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Histological structure of the gonads of hybrids of *Solea Aegyptiaca* and *Solea Senegalensis*: signs of fertility.

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Abstract
The histological study of male and female gonads, collected from the Bizerta lagoon (Northern Tunisia) where *S. aegyptiaca* and *S. senegalensis* are hybridizing, showed that all of the individuals (males and females) have functional histological structures. This could be interesting for aquaculture specialists who are trying to select and raise well-defined characteristics and raise less problems from a conservation point of view as this hybridization phenomenon is natural.

Keywords: Hybrid zone, *Solea senegalensis, Solea aegyptiaca, gonads*, histology, hybrid fertility.

1. Introduction
Hybrid zones are defined as areas where genetically distinct populations overlap, mate and produce offspring (Barton & Hewitt, 1985) [5]. Many of these contemporary hybrid zones are due to secondary contact zones (see Hewitt, 2001) [6]. Interspecific hybridization can, in theory, produce viable offspring that is either sterile, partially fertile or even fully fertile (Arnold, 1997) [3].
For interspecific fish hybrids, a total of 130 natural crosses between different species resulted in fertile F1 hybrids [2]. When hybrids with interesting intrinsic characters are fertile, it is possible to obtain several types of hybrids, such as F2 hybrids or triploids. Natural hybridization of fish may be perilous to the maintenance of genetic purity of natural fish populations (Legendre *et al*. 1992).
In addition to scientific interest, fish interspecific hybrids can be used in aquaculture to increase growth rates, transfer desirable traits and characters between species, increase tolerance towards the fluctuations of the environment elements and rise the overall robustness of individuals to the culture conditions (Bartley *et al*., 2000).
This utility for aquaculture is even more interesting in the case of hybrids between species like *Solea aegyptiaca* and *Solea senegalensis*, since these species are naturally predisposed to hybridize naturally without human control (nor artificial induction or initiation etc.) This means, implicitly, that this process is less problematic in terms of conservation and preservation of biodiversity. Moreover, some Mediterranean countries have already begun the cultivation of species of *Solea* especially along the southern coasts of Spain and Portugal (Drake, 1984 [9]; Rodriguez, 1984 [23]; Dinis, 1992) [8].
Assessing if hybrids are fertile or sterile is, thus, important. Abnormal gonadal development was already reported in hybrids of some species of fish while reaching sexual maturity (e.g. the case of Cyprinids *Notemigonus crysoleucas* x *Scardinius erythrophthalmus*) (Goodwin *et al*. 1994 [13]; Fishelson *et al*. 1996 [12]; Kopiejewska *et al*., 2003) [17] or while producing post-F1 generations (Legendre *et al*., 1992).
For all these reasons, and among the few marine hybrid zones that have been reported, we were interested in a reanalysis of the hybrid zone between *Solea aegyptiaca* and *Solea senegalensis* that was initially reported in 1987 by She *et al*., in the northern coast of Tunisia. Both species have similar life cycles (Quéro *et al*., 1986 [22]), are gonochoristic and their breeding seasons occur at the same time (the period from May to July) (Goucha, 1982 [14]).
The development, maturation of the gonads and fertility in hybrids between *S. senegalensis* and *S. aegyptiaca* has not been studied to date.

After genetically analysing this hybrid zone [21], our main endeavour during this work was to assess the fertility of hybrids through the analysis of the overall appearance of the histological organization of the gonads and the developing of male and female mature germ cells of gonads collected from hybrid individuals of the hybrid zone in the lagoon of Bizerta (Northern Tunisia). The genetic study confirmed the hybridization demonstrated previously by She *et al*. (1987) [24], report the existence of introgressed individuals of *S. aegyptiaca* previously undetected in the lagoon of Bizerte and highlight clines of introgression to the south and north of the lagoon, indicating the existence of a unimodal hybrid zone. The rate of introgression rate (36.4% vs 16% previously reported) found in the Bizerte Lagoon shows that the main area where hybridization occurs, but also indicates that genetic exchange between these two species have not stabilized and are still evolving.

2. Materials and Methods

2.1 Sampling

More than one hundred mature individuals were collected from the lagoon of Bizerta (Fig. 1 and Table 1) during the breeding seasons of both species (May-July) and over a period of two years (2005-2007).

The samples were classified according to their morphological aspects (i.e. *S. aegyptiaca* and *S. senegalensis*) and according to their gender. Freshly collected gonads were carefully preserved to be immediately dissected and analysed both macroscopically and microscopically for sex determination.

Only mature and genetically determined hybrid individuals (identified with the results of the genetic study based on EPIC-PCR and published in Ouanes *et al*., (2011) [21] and using allozymes and EPICs) were considered.

Table 1: Effective, morphology and sex of the studied individuals.

	Morphologically *S. aegyptiaca*		**Morphologically *S. senegalensis***	
Sex	Males	Females	Males	Females
Effective	23	26	19	24
Total	49		43	

Fig 1: The Bizerta lagoon: The site of hybridisation between *Solea aegyptiaca* and *Solea senegalensis*

2.2 Histology

Histological examination of gonads was performed. Gonads, collected at sexual maturity during the breeding season from hybrid individuals were fixed by Dubosq-Brasil alcohol.

Before incorporating them in paraffin, the fragments are fixed and dehydrated successively in alcohol baths of 70° and 95° then Butanol (Martoja & Martoja, 1967) [20]. They are then transferred to a balanced mixture of butanol and paraffin, and finally plunged in baths of paraffin to completely eliminate the intermediate solvents.

After impregnation with paraffin, the pieces are put into paraffin blocks in cubic molds. The blocks thus made are cut into a series of slices of 5 to 7 microns thick using a microtome. The cuts, were unfolded and spread, then glued on microscope slides by the albuminous water.

After dewaxing and removal of paraffin with two Toluene baths and moisturizing by successive baths of alcohol (decreasing degrees: 100 °C, 95 °C and 70 °C), the slides were rinsed with tap water.

As a staining technique, we used hematoxylin - eosin. The sections were dried and stained, staying in two baths of alcohol at 100 °C and then in toluene.

The slides are then dried in an oven set at 40 °C. Preparations are well prepared for microscopic observation. The sections representing different stages of maturation are photographed for onscreen analysis.

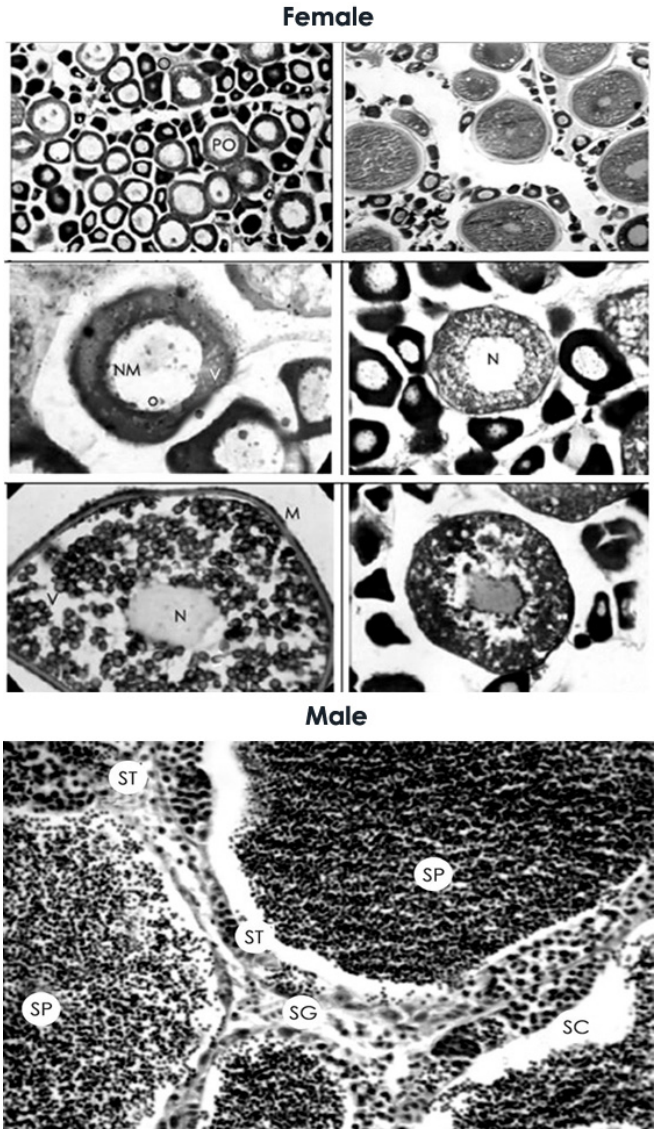

Fig 2: Histological observation of mature female (top) and mature male gonads (bottom).

3. Results

All of the 92 studied individuals had functional mature gonads

presenting all of the gametogenesis phases. No anomalies, deformations, tumours or necroses of the gonads were observed. We can confirm thanks to the obtained results that the gonadal structure and organisation is normal making the formation of normal gametes possible and therefore no potentially sterile individuals exist. Gonochorism was observed for every individual of the whole sample.

For females, Ovaries had filiform shapes and were located in the abdominal cavity. We were able to observe previtellogenic oocytes, oocytes at early vacuolisation, oocyte at early exogenous vitellogenesis, oocyte after complete exogenous vitellogenesis, oocyte at micropyle stage (Fig. 1).

For males, the bean-like testes are located in the anterior portion of the abdominal cavity The testis showed intense vascularization. A large amount of spermatozoa and a few spermatids were recorded in the lumina of seminal lobules and sperm duct. Spermatogonial divisions, germinal cysts containing spermatogonia and spermatocytes and further spermatogenesis were found in the seminal lobules (fig. 2).

4. Discussion

The histological sections showed that individuals in our sample are all fertile. Indeed, the specimens studied had matured, developed and functional gonads. These results showed structures and organizations of gonads similar to those of *S. senegalensis* studied by Fehri-Bedoui in Tunisia (1997) [10] during a study accompanying attempted breeding of the species.

It is widely accepted that natural hybridization is more common in fish than in other vertebrates (Campton, 1987; Smith, 1992). Moreover, cases of fertile interspecific hybrids between genetically different fish species have also been reported (Ferguson *et al*. 1985 [11]; Wood and Jordan 1987 [26]; Argue & Dunham, 1999 [2]; Kopiejewska, 2004) [18].

Once hybridization has begun, it is difficult to stop, especially if the hybrids are fertile and can breed with each other and with individuals of the parental species (Allendorf *et al*., 2001 [1]). This seems to be confirmed by molecular markers and allozymes used in our study to re-evaluate this hybrid zone [21]. Thus, viable and fertile hybrids that breed among themselves or with one or both parental taxa would allow the exchange of genes between existing groups and could contribute to the emergence of new recombinant taxa [15].

5. Conclusion

These results could be interesting for aquaculture specialists who are trying to select and raise well-defined characteristics in farmed fish. The hybridization in this case is a tool for improving aquaculture production. However, this requires knowledge of the genetic characteristics of the broodstock, a good broodstock management and the monitoring of the viability and fertility of the offspring.

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