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Review

Intersex in teleost fish: Are we distinguishing endocrine disruption from natural phenomena?

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ABSTRACT

Intersex is defined as the simultaneous presence of male and female gonadal tissue in a gonochoristic (fixed-sex) species. The intersex condition has been documented in both wild and laboratory animals, including fish, amphibians, and reptiles. In aquatic animals, intersex is often viewed as a signature effect of exposure to endocrine disrupting compounds. At least 37 fish species from 17 families have been identified with intersex gonads in 54 field survey studies. However, reports of the occurrence of intersex at reference sites have led to speculation that a baseline level of intersex is "normal". The objective of this critical review was to assess factors potentially associated with baseline levels of intersex in fish and to examine the mechanisms involved in the intersex condition in order to identify priority research areas. Based on current literature, the relationship between intersex and physiological parameters such as plasma sex steroids and vitellogenin is not well characterized or conclusive. Moreover, the literature is not definitive on whether field studies are distinguishing between natural intersex and intersex due to stressors. High throughput transcriptomics will improve understanding of how intersex condition manifests after exposure to aquatic pollution and it is recommended that studies consider both males with and without intersex that inhabit the same polluted site in order to differentiate pathways associated with xenobiotic responses versus molecular pathways associated with intersex. Other experimental design considerations for field studies examining intersex include data collection on life history (e.g. migratory patterns) and improved reference site characterization.

1. Introduction

With more than 20,000 species in 40 orders, teleost fish exhibit extensive biodiversity that is maintained by adaptation and niche exploitation; this is a result of diverse behaviors, physiological capacities, and genetic variability. Teleosts also display a wide variety of breeding strategies and plasticity that is reflected in their sexual development. Many teleosts are gonochorists, defined as those individuals that develop only as males or as females and remain the same sex throughout their life time (Devlin and Nagahama, 2002). Other species are hermaphroditic, producing both female and male gametes at some point in their life history. This includes synchronous hermaphroditic strategies, whereby individuals can develop male and female gametes at the same time, or sequential hermaphrodites; classified as protandrous (first mature as males) or protogynous (first mature as females) (Devlin and Nagahama, 2002). The simultaneous occurrence of male and female reproductive stages in the same gonad at the same time in species is atypical, and is usually referred to as intersex. However, this condition is also referred to as the presence of testicular oocytes, testicular follicles, testis–ova, or ovotestes.

Intersex has been documented histologically in an extensive and rapidly growing list of wild and laboratory animals, including ecologically vulnerable species of aquatic invertebrates, fish, amphibians, and reptiles (Hecker et al., 2006). In aquatic animals, intersex is often viewed as a signature effect of exposure to endocrine disrupting compounds (EDCs), most common being estrogenic chemicals (Metcalfe et al., 2010). The number of studies using intersex condition as an indicator of exposure to natural or synthetic steroid hormones (or chemicals that mimic such hormones) is increasing in the literature. Thus, there is also a concomitant increase in the detection of intersex in gonochoristic fish (Nolan et al., 2001). However, questions remain regarding the prevalence of intersex in natural populations of fish and how this might be related to the molecular signaling events underlying sexual development.

The objectives of this critical review were to summarize ecological and toxicological studies in wild fish in which intersex was detected in order to evaluate whether or not there is evidence for a natural background of intersex. Specific tasks were to (1) assess the current knowledge regarding intersex in fish; (2) describe the current issues with semantics when referring to intersex; (3)

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compare methods that score intersex condition and to point out additional considerations for intersex scoring in fish; (4) examine any associations between reproductive physiology and intersex; (5) investigate whether or not intersex is a natural condition or is a result of pollution, and (6) summarize the current knowledge regarding the molecular signaling cascades associated with intersex in fish.

2. What is intersex?

Intersex is defined as the simultaneous presence of male and female gonadal tissue in an individual of a gonochoristic (fixed-sex) species (Tyler and Jobling, 2008). The most frequently reported manifestation of intersex is the presence of single or multiple oocytes within the testes of sub-adult or adult males. However, several other manifestations, such as the presence of testicular tissue within ovaries or the feminization of male gonadal ducts, have also been documented (Nolan et al., 2001). In terms of aquatic contaminants, intersex presentation can vary according to the exposure and it could be a feminization process (i.e. the presence of oocytes in the testes (Nolan et al., 2001)) or a masculinization process (i.e. the presence of spermatozoa with previtellogenic oocytes (Hinck et al., 2007)).

To more fully evaluate the potential for the natural occurrence of intersex and its value as a potential indicator of chemical exposures, it is first necessary to address semantics and terminology, discuss how intersex is scored and presented, and describe how intersex may be related to seasonal changes in reproduction.

2.1. The semantics of intersex

The intersex condition has been described using several additional labels such as testicular oocytes (Blazer et al., 2007, 2012), testicular follicles, testis–ova, ovotestes, mixed sex, mixed gonad tissues, and many other synonymous terms (Hecker et al., 2006). Furthermore, according to the severity of the observation, studies describe the condition differently. For example, Getsfrid et al. (2004) made a distinction between ovotestis and testis–ova, and described ovotestis as a condition resulting from a mature ovarian tissue interspersed with scattered testicular tissue, whereas testis–ova was the presence of scattered ovarian follicles within a mature testicular tissue. Based on the current literature examined here, the term intersex was used in 84% of the studies ($n = 69$), followed by the term ovotestis at 12%. Despite differences in terminology, the description used in the majority of studies is the intersex condition, and we suggest that what is needed to minimize any confusion in terminology, intersex should be used in the literature for fish.

2.2. Current practices in assessing intersex severity

There are no clear standardized methods that quantitate the intersex condition and there are multiple methods that score the severity of the condition, including both alphabetical and numeric methodologies. In order to compare severity across species, it is recommended that researchers adapt one scoring system. Most of the scoring approaches are based on the number of oocytes observed per microscopic field and there are different criteria for developing the break points in the scale. For example van Aerle et al. (2001), used letters to categorize intersex severity in gudgeon (*Gobio gobio*): 'A' contained a maximum of five primary oocytes per section; 'B' contained five primary oocytes per section; and 'C' consisted of fish with both primary and secondary oocytes comprising a high proportion (50%) of the gonad. Anderson et al. (2003) scored intersex from levels of 1 to 3 in smallmouth bass (*Micropterus dolomieu*). The researchers accounted for the number of eggs in 20 hilar fields: if the number was <10 immature oocytes, the score was 1, if the number was >20, the score was 3. Score 2 was between 10 and 20. Conversely, Blazer et al. (2012) described an intersex severity index from 1 to 4 in the same species. Score 1 was a single oocyte within the field of view (200×); score 2 was more than one oocyte in the field of view, without a physical association with neighboring oocytes. A cluster distribution of oocytes was given a score of 3, and the zonal distribution (score 4) was considered to be five or more physically-associated oocytes or numerous clusters of oocytes within a field of view.

The diversity of approaches in scoring makes it challenging for comparisons between different fish species using intersex as an endpoint in studies investigating the effects of pollution in aquatic environments. Perhaps one of the most complete intersex indices was developed by Jobling et al. (1998). The researchers developed an index from a numerical range of 0–7, in order to evaluate the degree of feminization in each individual. A score of 0 indicated a histologically male gonad, a score of 1 or 2 indicated the presence of ovarian cavity in the testis, index score 3 was frequent clusters of primary oocytes within the testis with the sperm duct. Index 4 indicated that oocytes (primary and/or secondary) were frequent, although still interspersed with testicular tissue. Index score 5 indicated large, continuous areas of the histological section that were testicular while less than 50% as ovarian; oocytes were either primary and/or secondary. Index score 6 indicated that more than 50% of the gonadal tissue was ovarian, and oocytes were either primary and/or secondary. An index score of >4 but <6 indicated severe feminization, without the formation of a sperm duct and typically with a cluster of oocytes. Index score 7 indicated a histologically female gonad.

2.3. Seasonal differences in intersex

Seasonal variation in reproductive capacity is widespread throughout teleost species, and some species require a year or more to develop their gonadal tissue. However, it is not clear if the incidence of intersex in wild populations changes seasonally. Barrett and Munkittrick (2010) performed an extensive review of more than 60 fish species currently used in Canada's Environmental Effects Monitoring (EEM) program to evaluate reproductive impacts. They recommended standardizing sample sizes and sampling time for each species based on the reproductive strategy and timing of the spawning season. For synchronous spawners and multiple spawning species with a few spawns in a period of time, they recommended sampling two to three weeks before the spawning season starts to maximize sensitivity.

The recommendation for the timing to study intersex condition may be similar. Blazer et al. (2007) reported that the highest prevalence of intersex was during the prespawn season and that the incidence decreased with postspawn in smallmouth bass. At every site, intersex was significantly higher ($p = 0.001$) in the spring during the pre-spawn period (69–100%) than in the summer postspawn period (25–67%). The prevalence of intersex was also significantly higher in the fall than in the previous summer ($p = 0.018$); however, smallmouth bass were only collected at two sites in fall and sample sizes were small. The observation of seasonal dependence of intersex is rarely considered. In regards to the sampling time, and based upon 44 field studies, there were 21 studies that reported intersex that were conducted in the fall, 12 studies conducted in the spring and 11 studies conducted in the other two seasons (i.e. summer or winter). One concern at present is how to decide when it is optimal to sample for intersex condition, and that timing will vary with the reproductive strategies of the fish species being studied. Sampling at a suitable time point will increase the detection of intersex with less variability.