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Abnormal gonochoristic inter-sex in Silver hake (*Merluccius bilinearis*) from Div. 3N

by

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Abstract

Silver hake (*Merluccius bilinearis*) catches in NAFO Divisions 3Ps, 3O, and southern 3N have increased markedly since 2018, likely linked to ecosystem changes associated with ocean warming. To validate the macroscopic maturity ogive of this species, gonad samples were collected during Spanish scientific surveys conducted in Divisions 3NO in 2024 and 2025. Histological analyses were performed on 94 individuals ranging from 24 to 57 cm total length. Results revealed 22 males, 54 females, and 18 intersex individuals, representing a notably high intersex prevalence of 19%. Intersex prevalence was slightly higher in 2024 (21%) than in 2025 (16%). Three morphological intersex types were identified, differing in the distribution and maturity of male and female tissues within the gonads. Most intersex specimens were smaller than 38 cm. All sampled individuals were mature, preventing estimation of a microscopic maturity ogive. Previous reports of intersex in the genus Merluccius are extremely rare, making this the first documented case of such high prevalence. Potential causes may include environmental stressors, endocrine-disrupting contaminants, and climate-related changes. Future coordinated sampling and contaminant analyses are planned to investigate the underlying drivers and assess population-wide prevalence.

Introduction

Silver hake (*Merluccius bilinearis*) has been caught sporadically in scientific surveys and as by-catch in different commercial fisheries in Divisions 3Ps, 3O and in the southern part of Division 3N. Since 2018, catches of silver hake have been increasing (7686t in 2024), probably due to ecosystem changes linked to ocean warming (NAFO, 2026).
Furthermore, since 2019 there is a directed fishery for this species mainly in NAFO Regulatory Area (NRA) Div. 3O and to a lesser extent in the southern part of NRA Div. 3N (González-Costas et al., 2026).

In order to validate the macroscopic maturity ogive, ovary samples were collected during the Spanish scientific surveys delivered by the IEO in the Div. 3NO in 2024 and 2025.
The objective of this document is to present the results of the histological examination of gonads of silver hake from Div. 3NO.

Silver hake occur along the northwest Atlantic continental shelf from North Carolina to Newfoundland, with highest abundance between Nova Scotia and New Jersey. They usually inhabit depths of 50–400 metres, but may occur as deep as 900 metres. Despite migrations inshore during warmer months have been described in some areas (Frimodt, 1995; DFO, 2016), there is no evidence of inshore-offshore movement in Div. 3NO.

Studies of stock structure identify two U.S. stocks: a northern stock in the Gulf of Maine and northern Georges Bank, and a southern stock in southern Georges Bank and the mid-Atlantic Bight. A separate stock occupies the Scotian Shelf in Canadian NAFO areas 4VWX with likely certain leve lof mixing among the three populations.

Silver hake grow relatively quickly, reach sexual maturity at 2–3 years of age (20–30 cm), and can live up to 15 years, although fish older than six years are now rarely observed (Brodziak et al. 2001). Females grow faster, live longer, and attain larger sizes than males, reaching up to 60–64 cm and 12 years of age, compared with 34 cm and 6 years for males (Bigelow and Schroeder 1953; Nichy 1969).

Studies from the DFO summer 4VWX survey estimated 50% maturity at 20 cm for males and 21 cm for females during 2010–2012, lower than estimates from the 1970s (Stone et al. 2013).

Female silver hake are asynchronous spawners, releasing several egg batches during the spawning season. In Div. 4VWX, spawning occurs from July to September on the western Scotian Shelf, particularly around Emerald, Western, and Sable Island banks.

Material and Methods

Length and sex stratified sampling of silver hake was carried out during the Spanish scientific surveys delivered in Div. 3NO in Spring/Summer of 2024 and 2025. From each individual total length, total weight, sex and macroscopic maturity stage were recorded based on ICES Workshop on sexual maturity Staging of Hake and Monk (ICES, 2007 Annex 1), and *sagitta* otoliths were removed for growth analysis. Additionally, from a sub-sample of all these individuals, gonads were removed and preserved in 3.6% buffered formaldehyde solution for histological analysis.

A transversal slice from the central area of each gonad was embedded in paraffin, cut into 3 µm sections and stained with haematoxylin and eosin. All ovarian sections were examined with a Leica DM4000B light microscope with a Leica DFC420 camera incorporated and the LAS V3.1 software (Leica 2008). Microscopic maturity stages were assigned based on Lowerre-Barbieri scale (Lowerre-Barbieri et al., 2023, Annex 2).

Results

Gonads were collected from a total of 94 individuals between 24 and 57 cm of total length, from which 56 were collected in 2024 and 38 in 2025. Microscopic analysis of gonads revealed that, from the 94 analyzed individuals, 22 were males, 54 females and 18 intersex specimens, that means a sex proportion in the sample of 0.4:1 and a prevalence of intersex equals to 19% (Table 1). By year, intersex represented 21% from samples analysed in 2024 and 16% from those analyzed in 2025. From 31 specimens classified as males macroscopically, 9 of them showed also ovarian tissue presence (29%) and from 63 macroscopically classified as females, 9 present also testicular tissue (14%).

No males larger than 35 cm were collected while females' length range was between 20 and 57 cm. Most of intersex were smaller than 38 cm of total length, although one intersex of 42 cm was reported (Fig. 1).

According to the distribution of the male and female tissue in the gonad, three different morphological types of intersex were identified. The type I corresponded to gonads with the common morphology of testis but with dispersed oocytes (mature and immature) within the gonad tissue (Fig. 2A, 2B), the type II were morphologically ovaries with male gonadal tissue adjacent to the ovarian wall (Fig. 2C, 2D) and the type III were morphologically ovaries with mature oocytes and immature testicular tissue inside the gonad (Fig. 2E, 2F). In type II intersex, ovaries and testis were mature, i.e. functional sexes, while in type III ones only female tissue was mature and supposedly functional. In Type I intersex, it remains unclear whether both sexes are functionally active. In this case, the male tissue appears clearly mature and functional. However, although the oocytes scattered throughout the testicular tissue are morphologically mature, they are very scarce, preventing us from determining with certainty whether they will eventually be reabsorbed through atresia or continue their development.

All sampled individuals were mature, subsequently, microscopic maturity ogive could not be estimated.

Discussion

Only two cases of intersex in the genus *Merluccius* have been published before this report, specifically, they corresponded to one specimen of *M. productus* over 12000 individuals sampled during a routine biological examination (Millikan and Pattie, 1970) and another one individual of *M. merluccius* over 831 found in the SW Mediterranean Sea (Belhoucine et al., 2012). In the first case, the specimen presented a normal right ovary and an ovotestis in the left lobe, whereas the second one presented two testes and two contiguous mature ovaries.

Gonochoristic intersex (mixed male–female gonadal tissue in normally fixed-sex species) is increasingly reported in wild fish, but most mechanistic work is from rivers, estuaries, and coastal zones, as these ecosystems are typically subject to greater environmental stress in terms of pollution, temperature, salinity, and other factors. Nevertheless, evidence still helps identify likely drivers relevant to open ocean ecosystems. Despite some reviews emphasize that some baseline intersex occurs even at reference sites, suggesting natural anomalies (Bahamonde et al., 2013), stress has been reported to be one of the main causes underlying the occurrence of intersex individuals. Chemical stressors like Endocrine-Disrupting Chemicals (EDCs) has been extensily studied (Lee Pow et al., 2017; Carnevali et al., 2018; Grieshaber et al., 2018); Ramasre et al., 2024). However, although not linked to classic intersex, non-chemical stressors (temperature, hypoxia, salinity changes, crowding) can shift sex differentiation and skew sex ratios, usually toward masculinization as they interact with genetic and endocrine pathways (Devlin and Nagahama, 2002);(Yu et al., 2023); (Kitano et al., 2024). Climate-change factors (mainly warming, but also acidification and hypoxia) can modulate estrogenic effects and promote sex changes, but field evidence offshore is limited (Baroiller et al., 2009; Geffroy and Wedekind, 2020).

For truly open-ocean fish, direct evidence is sparse. Evidence accumulated over the past two decades indicates that fish inhabiting areas surrounding offshore oil and gas platforms may experience endocrine disruption associated with exposure to produced water and petroleum-derived contaminants. Produced water discharges contain complex mixtures of polycyclic aromatic hydrocarbons (PAHs), alkylphenols, and other compounds with documented estrogenic activity, capable of altering endocrine signaling and reproductive physiology in marine fish ((Boitsov et al., 2007)). Field and laboratory studies have reported elevated vitellogenin levels in male fish, altered steroid hormone regulation, impaired gonadal development, and reproductive dysfunction in species exposed to offshore petroleum effluents. Investigations in the North Sea, particularly in dab (*Limanda limanda*; (Scott et al., 2007) and Atlantic cod (*Gadus morhua;* (Balk et al., 2011), have demonstrated biomarkers consistent with feminization and endocrine modulation in fish collected near offshore installations. Although true intersex conditions, such as ovotestis formation, are less frequently documented offshore than in sewage-impacted estuarine systems, the presence of endocrine-active contaminants around oil platforms raises concern regarding chronic sublethal effects on fish reproduction and population health. Collectively, available evidence supports the conclusion that offshore petroleum activities could contribute to endocrine disruption in marine fish, although the prevalence and ecological significance of intersex phenotypes in offshore populations remain insufficiently characterized.

In this regard, it should be investigated whether the predominant current system in the area could be transporting contaminants generated by the oil platforms located in Div. 3L toward the area where intersex silver hake individuals were detected. The prevalence of these individuals in the analyzed sample is sufficiently high to suggest that they are caused by some environmental factor or a combination of them. Nevertheless, it would be desirable to expand the sampling effort toward the core distribution area of the silver hake stock within the Canadian EEZ in order to obtain a more accurate estimate of intersex prevalence across the entire population. For this reason, a coordinated sampling program between DFO and IEO will be carried out this year to expand gonad sampling for this species. The IEO will be responsible for conducting the histological analysis of these samples. Likewise, during the IEO scientific survey to be conducted in Div. 3N in 2026, samples of muscle and liver will be collected to analyze the concentration of heavy metals, PCBs and PAHs, with the aim of elucidating the potential causes underlying this high prevalence of intersex individuals in the study area.

Acknowledges

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TABLES

Table 1. Number of gonads collected for histological análisis by sex and year.

Sex	2024	2025	Total
Male	16	6	22
Female	28	26	54
Intersex	12	6	18
Total	**56**	**38**	**94**

FIGURES

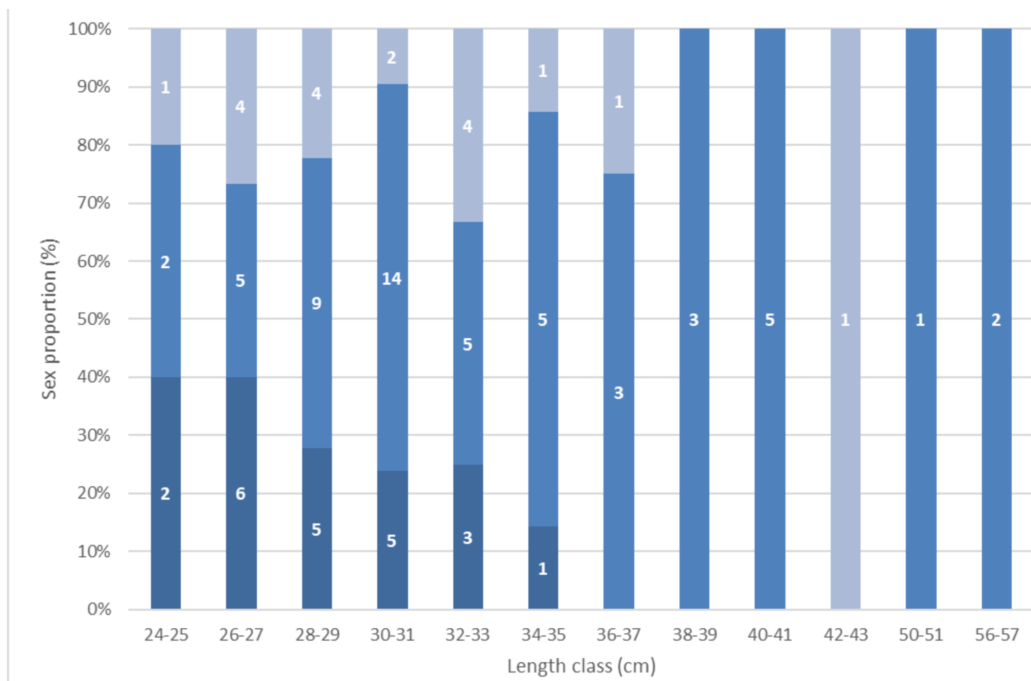

Figure 1. Proportion of individuals from each sex in the histological sample by length class (cm). White numbers inside bars indicate the number of specimens per sex in each length category. Dark blue: males, medium blue: females, light blue: intersex.

A) B)
C) D)
E) F)

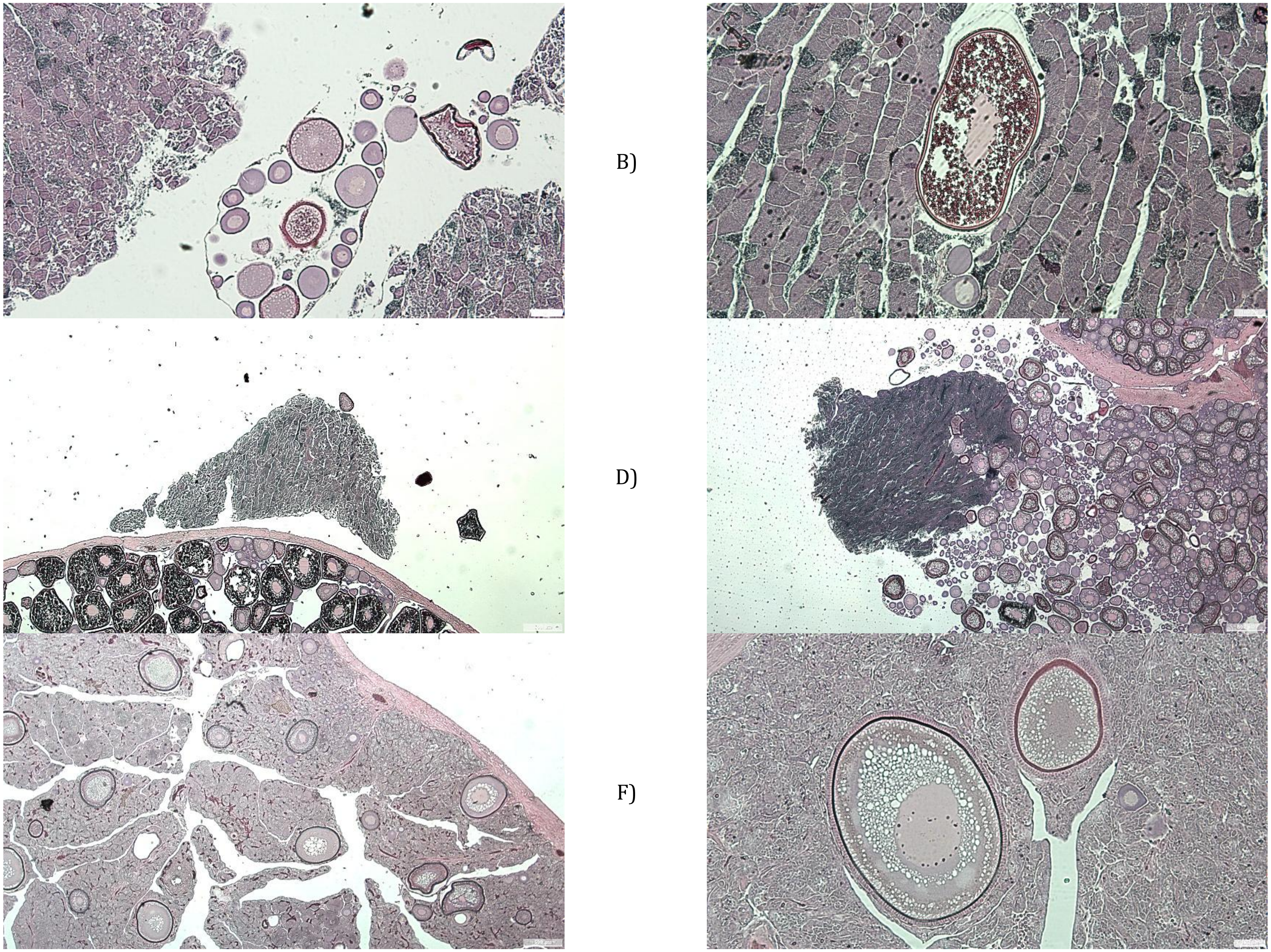

Figure 2. Histological images of the three types of intersex 0bserved in silver hake from NAFO Div. 3NO. A and B) type I, C and D) type II and E and F) type III.

ANNEX 1. Macroscopic maturity scale used to study the silver hake female maturity according to ICES (2007)

<table>
<tr><td>Stage</td><td>Stage</td><td colspan="2">Females</td><td>Male</td></tr>
<tr><td>Inmature</td><td>1-Inmature/resting</td><td colspan="2">Small-sized, transparent or pink-grey, firm consistency, minimal vascularization, opaque or hyaline oocytes absent.</td><td>Testis small and very thin, ribbon shaped without sign of development, no curling or a thin curling band, transparent or whitish, no sperm.</td></tr>
<tr><td rowspan="4">Mature</td><td>2-Developing/maturing</td><td colspan="2">Medium- or large-sized, pink or yellow to orange, firm consistency, vascularization variable, present and obvious, opaque oocytes present, hyaline oocytes absent.</td><td>Testis medium-sized and not thin, develop and curling, pink or whitish, no sperm.</td></tr>
<tr><td rowspan="2">3-Spawning</td><td colspan="2">Large-sized, pink or reddish orange, vascularization variable, present and obvious, opaque oocytes present</td><td rowspan="2">Testis large-sized and not thin, large band shape, white, sperm flows with pressure in the abdomen.</td></tr>
<tr><td>A (Hydrated): hyaline oocytes present, firm consistency.</td><td>B (Partial spawning): hyaline oocytes absent, flaccid consistency.</td></tr>
<tr><td>4-Regressing</td><td colspan="2">Small- or medium-sized, dark pink, orange or purple, flaccid consistency, vascularization high, present and obvious, opaque and hyaline oocytes absent or residual (few).</td><td>Testis large-sized and very thin in the spermduct, empty and deformed bands shape, redish to light pink, sperm absent or residual.</td></tr>
</table>

ANNEX 2. Microscopic maturity scale used to assess silver hake (*Merluccius bilinearis*) **female and male maturity, adapted from Lowerre-Barbieri *et al.* 2023.** Abbreviations: CW = coldwater, PG = primary growth, CA = cortical alveolar, Vtg =vitellogenic, OM = oocyte maturation, GVM = germinal vesicle migration, YC = yolk coalescence, GVBD = germinal vesicle breakdown, POF = postovulatory follicle, Sg1 = primary spermatogonia, Sg2 = secondary spermatogonia, Sc1 = primary spermatocyte, Sc2 = secondary spermatocyte, St = spermatid, Sz = sperm, rSz = residual sperm, OW = ovarian wall, CGE = continuous germinal epithelium, and NA = not aplicable.

<table>
<tr><td>Reproductive state</td><td>Phase</td><td>Females</td><td>Males</td></tr>
<tr><td>Immature</td><td>1- Immature</td><td>Oogonia and PG, no muscle bundles or large blood vessels. Lamellae are well organized. Thin OW.</td><td>Lobules contain Sg1 and Sg2, no lumens.</td></tr>
<tr><td>Sex change</td><td>T- Transitioning</td><td colspan="2">No sex assigned. Early transitioning: Sg, Sc, occasionally St, CGE, decreasing PG abundance; can have atretic oocytes. Late transitioning: Sg, Sc, and St, can have Sz in spermatocysts, clear male tissue proliferation; can have atretic oocytes</td></tr>
<tr><td rowspan="5">Mature</td><td>2- Early developing</td><td>PG and CA, no POFs; can be afew early Vtg and atresia</td><td>Sg2 and Sc1, sometimes Sc2. Lumens often obscure.</td></tr>
<tr><td>3- Late developing</td><td>Females with Vtg oocytes in any stage or combination and no spawning markers. Can have low levels of atresia.</td><td>All stages of spermatogenesis (Sg, Sc, St, Sz), but no Sz present in lobule lumens and/or sperm duct.</td></tr>
<tr><td>4- Spawning</td><td>OM, hydration, or POFs.

a) Imminent: Early OM (early GVM with Little YC).
b) Active: Late OM (GVBD, YC, hydrated), ovulating, or with newly collapsed POFs.
c) Spawned: POFs older than 2 h.</td><td>Sg, Sc, St, and Sz, Sz in lumens and/or sperm duct.</td></tr>
<tr><td>5- Regressing</td><td>≥50% of Vtg oocytes undergoing atresia (alpha and beta). Can have POFs.</td><td>Few to no spermatocysts, lumens contain Sz, rSz in ducts; spermatogonial proliferation.</td></tr>
<tr><td>6- Regenerating</td><td>PG, muscle bundles, blood vessels, thick OW. CW species can have POFs.</td><td>Sg present, some rSz can be present in lumens.</td></tr>
</table>