 1986, Smith, 1965)
Serranus cabrilla	SH	(Smith, 1965)
Serranus hepatus	SH	(Bruslé, 1983)
Serranus notospilus	SH	(Longley and Hildebrand, 1941)
Serranus phoebe	SH	(Smith, 1965)
Serranus psittacinus	SH	(Hastings and Petersen, 1986, Petersen, 1991)
Serranus scriba	SH	(Zorica et al., 2005)
Serranus subliganus	SHǂ	Hastings and Bortone (1980)
Serranus tabacarius	SH	(Smith, 1965, Petersen, 1991)
Serranus tigrinus	SH	(Smith, 1965)
Serranus tortugarum	SH	Petersen and Fisher (1996)
Epinephelidae (Grammistini)		
Pseudogramma gregoryi	SHǂ	Smith and Hafter (1969)
Gobiiformes		
Gobiidae		
Bryaninops natans	PG	(Fishelson, 1989)
Bryaninops yongei	PG	(Munday et al., 2002)

Coryphopterus alloides	PG	(Cole and Shapiro, 1990)
Coryphopterus dicrus	PG	(Cole and Shapiro, 1990)
Coryphopterus eidolon	PG	(Cole and Shapiro, 1990)
Coryphopterus glaucofraenum	PG	(Cole and Shapiro, 1990)
Coryphopterus hyalinus	PG	(Cole and Shapiro, 1990)
Coryphopterus lipernes	PG	(Cole and Shapiro, 1990)
Coryphopterus personatus	PG	(Robertson and Justines, 1982)
Coryphopterus thrix	PG	(Cole and Shapiro, 1990)
Eviota afelei	PG	(Cole, 1990)
Eviota albolineata	PG	(Cole, 1990)
Eviota saipanensis	PG	(Cole, 1990)
Fusigobius neophytus	PG	(Cole, 1990)
Gobiodon citrinus	BD	(Fishelson, 1989)
Gobiodon histrio	BD	(Munday et al., 1998)
Gobiodon oculolineatus	BD	(Nakashima et al., 1996)
Gobiodon okinawae	BD	(Cole and Hoese, 2001)
Gobiodon quinquestrigatus	BD	(Nakashima et al., 1996)
Gobiodon rivulatus	BD	(Nakashima et al., 1996)
Lophogobius cyprinoides	PG	(Cole, 1990)
Lythrypnus dalli	BD	(Mary, 1993)
Lythrypnus nesiotes	BD	(St. Mary, 2000)
Lythrypnus spilus	BD	(St. Mary, 2000)
Lythrypnus zebra	BD	(Mary, 1993)
Paragobiodon echinocephalus	BD	(Kuwamura et al., 1994)
Paragobiodon xanthosoma	BD	(Fishelson, 1989)
Priolepis cincta	BD	(Manabe et al., 2013)
Priolepis eugenius	SH**	(Cole, 1990)
Priolepis hipoliti	SH**	(Cole, 1990, Cole, 2010)
Rhinogobiops nicholsii	PG	(Yong and Grober, 2014)
Tigrigobius multifasciatus	PG	(Robertson and Justines, 1982)
Trimma caesiura	BD	(Sunobe et al., 2017)
Trimma okinawae	BD	(Sunobe and Nakazono, 1993)
Anguilliformes		
Muraenidae		
Echidna nebulosa	PG	(Fishelson, 1992)
Gymnomuraena zebra	PG	(Fishelson, 1992)
Gymnothorax fimbriatus	PG	(Fishelson, 1992)
Gymnothorax flavimarginatus	PG	(Fishelson, 1992)
Gymnothorax margaritophorus	PG	(Fishelson, 1992)
Gymnothorax griseus	SHǂ	Fishelson (1992)
Gymnothorax pictus	SH	(Fishelson, 1992)
Gymnothorax thyrsoidea	SHǂ	Fishelson (1992)
Cyprinodontiformes		
Cynolebiidae (Rivulidae)		
Kryptolebias hermaphroditus	SH*ǂ	(Costa et al., 2010)
Kryptolebias marmoratus	SH*	(Costa et al., 2010)

Kryptolebias ocellatus	SH*	(Costa et al., 2010)
Aulopiformes		
Alepisauridae		
Alepisaurus brevirostris	SH	(Baldwin and Johnson, 1996)
Alepisaurus ferox	SH	(Baldwin and Johnson, 1996)
Anotopteridae		
Anotopterus pharao	SH	(Baldwin and Johnson, 1996)
Bathysauridae		
Bathysaurus ferox	SH	(Davis and Fielitz, 2010, Sulak et al., 1985)
Bathysaurus mollis	SH‡	Nelson (1994)
Chlorophthalmidae		
Chlorophthalmus agassizi	SH	(Baldwin and Johnson, 1996)
Chlorophthalmus albatrossis	SH	(Smith, 1975, Ota et al., 2000)
Chlorophthalmus brasiliensis	SH‡	Mead (1959)
Chlorophthalmus mento	SH‡	Sulak et al. (1985)
Parasudis truculenta	SH	Mead (1959)
Evermannellidae		
Coccorella atlantica	SH	(Baldwin and Johnson, 1996)
Evermannella indica	SH	(Baldwin and Johnson, 1996)
Ipnopidae		
Bathypterois grallator	SH	(Baldwin and Johnson, 1996)
Bathypterois phenax	SH	(Baldwin and Johnson, 1996)
Notosudidae		
Ahliesaurus brevis	SH‡	(Baldwin and Johnson, 1996)
Scopelosaurus harryi	SH	(Baldwin and Johnson, 1996)
Scopelosaurus lepidus	SH	(Baldwin and Johnson, 1996)
Omosudidae		
Omosudis lowii	SH	(Smith and Atz, 1973)
Paralepididae		
Lestidium atlanticum	SH	(Baldwin and Johnson, 1996)
Lestidiops jayakari	SH	(Baldwin and Johnson, 1996)
Lestrolepis japonica	SH‡	(Baldwin and Johnson, 1996)
Lestidiops ringens	SH	(Baldwin and Johnson, 1996)
Lestrolepis intermedia	SH	(Baldwin and Johnson, 1996)
Macroparalepis johnfitchi	SH	(Baldwin and Johnson, 1996)
Magnisudis atlantica	SH	(Baldwin and Johnson, 1996)
Stemonosudis macrura	SH	(Baldwin and Johnson, 1996)
Sudis atrox	SH	(Baldwin and Johnson, 1996)
Scopelarchidae		
Benthalbella dentata	SH	(Baldwin and Johnson, 1996)
Benthalbella infans	SH	Merrett et al. (1973)
Benthalbella macropinna	SH	(Baldwin and Johnson, 1996)
Scopelarchus analis	SH‡	(Baldwin and Johnson, 1996)
Scopelarchus guentheri	SH‡	(Baldwin and Johnson, 1996)
Scopelarchoides nicholsi	SH‡	(Baldwin and Johnson, 1996)
Rosenblattichthys volucris	SH‡	(Baldwin and Johnson, 1996)

Cypriniformes		
Leuciscidae		
Tropidophoxinellus alburnoides	SH**	(Matos et al., 2010)

Abbreviations: G, gonochorism, PG, protogyny, BD, bi-directional, SH, simultaneous hermaphroditism. * Androdiocey; ** Simultaneous hermaphroditism questioned; ǂ not present in the Rabobsky et al. (2018) tree (and thus not used for phylogenetic ancestral state reconstruction).

Table S2. Notes on phylogenetic relationships and inclusion of some species in this study

Taxa	Notes on phylogenetic relationships
F. Serranidae	The Serranidae is the fish family where we found the highest number of documented SH species. Serranidae phylogeny is not devoid of controversy. Thus, for example, in the Rabosky et al. (2018) tree the phylogenetic relationships of *Serranus tigrinus* is different when compared to more family-specific trees (Smith and Craig, 2007, Craig and Hastings, 2007). However, in our opinion, using the Rabosky et al. (2018) tree was the best option, not only because it allowed us to include information on the sexual system of more species for analysis, but also because the transitions in the species with more interest for this study were the same as in the Smith and Craig (2007) tree.
S.F. Gobiinae	This is a family with ~1900 estimated species (Fricke et al., 2019). Complete agreement in different trees is almost impossible although the major groupings are conserved when comparing the Rabosky et al. (2018) and other phylogenies (Agorreta and Rüber, 2012). The major difference is that the relationships between *Trimma*, *Lythrypnus* and *Priolepis* are different in the Rabosky et al. (2018) tree. The Gobiinae is the only fish family where bi-directional sequential hermaphroditism (BD) has been documented in the field. There is an inherent difficulty in the diagnosis of SH, based in some cases on the presence of gametes in advanced stages of development of both sexes in the same individual. Thus, diagnosis of SH is difficult because the gonads can be confused with those of a sequential hermaphrodite with remains of gametes of the first sex during sex change or, worse, the presence of remaining mature gametes in the gonads of bi-directional sequential hermaphrodites. Thus, distinguishing true SH from BD gobies can be challenging (Cole, 1990, St. Mary, 1998, St. Mary, 2000).
O.Aulopiformes	Erisman et al. (2013) citing Baldwin and Johnson (1996) stated that two thirds of the 236 species of aulopiform fishes (i.e., about 158 species) were reported to be SH. However, we could not find evidence for that. Based on the primary literature we could only confirm SH in 34 species. For ancestral state reconstruction and MBS evolution, we used the Rabosky et al. (2018) tree instead of the Davis and Fielitz (2010) tree specific for Aulopiformes, because, like the situation in the Serranidae discussed above, the number of species was higher in the former (47 vs. 38), and because there were no major discrepancies in phylogenetic relationships between the

	two trees. The two differences perhaps worth mentioning being that *Aulopus filamentosus* and three species of the *Benthalbella* genus had different relationships between the two trees (Baldwin and Johnson, 1996, Sato and Nakabo, 2002), although this did not affect the estimation of transitions.
F. Muraenidae	This family presents also several difficulties. First, protogyny had not been confirmed in *Muraena pavonina, Gymnomuraena zebra, Gymnothorax fimbriatus, G. margaritophorus, G. gracilicauda, G. flavimarginatus* and *Echidna nebulosa* (Sadovy de Mitcheson and Liu, 2008, citing Fishelson, 1992) and, to the best of our knowledge, confirmation of whether these species are protogynous is not available. Second, the Muraneidae tree of Tang and Fielitz (2013), which should be considered the best available tree for this family, includes 38 species. However, we had information on the sexual system of only seven of them. In contrast, the Rabosky et al. (2018) tree contains 57 species — of which 30 coincide with the Tang and Fielitz (2013) tree — but in this case we had information on the sexual system of 12 of them, not seven. Fortunately, , the phylogenetic relationships between the only SH species in this family, *G. pictus,* and the protogynous *E. nebulosa* are identical in both phylogenetic trees (Tang and Fielitz, 2013, Rabosky et al., 2018). However, due to the unconfirmed protogyny of *E. nebulosa* and the absence of two other SH species, *G. griseus* and *G. thyrsoidea* (Fishelson, 1992), in both trees, we cannot completely rule out the possibility that SH evolved from gonochoristic species since the *Gymnothorax* genus shows a great diversity between protogynous and gonochoristic sexual systems.
F. Rivulidae	Androdioecy evolved in *Kryptolebias ocellatus, K. marmoratus* and *K. hermaphroditus.* The latter species is not present in the Rabosky et al. (2018) tree. Since it has been argued that another species, *K. caudomarginatus*, is in fact the same species as *K. ocellatus* (Costa, 2006) we did not consider *K. caudomarginatus* as a separate species. *K. marmoratus* and *K. hermaphroditus* are the only known vertebrate species that engage in self-fertilization (Harrington, 1961, Costa et al., 2010).
F. Sparidae	We did not include *Pagellus bogaraveo* (Perciformes: Sparidae) being SH, as done in Sadovy de Mitcheson and Liu (2008), based on the report of Buxton and Garratt (1990), in agreement with the view that is a protandrous species (Micale et al., 2002, Lechekhab et al., 2010).

F. Cyprinidae	SH was reported in *Squalius alburnoides* (Matos et al., 2010, Collares-Pereira et al., 2013), a species endemic to the Iberian peninsula originated by hybridization and capable of exhibiting different reproductive modes (Matos et al., 2010, Collares-Pereira et al., 2013). The simultaneous presence of mature gametes of both sexes (i.e., SH) was diagnosed in a few individuals based on morphological criteria. However, despite the elevated number of species in the family Cyprinidae, SH concerns so far only this single species, and since the influence of endocrine disrupting individuals could not be completely excluded in that survey, we tentatively did not include the family Cyprinidae as harboring true SH species.
F.Epinephelidae	The family Epinephelidae contains one species where SH has been claimed, *Pseudogramma gregoryi* (Smith and Hafter, 1969). This species belongs to the Grammistini tribe (Johnson, 1983, Baldwin and Johnson, 1993) and was not considered for analysis because, first, the low number of species in the tribe (Craig and Hastings, 2007, Smith and Craig, 2007) and, second, the scarce information on their sexual systems. Nevertheless, it is worth pointing out that since *P. xantha*, a close relative of *P. gregoryi*, is protogynous (Randall and Baldwin, 1997), if SH is confirmed in *P. gregoryi* then the transition would be from protogyny to SH, as in Serranidae.

Table S3. Ape-fitted comparative reconstruction models of sexual systems

	ER model		**ARD model**	
Taxa	**AIC**	**Log-likelihood**	**AIC**	**Log-likelihood**
F. Serranidae	21.73	-9.865	29.461	-8.730
S.F. Gobiinae	80.923	-39.461	91.435	-33.717
O. Aulopiformes	9.102	-3.551	11.078	-3.539
F. Muraenidae	26.169	-12.084	31.841	-9.910

Abbreviations: ER, equal transition rates and ARD, all rates different. AIC, Akaike information criterion. Note that shaded values indicate the best fit after likelihood ratio test.

General discussion

Some aspects that either could not be fully addressed within the length and scope of individual chapters, or that were not appropriate within a chapter are discussed below. They are worth being dealt with as a sort of wrap-up and connection between chapters.

1. Source of data and the material and methods used

1.1. Challenges in the diagnosis of the actual sexual system

1.1.1. Problems in distinguishing between juvenile or false hermaphroditism from true hermaphroditism

Recurrently, certain discrepancies and contradictions were found in the diagnosis and assignment of sexual systems in both FishBase, our primary source, and the primary literature. Some of these problems involve the distinction between false and true hermaphroditism or the confusion between gonochorism and protogyny. Species that sometimes are considered protogynous in the literature but that are in fact gonochoristic include, for example, *Mycteroperca rosacea*, *Paralabrax masculofasciatus* and the zebrafish, *Danio rerio*. This is because there are individuals of these species who in the juvenile stage present an immature intersex state, i.e., an immature bisexual gonad, which can be misinterpreted as undergoing sex change and thus being classified as hermaphrodite. However, despite having a bisexual stage, only one sex is functional throughout life in a given individual and therefore the species is not hermaphrodite. These peculiarities can make the diagnosis of sexual systems extremely challenging (Buxton and Garratt, 1990, Sadovy de Mitcheson and Liu, 2008).

Functional hermaphroditism is only accepted when all individuals of the population express both sexes in a functional manner, either sequentially or simultaneously (Sadovy de Mitcheson and Liu, 2008). Therefore, a specific diagnosis should be made based on the criteria of Sadovy and Shapiro (1987), which includes careful histological examination of the gonads, in different individuals and at different stages and ages. In addition, it must persist in the entire population and not just in one fraction of the individuals (Sadovy and Shapiro, 1987). When contradictory references were found, the issue was resolved by considering these criteria, particularly gonadal studies, rather than bimodal length-frequency distributions and biased population sex ratios, a common source of error (Sadovy and Shapiro, 1987).

1.1.2. Problems in distinguishing between protogyny and protandry

Similar to the above confusion, there have also been problems distinguishing between protogyny and protandry. There are some species that, like the undifferentiated type of species in gonochorism (Piferrer, 2001), may begin to differentiate as females, with ovaries in the early stages of development, although they quickly revert and then develop first as males (Lee et al. 2001; Miura et al. 2008). Therefore, the presence of testicular oocytes may lead to think that the species in question is a protogynous hermaphrodite. However, if one delays the examination of the gonads until first maturation it will become apparent that female-to-male sex change does not occur and that the species in question first matures as a male, indicating that is a protandrous, not protogynous species.

1.1.3. Problems in distinguishing bi-directional sequential hermaphroditism from simultaneous hermaphroditism

This problem arises due to the presence of remains of gametes of the first sex during sex change, or even by maintaining remaining mature gametes of the first sex in the gonads of the second sex in bi-directional sequential hermaphrodites. This may lead to classify a species as simultaneous hermaphrodite when in fact it is a bi-directional sequential hermaphrodite. Examples of species in which the literature has confused these two systems are the bluebanded goby, *Lythrypnus dalli*, considered a simultaneous hermaphrodite in St. Mary (1993) but a bi-directional sequential hermaphrodite in Lorenzi et al. (2006). In addition, in the genus *Priolepis*, the distinction between bi-directional sequential hermaphrodites and true simultaneous hermaphrodites can be challenging (Cole 1990; St. Mary 1998, 2000). Species of this genus have been assigned as bi-directional sequential hermaphroditism although two particular species, *Priolepis eugenius and P. hipoliti*, have been described as having vitellogenic oocytes and free sperm at the same time in the ovotestis. However, it remains unverified whether this state was transient or indicative of simultaneous hermaphroditism (Cole, 2010). After consulting with Dr. Kathleen Cole, in our study on simultaneous hermaphroditism we have considered them simultaneous hermaphrodites although it remains to be fully confirmed. In fact, there is no agreement among the community on how long gametes of the first sex should remain in the gonad to distinguish between bi-directional sequential hermaphrodites from a simultaneous hermaphrodite. Incidentally, it is interesting to note another similarity between these two types of sexual systems: the models that explain their appearance, the risk-of-movement in the bi-directional sequential hermaphroditism (Kuwamura et al. 1993; Nakashima et al. 1995) and the low-density in simultaneous hermaphroditism (Ghiselin 1969), are ultimately rooted in the same cause, which is the low probability of finding a mate.

1.2. Source of the data

Most of the data used come primarily from FishBase, www.fishbase.org (Froese and Pauly, 2019), the largest public database about fish in the world, which has been very convenient for this study, although it was verified as much as it was possible. But one, of course, can ask how reliable is the information contained there, particularly regarding sensitive issues such as the proper assignment of sexual systems, as discussed above. The Web of Science (http://wos.fecyt.es/) returns 254 documents when searching for the term "FishBase" in the ‘Topic’ field. Most studies (~90%) are about length-weight relationships of particular species but some use information about several variables in many species, as done here. For example, recent studies that have used body size and trophic level data have been published in well-respected journals such as *Science of the Total Environment* (Maceda-Veiga et al 2018) or even high-impact journals such as *Nature Communications* (Rabosky et al. 2013), indicating that the data obtained from FishBase in these studies has been accepted as reliable.

It should be mentioned, nevertheless, that FishBase updates its contents periodically as new studies become available. However, many data come from old studies that are not possible to verify or do not contain the information they refer to. For example, there are some sexual systems of fish that are referenced in the encyclopedic works by Breder and Rosen (1966) or in Heemstra and Randall (1993) but without providing the source. This is why here it was required to verify the sexual systems with the primary literature or through revisions, at the family level such as in Epinephelidae (Erisman et al 2009), Serranidae (Erisman et al, 2011), Sparidae (Buxton and Garratt, 1990) and Labridae (Kazanglu and Alonso, 2010).

In this regard, it has to be emphasized that, as mentioned in chapters 1 and 2, an attempt was made to validate the 552 hermaphrodite species identified with the primary literature. However, only 380 species could be validated, of which 360 coincided in the assignment of a particular type of hermaphroditism (~95% coincidence). On the other hand, information on the sexual system of 172 hermaphrodite species (31%) were not found in the primary literature and thus could not be verified. Assuming the same level of error, that is ~5% (20 of 360 species), it can be estimated that about 9 of the 172 species that could not be independently verified had an erroneous sexual system assignment. Thus, the maximum possible error in the set of the hermaphrodite species is 1.63% (9 of 552 species). Therefore, despite not being able to verify all sexual systems in fish, the error is very low, providing confidence that it did not affect the results obtained and thus the conclusions reached.

1.3. Methods used

Results cannot only be influenced by the reliability of the data source used, as just discussed above, but also by the phylogenetic tree used. Importantly, modern comparative phylogenetic methods have been used throughout.

Chapter 4, where the evolution of sexual transitions between different families of simultaneous hermaphrodites was studied, could have, in principle, benefited from the use of specific trees for each family because the relationships between species are likely well resolved in these type of trees. However, it was chosen not to use family-specific phylogenetic trees, the main reason being that they did not contain enough hermaphrodite species of interest for the study. The type of studies that were planned require the use of the highest possible number of species to reach robust conclusions. For this reason, the tree of Rabosky et al. (2018) was used. This is the most comprehensive molecular, dated, fully bifurcating phylogeny of Actinopterygians fishes to date that includes 11,638 extant species. Importantly, it was found that, within this tree, the phylogenetic relationships between species of particular interest was similar to that of the respective family-specific trees. This means that the results obtained are reliable not only because of the small error in the assignment of the sexual system but also because the phylogeny was also reliable. In this regard, results in phylogenetic analysis are, logically, sensitive to the topological representation of the species (Nunn, 2011). Our choice, nevertheless, did not alter the estimation in the transitions among sexual systems. In summary, the use of the Rabosky et al. (2018) phylogenetic tree was the best option, not only because it allowed us to include information on the sexual system of more species for analysis, but also because the transitions in the species of greatest interest for this study were the same as in specific phylogenetic trees.

Comparative phylogenetic methods assume that the relationship between species are not independent but influenced by their position in the phylogeny (Blomberg and Garland, 2002). Therefore, their use vs. conventional statistical methods can lead to radically different results and conclusions (Nunn, 2011). A comparison among the two types of methods involving 556 studies where differences were reported as significant between species (different questions being asked, not relevant for this discussion) revealed that, from all cases in which statistical differences were found with the use of non-phylogenetic methods, in 27% of these cases differences were no longer significant when phylogenetic methods were used (Carvalho et al. 2006). Therefore, a type I error (i.e., false-positive that occurs when the true null hypothesis is incorrectly rejected) can occur in 44-70% of cases when phylogeny is not considered (Harvey and Rambaut 1998; Lindenfors et al. 2010). In addition, type II errors (i.e., false negatives that occur when the null hypothesis is really false and is not rejected) can also occur. Thus, even the use of incorrect phylogeny may provide a better picture of the reality than the use of non-phylogenetic methods (Purvis et al. 1994).

For the ancestral reconstructions of sexual systems, two phylogenetic methodologies considered appropriate for the questions being asked were used: maximum likelihood (ML) and Bayesian approaches. These methodologies consider branch length (the longer the branch, the more opportunities for change) and offer a stronger statistical framework for quantifying uncertainty and exploring alternative evolutionary models (Nunn, 2011). ML models estimate the maximum probability of the ancestor using the Markov model, where branch change is considered independent of other branches and only depends on their start and length (Pagel 1994a, 1999b). On the other hand, Bayesian approaches are more recent methodologies (Pagel et al 2004) that take into account not only branch length but also topographic error (Pagel et al 1994), the latter also called uncertain phylogeny (Roquist, 2004). This method follows a Markov Chain Monte Carlo sampling procedure (MCMC), where parameters are proposed and accepted or rejected by means of an algorithm. These two methodologies are considered much better than the parsimony methods used in previous studies referred to throughout (Erisman et al 2009; 2011, 2013) that ignored branch length, which increases the imprecision of the estimates and does not have a statistical support to evaluate the model (Nunn, 2011).

Finally, to analyze whether there is a relationship between two or more continuous and/or discrete predictor variables on a single response variable in a single model, the most common comparative phylogenetic method used nowadays, called generalized phylogenetic least squares (PGLS), was used. This method estimates an evolutionary parameter that incorporates the phylogenetic relationships between species in the error term (Grafen 1989; Martins & Hansen 1997). What differentiates PGLS from other multiple standard regression methods is that it allows to control that the residuals of the model are non-independent, i.e., they depend on the phylogeny of the taxa being studied (Mundry, 2014).

2. Abundance and distribution of hermaphroditism

In order to understand the distribution and evolutionary transitions into and out of hermaphroditism, it is necessary to study a group of organisms that present a great diversity of sexual systems. The teleosts is a suitable group to study the plasticity of sexual systems because are considered the most derived group of fishes and exhibit a great diversity in life-history traits, morphology, behavior, habitats, trophic levels and, of course, sexual systems among the vertebrates (Smith and Wootten, 2016). The features that make fish unique with respect to other vertebrate groups are their third whole-genome duplication (Van de Peer et al., 2003; Smith and Wootten, 2016) that lead to phenotypic complexity (Hoegg et al., 2004). No less important are the anatomical peculiarities regarding the loss of the Müllerian ducts and the unique origin of their gonads as opposed to the rest of vertebrates. These morphological peculiarities facilitate the transition between sexual roles (Adolfi et al., 2018), already discussed in the general introduction.

Of the 33,822 existing species of teleost fish known to date (Eschmeyer et al 2019), information could be collected only for 10,914 of them, which represents exactly one third of the total. Hermaphroditism was found present only in ~5% of fish species for which there is information of their sexual system, of which 0.6% are simultaneous hermaphrodites (Chapter 1). This figure represents a very low incidence compared to the situation in other taxa such as angiosperms (84-90%, Barrett, 2002), gymnosperms (34%, Walas et al., 2018) or invertebrates, where ~30% of the species are hermaphrodites if the insects are excluded (Jarne and Auld, 2006). This low incidence in fish may be due to the presence of opposing hormonal systems and antagonizing signaling pathways within the same individual or gonad and along the hypothalamus-pituitary-gonadal axis (Bull and Charnov, 1985). Further, in contrast to the situation in other taxa, where simultaneous hermaphroditism is the most common form of hermaphroditism, in teleosts sequential hermaphroditism predominates (4.4%). Protogyny is the most common system of sequential hermaphroditism in fish and this may be due not only to the fact that female is the “default” sex in vertebrates but also that it might be easier to develop a female gonad and change later sex to male that the other way around, which would imply male development in a background of female sex tendency.

Here, it has been determined for the first time that, in addition to gonochorism, protogyny and, to a lesser extent, simultaneous hermaphroditism also constitute what Maynard Smith (1975) in his book *The Evolution of Sex* called an evolutionarily stable strategy. This means that the 'into' transition rates of protogyny and simultaneous hermaphroditism are higher than the 'out' rates, i.e., when a character is lost more slowly than it is gained it persists in a species and its descendants. Conversely, protandry is unstable, that is, it is evolutionarily lost faster than it is gained. Therefore, it has a short evolutionary lifespan. It is interesting to note that the evolutionary transitions among the different sexual systems have a sort of “fractal properties”, in the sense that they are, essentially, consistent no matter from how “far” or from how “close” one looks into the phylogeny. Thus, the sexual transitions found when exploring the Actinopterygians as a whole (Chapter 2) coincided with those found when focusing on specific families, e.g., Sparidae (Chapter 3), Serranidae, Muraenidae, etc. (Chapter 4). This means that, at a general level, transitions among sexual systems in teleost fish show the same predictions that Leonard (2013; 2018) suggested in Metazoans, where gonochorism and simultaneous hermaphroditism are endpoints while sequential hermaphroditism constitutes an intermediate state. Here, it was found that the transitions from sequential to simultaneous hermaphroditism can occur as observed in the families Muraenidae and Gobiidae but the reverse does not happen. In gastropods, it does not happen either (Collin, 2013). However, in the Serranidae family a transition from simultaneous hermaphroditism to gonochorism occurs in the genus *Paralabrax,* while from gonochorism to simultaneous hermaphroditism takes place without intermediate states in several families such as Aulopiformes and Rivulidae.

3. Aspects of evolutionary biology: Williams' Paradox and Bateman's principles

The research carried here touches upon two important principles of evolutionary biology: Williams’ paradox and Bateman’s principles.

By the 1970s, the prevailing theories on the distribution of sexual systems stated that such distribution was better explained by ecological factors (e.g., by the low encounter probability (Ghiselin, 1966; 1969). Later Williams (1975), challenging the theoretical models of that time, pointed out that what actually explains the distribution of the different sexual systems is phylogeny, not ecology. George Williams realized that sexual systems are conserved at high taxonomic levels. He reasoned that since the ecological forces that determined their origin would not be longer the same for millions of years and yet the sexual system remained stable, then phylogeny rather than ecology must be the better predictor (Williams, 1975).

Eric Charnov (Charnov, 1982) developed in his Sex Allocation Theory the mathematical model explaining when dioecy or simultaneous hermaphroditism is favored based on the fitness and reproductive success in male and female functions (more details in section 3.3.2 of the General Introduction) conditioned by the ecological factors and the theories of the 70s. Leonard (1900) defined the discrepancy between theory and actual data a paradox because she agrees with Williams (1975) in that the models that tried to explain the distribution of sexual systems with ecological factors do not really explain such a distribution. An example would be two closely related species living in very similar habitats but exhibiting with different sexual systems. However, once a sexual system evolves, it remains very stable evolutionarily. An example is the insects, which live in a multitude of habitats and thus under very different sorts of ecological conditions, yet they are almost exclusively dioecious (= gonochoristic). Therefore, the study of groups such as fishes, with great sexual diversity, can help clarify the adaptive advantages of the different types of sexual systems (Williams, 1975).

In fish, hermaphroditism is present in thirteen orders (Chapter 1), corresponding mostly with recent lineages, with the order Perciformes at the top of the podium in number of species. This indicates that hermaphroditism in fish is a relatively recent sexual system as a whole and a study focusing in several hermaphroditic families suggested that sexual systems evolved by exogenous factors (Erisman et al, 2009; 2013) in disagreement with Williams (1975). In this study, it was concluded that the families Sparidae, Gobiidae and Muraenidae, the sequential hermaphroditism is a labile sexual system that could be also influenced by ecologic factors. However, the distribution of simultaneous hermaphroditism is depending on phylogeny because once it appears it is maintained in all derived extant species of the same family or genus. This occurs in several families of the Aulopiformes and in the genera *Serranus*, *Dipectrum*, *Hypoplectrodes* of Serranidae. Previous theories not included sequential hermaphroditism system, but only compared gonochorism versus simultaneous hermaphroditism. That is why we consider that simultaneous hermaphroditism shows phylogenetic inertia, as Williams (1975) suggested. However, sequential hermaphroditism as a whole, which is an intermediate system between gonochorism and simultaneous hermaphroditism (Leonard, 2013; 2018), it could be explained by changes not only by abiotic factors but by mating systems as already seen in Epinephelidae (Erisman et al, 2009).

Regarding Bateman’s principles, in sexually reproducing animals with two sexes males produce large amounts of sperm with little effort and cost, while females invest much more energy to nourish a small number of eggs. Therefore, females have an important role in the selection of the male to mate with, while males must compete against other males to copulate successfully. Thus, females are the limiting factor regarding parental investment since male reproductive success (RS) is limited by the number of females with which males can mate, while female RS is limited by the number of eggs they can produce. Consequently, the sex with the greatest variance in RS is the sex with the greatest sexual selection (Bateman, 1948). Thus, Bateman's principles can be summarized that due to the smaller cost of producing sperm when compared to eggs: (i) male RS increases with mate number whereas female RS does not; (ii) males have greater variance in RS than females, and (iii) the sex with the greater variance in RS undergoes stronger sexual selection (Tang-Martinez, 2012).

Although the presence of sexual selection in sequential hermaphrodites has been recognized with the assumption that it is stronger on the most abundant sex (Leonard, 2006), Bateman's principles have not been formally tested. Here, through the study of the Sparidae family (Chapter 3), it was discovered that the small protandrous males have developed a strategy to increase their reproductive success, since they are usually pair-spawners with only one female. Therefore, their large gonad size is not related to sperm competition environments, but sexual selection is what presumably led males to develop a larger GSI than expected and thus be able to fertilize the eggs of a large female. In summary, one of the predictions of the size advantage model is that protandrous males should have a low GSI because little or no sperm competition. However, the unusually high GSI of these small-sized males can be regarded as a consequence of sexual selection —here is the integration of Bateman principles— acting directly on their capacity to produce larger amounts of sperm and thus enabling them to be able to fertilize the eggs of large females.

4. Applications of the results of this thesis

As it was mentioned elsewhere (Chapter 1), the research carried out can serve as a basis for future research on specific groups for different purposes, including ecological and evolutionary issues, as well as the conservation and management of exploited species. Below, these ideas are further developed and some examples are provided.

4.1. Applications to general knowledge and evolutionary biology

We studied how different abiotic factors and life-history traits correlated with the distribution of the different sexual systems present in fish, something that, to our knowledge, had never been attempted before or at least not attempted at the large scale as done here. This task was considered of great relevance to advance the field (Sadovy and Liu, 2008; Leonard, 2018).

Similar efforts on the evolution of sex have been carried out in plants (Bawa, 1981; Berrett, 2002). Regarding the influence of external factors, it was found that abiotic factors strongly constrain the distribution of species in plants (e.g., Dubuis et al., 2013) and that both abiotic and biotic interactions constrain individual species ranges and, thus, the spatial variation in species assemblages (Wisz et al., 2013). In invertebrates, particularly in mollusks, the evolution of sex was studied but comparative analyses of the associated ecological factors were not solid due to the scarce data in some groups (Collin, 2013).

In fish, data on reproductive and breeding behavior are also needed to corroborate some of the conclusions. First, in Sparidae (Chapter 3), data in a higher number of species would be needed on the mating system to confirm the prediction that protandry increases the gonadosomatic index by compensating for the small size of the male and not because they experience high sperm-competitive environments. Secondly, regarding the families where simultaneous hermaphroditism is present (Chapter 4), more information on mating systems would allow to confirm our hypothesis linking reduced body size with simultaneous hermaphroditism only under certain situations. Similarly, knowledge of the mating system and sex determining mechanism in *Paralabrax* would help to understand the transition between simultaneous hermaphroditism and gonochorism, first seen in fish. In general, for simultaneous hermaphrodites there is very little information about life-history traits such as longevity, maturity or mating systems in species that do not belong to the genus *Serranus*, which did not allow us to include them in the analyses carried out in chapters 2 and 4. On the other hand, another variable of interest that limited our efforts is body length in females. For this reason, the magnitude of the size-sexual dimorphism could not be incorporated as a covariate in the GSI analysis.

Sex change in sequential hermaphrodites involve profound organizational and functional transformations not only in the gonads but also in the areas of the brain involved in the control of reproduction. Sex change offers interesting opportunities to study the central control of alternate reproductive behaviors at many levels of organization (genomic, epigenetic, transcriptomic, etc.). In this regard, transcriptomic analysis in the brain has been carried out during the process of sex change in clownfish, *Amphiprion bicinctus*, identifying a large number of well-known and some novel candidate genes involved in the process (Casas et al 2016).

4.2. Applications to fisheries and aquaculture

In fish, knowledge on the biology and physiology of hermaphrodites is, in general, scarce, probably due to the low number of hermaphrodite species when compared to that of gonochoristic species. Nevertheless, there are hermaphrodite species that are of great economic importance in fisheries (e.g., *Epinephelus aeneus*, *Labrus bergylta*, *Pagellus bellottii*, etc.), aquaculture (e.g., gilthead seabream, *Sparus aurata*, blackspot seabream, *Pagellus bogaraveo*, white seabream, *Diplodus sargus*, etc.) and ornamental fish trade (e.g. *Amphiprion spp.* or *Gobiodon spp.*).

The attempt to domesticate hermaphrodite species has led to some complications in aquaculture due to the challenge of controlling sex ratios in species that have the natural tendency of changing sex as they grow. This was not a big problem before (actually, the aquaculture of species such as the gilthead seabream has developed well) but now with long-term genetic selection programs, where keeping certain fish with desired characteristics is required, presents a problem since due to sex change broodstock may not have the required sex ratio for optimal egg production (Grigorrksi et al. 2002, Flores et al 2019). This is one of the reasons why there is interest in sex control in aquaculture (Wang et al., 2019). This requires knowledge of the biology and physiology of the species as well as their reproductive behavior including the social factors that lead to sex change (Flores et al., 2019). Here, the situation in some of the species mentioned above (*Sparus aurata*, *Labrus bergylta* or *Amphiprion ocellatus*) will be discussed.

First, the gilthead sea bream, *Sparus aurata*, is an important aquaculture fish in the Mediterranean area in general, with a world production of 9,430 Tm from capture fisheries and 185,980 Tm from aquaculture, where Greece is the main European producers followed by Turkey and Spain (FAO, 2016). In the wild, this protandrous species forms small groups consisting of a large female, a small male and several immature males during the breeding season. Therefore, their spawning is of a monogamous system, so the accumulation of several mature males or females in the same tank can complicate their reproduction by inhibiting their spawning under normal conditions or reducing their gonadosomatic index (Grigorakis et al 2002).

The protogynous ballan wrasse, *Labrus bergylta* is widely distributed in temperate latitudes of the North Atlantic, from 64°N to 27°N, and a modest species for fisheries, with a production of 149 Tm in Norway and 11 Tm in Portugal in 2011 (Froese and Pauly, 2018). Due to climate change, temperatures can easily increase between 2 and 4ºC in some areas by the end of this century (IPCC, 2014). Thus, knowledge of abiotic correlations in protogynous species can help to forecast possible future distribution shifts.

Another example of protogyny in aquaculture are species of the genus *Epinephelus* for their high commercial and nutritional value (Froese and Pauly, 2018). The white grouper, *E. aeneus*, is of great economic importance for Mediterranean fisheries, with a catch production of 6,837 Tm in 2016 (FAO, 2016). The orange-spotted grouper, *E. coioides*, is distributed in Asia and has an aquaculture production of 317 Tm (FAO, 2016). Efforts have been made to develop the aquaculture of the dusky grouper, *E. marginatus*, in the Mediterranean (Roig et al., 2010). In fact, it was not until its reproduction was observed in the wild, with the courtship involving a upward movement of the breeding pair swimming around each other forming a double helix (Dantard et al 1997), that it was realized that reproducing this and related species would require the construction of breeding tanks of sufficient depth and diameter to facilitate their courtship and reproduction.

Ornamental (or aquarium) fish trade is very important and it moved more than US$ 347 million in 2014 worldwide (Dey, 2016). About 3.2 million people in the European Union have an aquarium at home, a figure that keeps increasing (Ghosh et al., 2014). An increasing number of aquarium fishes are saltwater. For example, many hermaphrodite gobies such as *Gobiodon histrio, G. okinawae* and *Cryptocentrus cinctus* are now popular aquarium fish. However, one of the most popular marine aquarium fishes is *Amphiprion* *ocellaris* (Pomacentridae) and *Centropyge bicolor* (Pomacanthidae), which inhabit the tropical Indo-West Pacific area and are commonly exported through the aquarium trade. Thus, knowledge on the sexual system, particularly sex change dynamics, appropriate population density and sex ratio can contribute to better health and reproductive performance of these fishes.

In summary, many hermaphrodite species are of great economic importance for fisheries, aquaculture and ornamental fish trade. Thus, efforts towards increasing the knowledge about their sexual systems and reproductive biology are justified not only for the obvious scientific interest, but also because they can hopefully find translation into better management and exploitation practices. The work presented here has been ultimately aimed at contributing to this goal.

Conclusions

1. Hermaphroditism is a sexual system that, in sharp contrast with other taxa where is present, is a rare condition in fish, the only group of vertebrates where it has evolved. The number of hermaphrodite species identified so far in fish has been quantified for the first time. This number, 552, roughly represents between one third and one fourth of the maximum number of estimated hermaphrodites, which can be between 1,500 and 2,000 species.

2. Hermaphroditism is a derived character that has evolved multiple times in fish, with sequential hermaphroditism predominating over simultaneous hermaphroditism. Within sequential hermaphroditism, protogyny predominates over protandry. Regarding George Williams' statement about whether the distribution of sexual systems is better explained by phylogeny than by ecological factors, analysis of the distribution of sexual systems in several families shows that sequential hermaphroditism seems to be more influenced by ecology rather than by phylogeny. In contrast, simultaneous hermaphroditism is best explained by the phylogeny given its conservation along lineages once it has evolved.

3. Gonochorism is the ancestral sexual state of the Actinopterygians, while hermaphroditism as a whole is an evolutionarily unstable derived condition. However, within hermaphroditism protogyny and simultaneous hermaphroditism are evolutionarily stable strategies, while protandry is unstable.

4. Based on the study of global evolutionary transitions between the different sexual systems in actinopterygian fishes, it can be concluded that sequential hermaphroditism is an intermediary state between gonochorism and simultaneous hermaphroditism, thus supporting Janet Leonard's hypothesis. This also holds for the families Muraenidae and Gobiidae. However, there are no sequential hermaphrodite intermediates in the transition from gonochorism to simultaneous hermaphroditism (Aulopiformes) and in the reversion back to gonochorism (Serranidae).

5. Protogynous species tend to live longer than their gonochoristic relatives. This is not due to the complex social systems typical of many protogynous species living in coral reefs that, theoretically, could favor from higher longevity, as initially surmised, since this is observed irrespective of habitat. Instead, higher longevity seems to be an inherent property of protogyny, probably driven by selection for higher fecundity associated with larger, and hence older, females to mate with even older males.

6. The family Sparidae remains a challenging one. Their ancestral sexual state is still not clear. In this particular family, protogyny and protandry are unstable conditions that rapidly revert to gonochorism, and transitions between them are almost non-existent.

7. In sparids, gonochoristic and protogynous species support the predictions of the size-advantage model but protandrous species do not, since they exhibit higher gonadosomatic index values than expected, even after accounting for the mating system. Small males of protandrous species may have a compensatory mechanism allowing them to invest disproportionally more in sperm production than predicted due to the need to fertilize highly fecund females, much larger than themselves. This mechanism, together with Bateman's principles in sequential hermaphrodites, should be formally incorporated in the size-advantage model.

8. In fish, simultaneous hermaphroditism can evolve from gonochorism and protogyny, but not from protandry, as determined from the study of the evolutionary transitions among sexual systems in the families where simultaneous hermaphroditism is present.

9. Body size reduction in simultaneous hermaphrodites only occurs in association with particular mating systems such as egg-trading in the Serranidae. We provide a framework for making predictions about the selective pressures that limit body size based on mating systems that can be tested in other taxa that contain species of simultaneous hermaphroditism.

10. These results contribute to our understanding of the distribution, biotic and abiotic correlates, and evolutionary transitions of the different sexual systems in fish. These results are of interest not only for ecology and evolutionary biology but also for applied biology since they can assist in the forecast of possible changes in the phenology of hermaphrodites and they could have translation into better management and exploitation practices in a context of global change.

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