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# Evidence of a high percentage of intersex in the Mediterranean swordfish (*Xiphias gladius* L.)

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**Abstract**

The first evidence of the presence of intersexuality in a wild population of Mediterranean swordfish (*Xhiphias gladius* L.) is reported. Forty of 162 specimens (25%) microscopically classified as males, showed the presence of female germ cells within the testes. In two specimens grouped previtellogenic oocytes were present; all the other specimens possessed single scattered previtellogenic oocytes. The presence of vitellogenin was demonstrated immunohistochemically in the liver of both intersex and normal males. These findings could be due to the exposure to oestrogen-mimicking substances.


*Keywords:* Intersex; *Xiphias gladius* (swordfish); Endocrine disruption; Pollution effects; Mediterranean sea; Vitellogenin

Exposure to natural and synthetic hormones (estrogens and androgens) or aromatase inhibitors can affect sexual differentiation in gonochoristic species and induce sex reversal and/or intersex (Piferrer and Donaldson, 1992; Blázquez et al., 1995). In addition, man-made chemicals, including organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, surfactants and plasticisers, present in the environment can mimic endogenous hormones, disrupt reproductive functions and cause developmental abnormalities (such as intersexes) in wild animal populations (Colborn and Clement, 1992; Sumpter et al., 1996). Endocrine disruption caused by environmental pollution has been documented or hypothesised in fish (Howell et al., 1980; Gimeno et al., 1996; Nolan et al., 2001), molluscs (Bauer et al., 1995; Svavarsson, 2000), piscivorous birds (Fry and Toone, 1981), alligators (Guillette et al., 1994), and polar bears (Wiig et al., 1998).

Recently, high plasma levels of vitellogenin (Vtg), a popular biomarker for measuring exposure of oviparous animals to oestrogen or oestrogen mimics (Kime et al., 1999), have been reported in three wild adult male Mediterranean swordfish taken from the Straits of Messina (Fossi et al., 2001).

For this study, testis and liver samples from a total of 162 swordfish were collected on board commercial vessels operating in the Gulf of Taranto (North Ionian Sea) ($n = 121$) and in the Western Mediterranean (Spanish seas) ($n = 41$). The fish were captured, between April and December, during the fishing seasons 2000 and 2001. Soon after capture, testes and liver were removed and processed. All the specimens were measured to the nearest cm in lower jaw fork length (LJFL). LJFL of sampled fish ranged from 71 to 202 cm.

Fragments from testis and liver were cut and fixed in Bouin's solution for 4–6 h. The samples were dehydrated in increasing ethanol concentrations and embedded in paraffin wax. Sections (5 μm thick) were processed for haematoxylin–eosin or immunohistochemical staining.

The immunohistochemical detection of Vtg was performed on testis, intersex gonad and liver sections using rabbit anti-swordfish Vtg serum (abSwo-Vtg) obtained by Eicker (2001) using the methodology of Susca et al. (2001).

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Histologically, the testis was constituted by seminipherous tubules, interspersed in the connective stroma, radiating from the longitudinal main sperm duct toward the testicular periphery. The wall of the seminipherous tubules was formed by cysts consisting of synchronously developing germ cells enveloped in Sertoli cell processes (Fig. 1A). Spermatogonia, with diameter $9.49 \pm 0.54$ (SEM) μm, had a roundish nucleus with an eccentric nucleolus and a high nucleo-cytoplasmic ratio. Primary spermatocytes and secondary spermatocytes measured $6.17 \pm 0.27$ and $4.40 \pm 0.25$ μm respectively. Spermatids ($2.61 \pm 0.13$ μm in diameter) were characterized by a darkly stained nucleus. Mature spermatozoa were flagellated cells, which had been released into the tubule lumen after the breakdown of cysts.

The testes of 40 of 162 specimens (25%) revealed the presence of oocytes. The percentage of intersex was different in the two areas investigated. In the North Ionian Sea 33 of 121 specimens (27%) were intersex; while in the Western Mediterranean 7 of 41 (17%) were intersex.

All the oocytes observed were at the perinucleolar classification stage of Arocha (2002) classification (Figs. 1B and C, and 2A and B) and their diameter ranged from 20 to 105 μm.

Thirty-eight specimens showed a few isolated oocytes, which were sparsely scattered among the seminipherous tubules (Fig. 1B), while they were more frequently found within the lumen of tubules (Fig. 1C). Occasional round cells larger than spermatogonia ($16.25 \pm 0.68$ μm vs $9.49 \pm 0.54$ μm) with a vesicular nucleus containing an eccentric nucleolus and a basophilic cytoplasm (presumptively oogonia) were seen along the wall of seminipherous tubules as well as in interstitial tissue (Fig. 1D).

Only two specimens contained testes with oocytes grouped in the interstitial tissue (Fig. 2A). A few of these oocytes showed degenerative features (probably re-

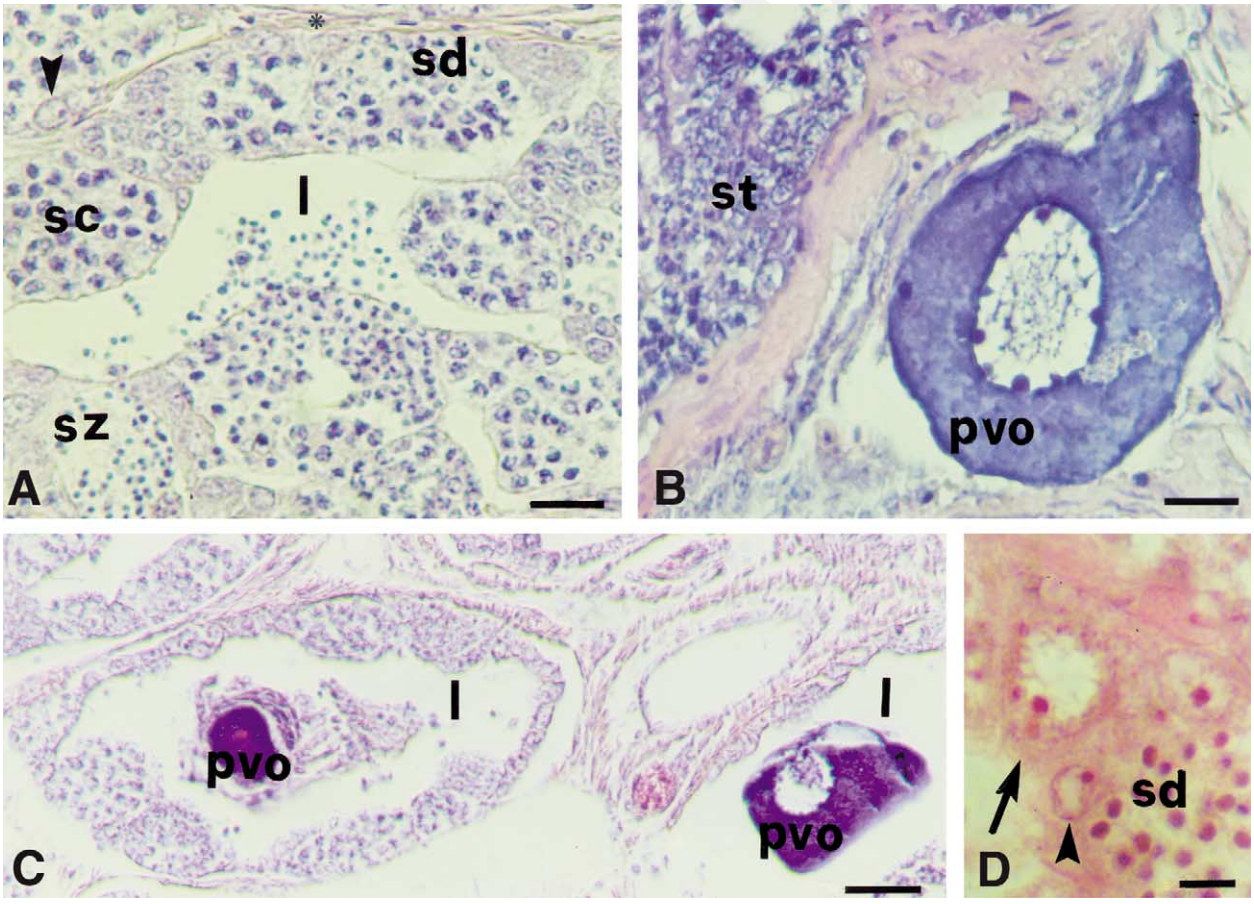


Fig. 1. (A) Testis of a maturing swordfish showing the normal testicular organization. The wall of seminipherous tubules is formed by cysts consisting in synchronously developing germ cells enveloped in Sertoli cell processes. Magnification bar = 15 μm. (B) Intersex gonad with an isolated interstitial previtellogenic oocyte. Magnification bar = 15 μm. (C) Intersex gonad with two isolated previtellogenic oocytes in the lumen of seminipherous tubules. Magnification bar = 35 μm. (D) Intersex gonad showing the presence of a putative oogonium, with a vesicular nucleus containing an eccentric nucleolus and a basophilic cytoplasm, along the wall of a seminipherous tubule. Magnification bar = 5 μm. Haematoxylin–eosin staining. Arrow: putative oogonium; arrowhead: spermatogonium; asterisk: connective stroma; l: lumen of seminipherous tubule; pvo: previtellogenic oocyte; sc: spermatocyte cyst; sd: spermatid cyst; st: seminipherous tubule; sz: sperm cyst.

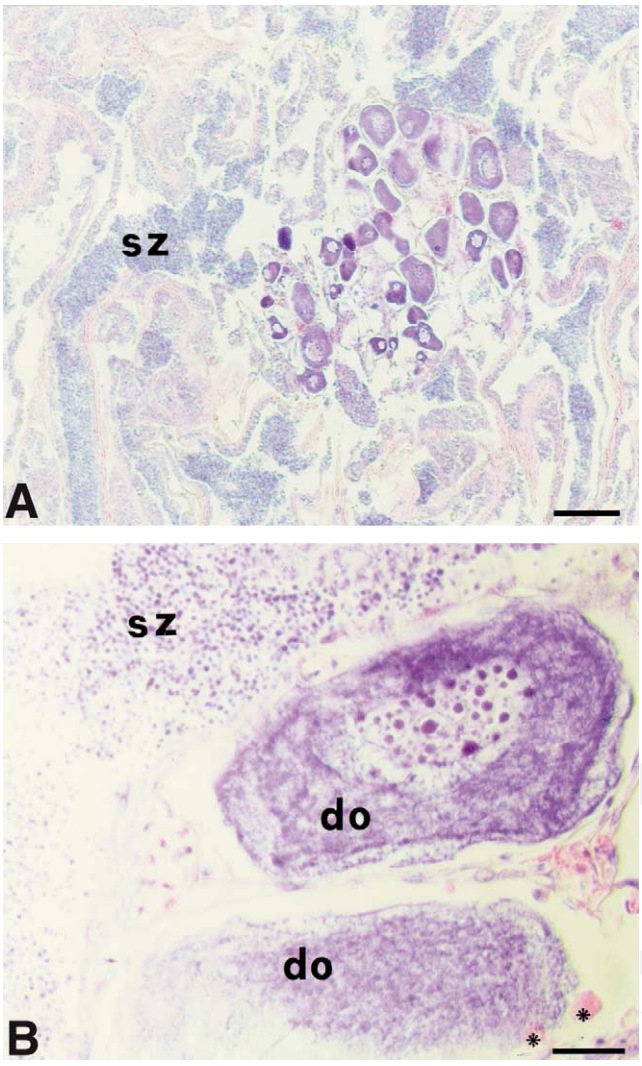


Fig. 2. (A) Intersex gonad with previtellogenic oocytes grouped in the interstitial tissue. Magnification bar = 150 μm. (B) Intersex gonad showing two degenerating oocytes. Cells containing eosinophilic granules can be observed at the periphery and inside a degenerating oocyte. Magnification bar = 20 μm. Haematoxylin–eosin staining. Asterisk: cell containing eosinophilic granules; do: degenerating oocyte; sz: luminal spermatozoa.

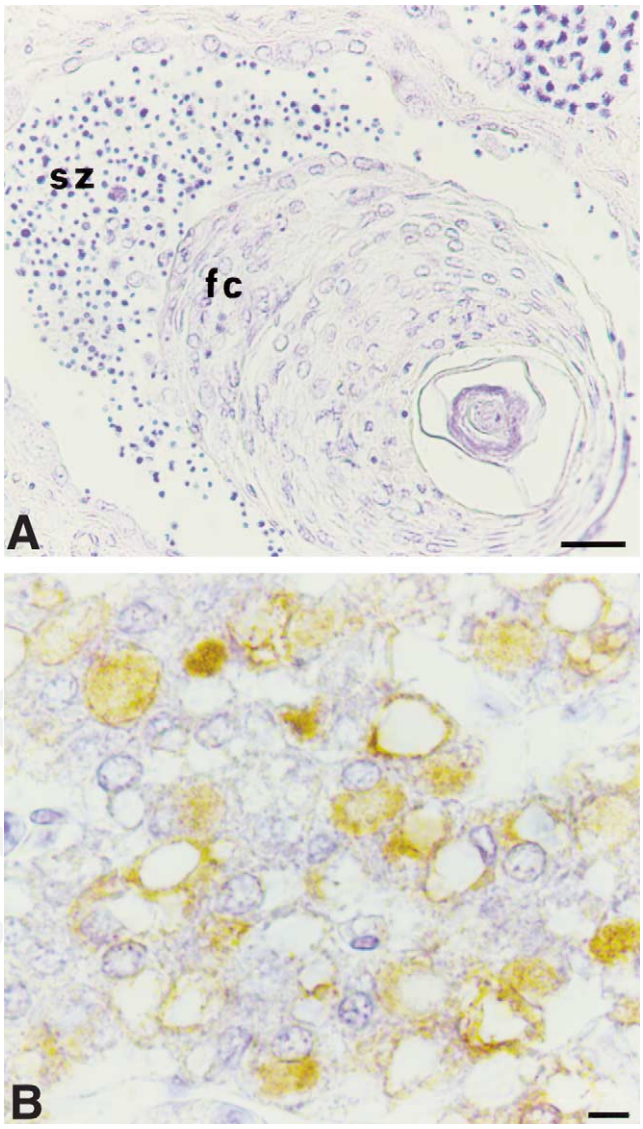


Fig. 3. (A) Encystation made up of concentric layers of fibroblast-like cells containing basophilic material in an intersex gonad. Magnification bar = 15 μm. (B) Section of the liver from a male swordfish immunostained with abSwo-VTG showing abundance of positive hepatocytes. The immunoreactive cells are charectarised by the presence of a lipid droplet observed in sections as a vacuole. Magnification bar = 5 μm. Haematoxylin–eosin staining. fc: fibroblast-like cells; sz: spermatozoa.

sorption) and were surrounded and/or infiltrated by cells containing eosinophilic granules (Fig. 2B). This phenomenon has already been associated with sex change in hermaphrodite teleosts (Besseau and Faliex, 1989; Kokokiris et al., 1999).

The general structure of the testes containing oocytes was not damaged except at the site where these abnormal cells were found, so that the functionality of the testes did not seem to be impaired: cysts at all the stages of spermatogenesis and luminal spermatozoa were as in normal testes. In addition, $I_G$ (gonadosomatic index) values of intersex fish did not show significant differences when compared with those from normal males having similar size and captured during the same period (data not shown).

Encystations, made up of concentric layers of fibroblast-like cells containing basophilic material, were present in some intersex gonads and normal testes (Fig. 3A). Nolan et al. (2001) reported similar findings in intersex roach and hypothesised them to be oocytes undergoing atresia or resorption.

AbSwo-Vtg did not label any germinal cell in either normal or intersex testes. AbSwo-Vtg positive cells were found in the liver of both normal male and intersex fish without apparent difference in their distribution, localization, and density. The immunoreactive hepatocytes

were characterised by the presence of a lipid droplet observed in the sections as a vacuole (Fig. 3B).

The presence of specimens with intersex gonads is well documented in wild population of gonochorist teleosts (Allen et al., 1999; Nolan et al., 2001; Viganò et al., 2001) and in reptiles (Guillette et al., 1994) living in polluted environments.

The high percentage of intersex together with the Vtg liver production of males and intersex, shown in the present study, suggest that Mediterranean swordfish could be exposed to estrogen-mimicking substances and may therefore increase concern regarding the damage caused by accumulation of toxic compounds in top predator organisms living in the Mediterranean. Such effects may be particularly important to the future of commercial fisheries of already overfished populations if they lead to decreased fertility as recently demonstrated for intersex roach by Jobling et al. (in press). In addition, it opens the question about the risk to human health by the consumption of fish containing bio-accumulated endocrine disrupters.

Further work on a global scale is now necessary in order to understand if the observed phenomena are just limited to the Mediterranean Sea or are widespread in other geographical areas.

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