INFLUENCE ENVIRONMENTAL FACTORS IN SEX TELEOST FISH DETERMINATION

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1- Objective: To explain which environmental factors can affect sex teleost fish determination and how, providing examples of these species.

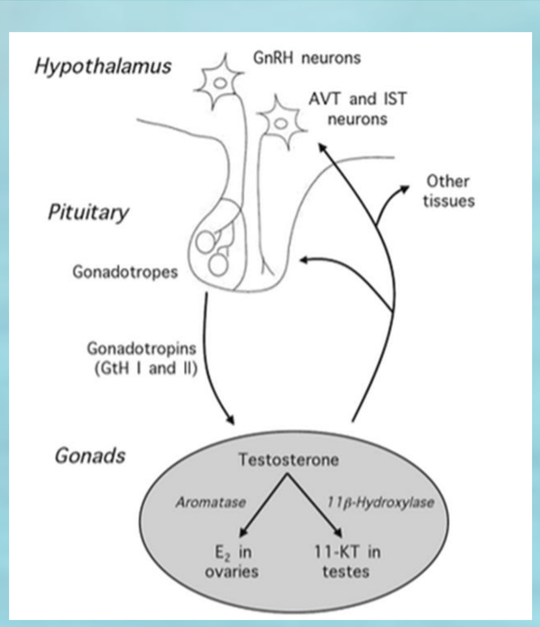

Fig.1 HPG axis

2- HPG axis

The Hypothalamic–pituitary–gonadal axis (HPG) (fig.1) is a set of endocrine glands working in cooperation in the following way:
1-Hypothalamus neurons secrete gonadotropin-releasing hormones (GnRH) according to internal and external signals.
2-GnRH regulates pituitary gland gonadotropin secretion.
3-Gonadotropin regulates gonadal development and functioning by modulation of steroid production and conversion.

3- Aromatase

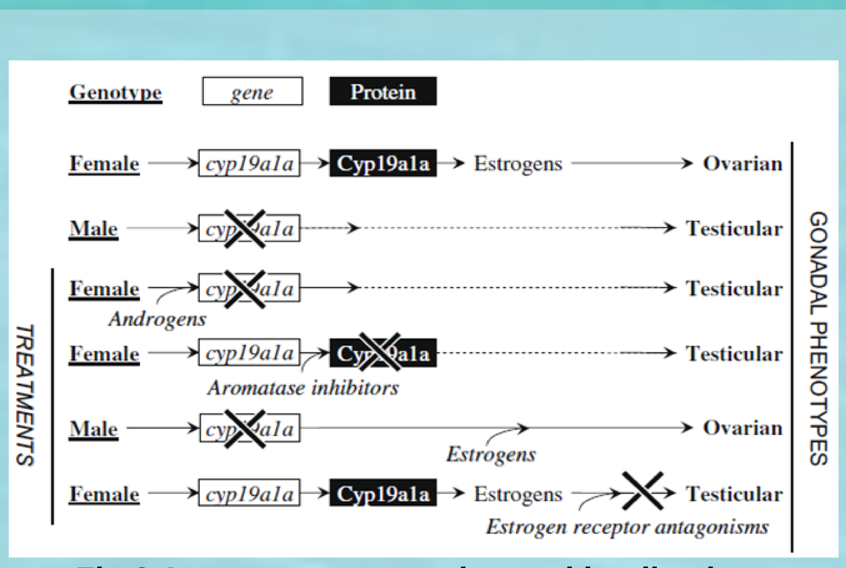

Fig.2 Aromatase expression and implication

Aromatase enzyme (*cyp19a1*) catalyzes the androgen irreversible conversion to estrogen. The ratio of androgens and estrogens determine the sexual phenotype of each individual.
Although aromatase gene is the most important and well-studied, other genes that contribute to fish sex differentiation and determination can be influenced by environmental factors. In general, ovarian differentiation correlates with increased expression of *cyp19a1, foxl2, fst* and *figa* while testicle differentiation is correlated with increased expression of *dmrt1, sox9, nr5a1* and *amh*.
Different factors can alter the normal sexual steroid hormone ratios (fig.2) causing differences between the sexual genotype and phenotype. Environmental factors can act as signals that induce epigenetic changes in these enzymes or in their gens, altering their production or activity.

The following scheme shows examples of species influenced on sex determination by environmental factors. The colored arrows indicate the resulting sex ratios towards males (blue) or females (red) with increased intensity of the environmental factors. (Increasing the factor intensity increases males proportion when it's red arrowed or females proportion when it's blue arrowed).

Temperature ⬆ ⬇⬆

Endocrine disruptors pollution (TBT, DDT)

Hypoxia

Density ⬆

Fig.3 Sea bass, *Dicentrarchus labrax*

Fig.4 Channel catfish, *Ictalurus punctatus*

Fig.5 Olive flounder , *Paralichthys olivaceus*

Fig.6 Medaka, *Oryzias latipes*

Fig.7 American eel, *Anguilla rostrata*

Fig.8 Paradise fish, *Macropodus opercularis*

4- Environmental factors

Primordial germ cells can interpret environmental signals and become spermatogonia or oogonia. It's in the early critical period of development when the gonad has the potential of taking any direction, according to gonadal plasticity. It's usually in this period when environmental factors influence the sex determination process in some species, called ESD (environmental sex determination).
Temperature is the main environmental factor in ESD fish, affecting more than 50 studied species. The most common effect is that as temperature rises, so does males proportion, as it occurs in sea bass (*Dicentrarchus labrax*) (fig.3). In this case, high temperatures inhibit aromatase expression due to its promoter methylation. The sex ratio variation can be significant. For example Nile tilapia, *Oreochromis niloticus*, has a 1:1 sex ratio in water at 27ºC, but at 36ºC the sex ratio become 3:1. However, temperature can also acts in the opposite way, with more males when it's low, such as in channel catfish (fig.4), or even when it's extreme, high or low, such as in *Paralichthys* genus flounders (fig.5). These flounders regulate aromatase quantity through the transcription factor foxl2, and extreme temperature cancels its expression

pH ⬇

Social factors (♂ → ♀, ♀ → ♂)

Fig.9 Red and yellow operculum males of purple cichlid, *Pelvicachromis pulcher*

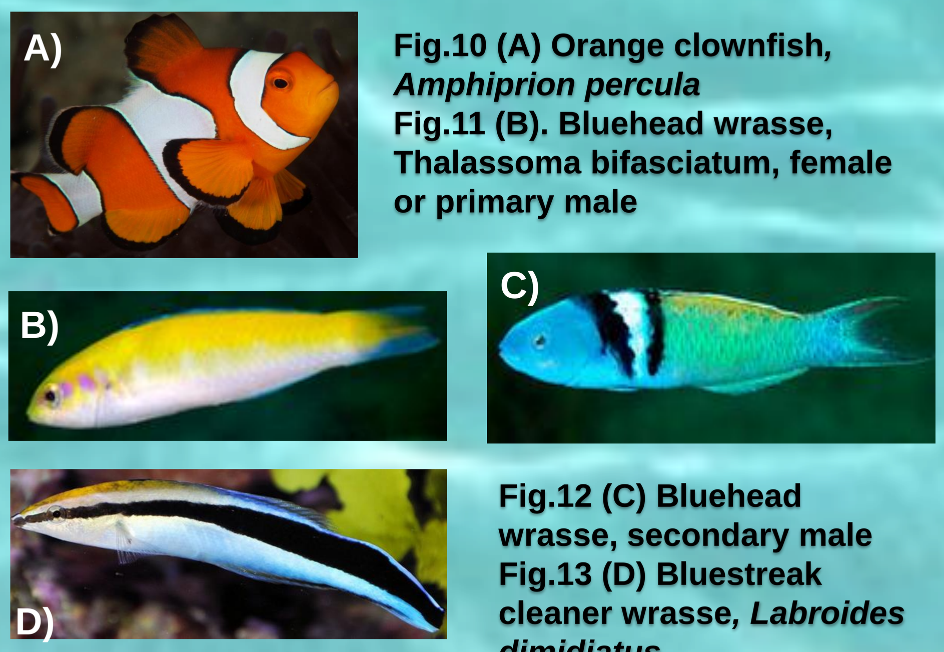

Fig.10 (A) Orange clownfish, *Amphiprion percula*
Fig.11 (B). Bluehead wrasse, Thalassoma bifasciatum, female or primary male
Fig.12 (C) Bluehead wrasse, secondary male
Fig.13 (D) Bluestreak cleaner wrasse, *Labroides dimidiatus*

There are other environmental factors influencing sex determination such as hypoxia, density, pH and social factors. There are also pollutants which may cause chemistry interferences on sex differentiation physiological processes. Endocrine disruptors are the most common and can be found in pesticides, synthetic compounds and some metals. They attach with hormone receptors causing the same signal or blocking it. For example, TBT (tributyltin) can block aromatase expression (fig.5) or DDT (dichlorodiphenyltrichloroethane) can act as estrogen (fig.6). Under hypoxia conditions (0.8 mg/L), ¾ of medakas (fig.6) with female genotype obtain male phenotype. Studies with zebra fish, *Danio rerio*, concluded that in female genotype individuals undergoing these conditions testosterone levels are increased while estradiol ones are decreased due to some sexual genes such as aromatase are not expressed.

A high density of individuals generates stressful conditions and consequently cortisol secretion is activated. Cortisol is a steroid hormone, and changes in its levels can cause masculinization or feminization in some species, such as American eel (fig.7) and paradise fish (fig.8).
PH has influence on some species of two families, *Poecilidae* and *Cichidae*, causing more males under acid conditions. The most affected species are freshwater ones due to in this environment pH variations occur easily. A curious example is the purple cichlid fish. The males exhibit two different morphs with different color of the operculum: yellow operculum males (YO) and red operculum males (RO). The former is monogamous and satellite of RO. However, RO are polygamist, more aggressive and territorial. Low pH increases male proportion and also RO number, affecting male behavior. It could be because of YO needing certain levels of aromatase.

In many hermaphrodite species of coral reefs the fish can change their sex depending on the presence or absence of socially dominant individuals. In some species, fertility increases with body size, and their sex change is favored by size increase which represents greater reproductive success. The orange clownfish (fig.10) society is formed by one dominant female, one reproductive male and sterile males. Females are the biggest and the reproductive male is bigger than the sterile males. When the female dies, the reproductive male becomes a female and the biggest of sterile males becomes a reproductive male, increasing in size. The females and primary males of bluehead wrasse (fig.11) have the potential to become secondary male (fig.12), which are bigger and with different color, if it isn't present. In some species the sex change can be bidirectional, such as bluestreak cleaner wrasse (fig.13). A big dominant male has a harem. In the absence of a dominant male, the biggest female becomes male. When the dominant male does not have a harem it can become female, increasing its reproductive success.

5- Conclusion

- Specially during the early critical period of development an environmental factor can affect some steroid enzyme genes expression and disarrange HPG axis in some species, called ESD.
- Aromatase is the most important steroid enzyme and it catalyzes the androgen irreversible conversion to estrogen. The proportion of steroid hormones usually decides sex phenotype.
- Temperature is the most influence factor. The most common is that as temperature rises male proportion is increased. With low oxygen or pH levels, male proportion also increases in some species. However high individuals density can change the proportion of sex phenotype, depending on the effect of stress cortisol secretion on each specie.
- Some pollutants can be influenced on sex determination, specially endocrine disruptors.
- The presence or absence of socially dominant individual can lead to sex change in many hermaphrodite species from coral reefs, usually getting larger.
- Knowing how these factors affect fish is important for the ecosystem and their conservation. It also generates benefits in marketable fish production due to lot of them have size sexual dimorphism.