NOTE / NOTE

Incidence of male intersex in adult Pacific lamprey (*Entosphenus tridentatus*), with a brief discussion of intersex vs. hermaphroditism in lampreys (Petromyzontiformes)

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Abstract: We report the incidence of male intersex in adult Pacific lamprey (*Entosphenus tridentatus* (Richardson, 1836)) during their pre-spawning migration in fresh water. Although "hermaphrodites" have been suggested in other adult lampreys, this is the first detailed description and discussion of this phenomenon. A total of 0.5% of our adult Pacific lamprey from Willamette Falls (2 out of 427 adults) were intersex, with oocytes in the testes. This phenomenon was identifiable only by histological examination. The testes of the intersex males were immature, in the beginning stages of meiosis. One intersex male possessed primary growth or perinucleolar stage oocytes loosely interspersed throughout the testes, and the other possessed at least 6 mid-vitellogenic oocytes (0.6 mm, mean long diameter) separate from the testes. Because premetamorphic lamprey can possess both female and male gonial cells, we hypothesize that intersex is a remnant larval trait and that these fish failed to fully develop into males during metamorphosis.

Key words: intersex, gonochoristic, primitive fish, reproduction, maturation, cyclostomes.

Résumé : Nous rendons compte de l'incidence d'intersexualité chez des mâles adultes de lamproie du Pacifique (*Entosphenus tridentatus* (Richardson, 1836)) durant leur migration en eau douce précédant le frai. Bien que la présence « d'hermaphrodites » ait été suggérée chez d'autres lamproies adultes, il s'agit des premières description et discussion détaillées de ce phénomène. Au total, 0,5 % des lamproies du Pacifique adultes de Willamette Falls recensées (2 individus sur 427 adultes) étaient intersexuelles, présentant des oocytes dans les testicules. Ce phénomène n'est observable que par un examen histologique. Les testicules des mâles intersexuels étaient immatures, aux stades initiaux de la méiose. Un mâle intersexuel possédait des oocytes au stade de croissance primaire ou périnucléolaire disséminés çà et là dans les testicules et l'autre possédait au moins six oocytes à des stades intermédiaires de la vitellogénèse (diamètre long moyen de 0,6 mm) séparés des testicules. Étant donné que la lamproie prémétamorphique peut présenter des cellules goniales femelles et mâles, nous avons émis l'hypothèse que l'intersexualité est un caractère larvaire résiduel et que ces poissons ne se sont pas développés complètement en individus mâles durant la métamorphose.

Mots-clés : intersexualité, gonochoriste, poisson primitif, reproduction, maturation, cyclostomes.

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Introduction

Hermaphroditism, either sequential or synchronous, exists in approximately 5% to 6% of all animal species (Grober and Rodgers 2007) and approximately 2% of all extant teleost fishes, across 20 families (Avise and Mank 2009). Grober and Rodgers (2007) noted that "*Ancestral fish lineages (e.g.,*

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lamprey, sharks/rays, gars, paddlefish, sturgeon, lungfish…) are remarkably devoid of hermaphroditic species, whereas the more recently diverged percomorph fishes (advanced teleosts) boast a large variety of hermaphroditic forms, suggesting that hermaphroditism is on the rise, not in decline…". However, "ancestral" or primitive fish such as the hagfishes (Myxiniformes) can be hermaphroditic or sexually dimorphic (Powell et al. 2004 and references therein), and so hermaphroditism is not absent in this ancient, basal vertebrate. There have also been obscure reports on "hermaphroditism" in adult lampreys (Petromyzontiformes; Breder and Rosen 1966; Holcik and Delic 2000), a cyclostome like the hagfishes (Janvier 2008).

Here we describe the occurrence of male intersex in adult Pacific lamprey (*Entosphenus tridentatus* (Richardson, 1836)), where we define "adult" as the postparasitic fish returning from the ocean to fresh water to spawn (Pacific lamprey are semelparous; Clemens et al. 2010). We discuss the intersex condition in larval and adult lampreys in relation to hermaphroditism. We define intersex as the co-occurrence of cells of both sexes in the gonad, with one sex cell type predominating in a species that is otherwise gonochoristic (e.g., see Devlin and Nagahama 2002; Hinck et al. 2009; Iwanowicz et al. 2009; Schwindt et al. 2009). Intersex in otherwise gonochoristic species is an abnormal condition associated with environmental exposure to xenbiotics (mercury, PCB, DDT, and its derivatives, etc.). The case is often made that exposure to xenobiotics causes endocrine disruption, resulting in abnormal sexual development (Hinck et al. 2009; Iwanowicz et al. 2009; Schwindt et al. 2009). However, the incidence of intersex in pristine environments (i.e., no xenobiotics) is unknown (Hinck et al. 2009; Schwindt et al. 2009). We define hermaphroditism as the condition in which fish generate mature, viable sperm and oocytes, either simultaneously (synchronous hermaphroditism) or at different times (sequential hermaphroditism) (Devlin and Nagahama 2002). We conclude by discussing potential causes of the intersex phenomenon in lampreys.

Materials and methods

Lamprey were collected during their pre-spawning migration in the Willamette River at Willamette Falls, Oregon, USA (approximately 204 km of river from the Pacific Ocean). The fish were collected during April–September of 2007 and 2008. During April–June, fish were collected from a fish trap in the fish ladder of the Portland General Electric hydroelectric dam that flanks the 12 m high basaltic falls. During June–September, fish were collected by hand from the base of the falls, where they congregate prior to going upstream or back downstream to spawn. Several different morphological and physiological indices were measured on the fish as part of efforts to monitor the maturation characteristics of Pacific lamprey (Clemens 2011). We collected a small piece of gonad (approximately 1–3 cm in diameter) immediately posterior to the posterior tip of the liver, following the protocol of Sower et al. (1985). The tissues were fixed in buffered formalin, processed for gonad histology and stained with hematoxylin and eosin, and viewed under a compound light microscope (Leica DMLB). Images were taken with a digital camera (SPOT, Diagnostic Instruments, Inc.).

The stage of maturity for oocytes was identified through a modified scale used for sea lamprey (*Petromyzon marinus* L., 1758) from Lewis and McMillan (1965) and McMillan (2007), and cross-verified with Bolduc and Sower (1992). The long diameter (animal pole to vegetable pole) of the oocytes was also measured with the digital camera software mentioned above and these diameters were used in conjunction with other morphological features to aid in the categorization of the stage of maturity of the oocytes.

The stage of maturity for the testes was identified with the aid of keys for southern hemisphere lamprey (*Geotria australis* Gray, 1851) (Potter and Robinson 1991) and for salmon (genus *Salmo* L., 1758) (Dziewulska and Domagala 2003). Because the immature testes showed more complicated and nuanced morphological differences than suggested in the aforementioned papers, and there is a growing literature of complicated testicular cell line iterations in fish and other animals (e.g., see de Rooij and Russell 2000; Leal et al. 2009; Schulz et al. 2010), we opted for identification of more general maturation stages of the testes, akin to Van Eenennaam and Doroshov (1998). This amounted to four stages: (1) before meiosis, or an inscrutable proportion of spermatogonia and spermatocytes; (2) beginning meiosis, or the obvious presence of what many authors refer to as secondary spermatocytes (e.g., see Potter and Robinson 1991; Fahien and Sower 1990); (3) the second meiotic division, resulting in a prevalence of spermatids; and (4) differentiated spermatozoa.

Results

A total of 0.5% of all adult lamprey (2 out of 427) collected from the Willamette River at Willamette Falls were intersex fish, primarily possessing testicular tissue. This includes 1 out of 210 fish collected from Willamette Falls during 2007 (Figs. 1A–1E) and 1 out 217 fish collected from the same location during 2008 (Fig. 2). These fish were 520 mm (2007) and 601 mm (2008) in total body length, respectively.

The intersex lamprey were collected late in the year (during September of 2007 and August of 2008), long after the spawning season. Our gross identification of the sexes of both of these lamprey indicated that they were male; the oocytes were only evident from histological examination of gonad tissue.

The intersex fish were immature and although they possessed mostly spermatogonia or early stage spermatocytes, the few oocytes that were apparent were in vastly different stages of maturation between the two fish. The intersex fish from 2007 possessed at least six mid-vitellogenic oocytes (0.6 mm, mean long diameter) separate from the testes (Figs. 1A–1E). We have included an example of "normal" mid-vitellogenic oocytes from a non-intersex female for comparison (see Figs. 1C and 1D vs. 1E). By contrast to the intersex fish from 2007 that exhibited oocytes separate from the testes, the intersex fish from 2008 possessed primary growth or perinucleolar stage oocytes interspersed throughout the testes (Fig. 2). This latter fish also showed signs of testicular atresia, indicated by eosin-stained voids where other gonial cells used to be (Fig. 2).

Fig. 1. Gonad histology of a male intersex adult Pacific lamprey (*Entosphenus tridentatus*) (total body length = 520 mm) collected from Willamette Falls, Oregon, USA, during September of 2007. (A) Mid-vitellogenic oocytes towards the top left (top arrowhead) and the immature testes towards the bottom right (bottom arrowhead). The eosinophilic staining tissue spanning the left of the panel is connective tissue that formerly attached the gonad to the dorsal side of the body cavity. The remaining, diffuse tissue is primarily gonadal mesentery with some lipid cells near the testes (refer to the top of B). (B) Testes from A with spermatogonia and (or) spermatocytes. (C) Mid-vitellogenic oocytes from A. Arrow indicates oocyte with a visible nucleus. (D) Expanded image (2.3×) of oocyte with a visible nucleus from C. (E) Mid-vitellogenic oocytes from a "normal" female (i.e., non-intersex; total body length = 754 mm) shown for comparison.

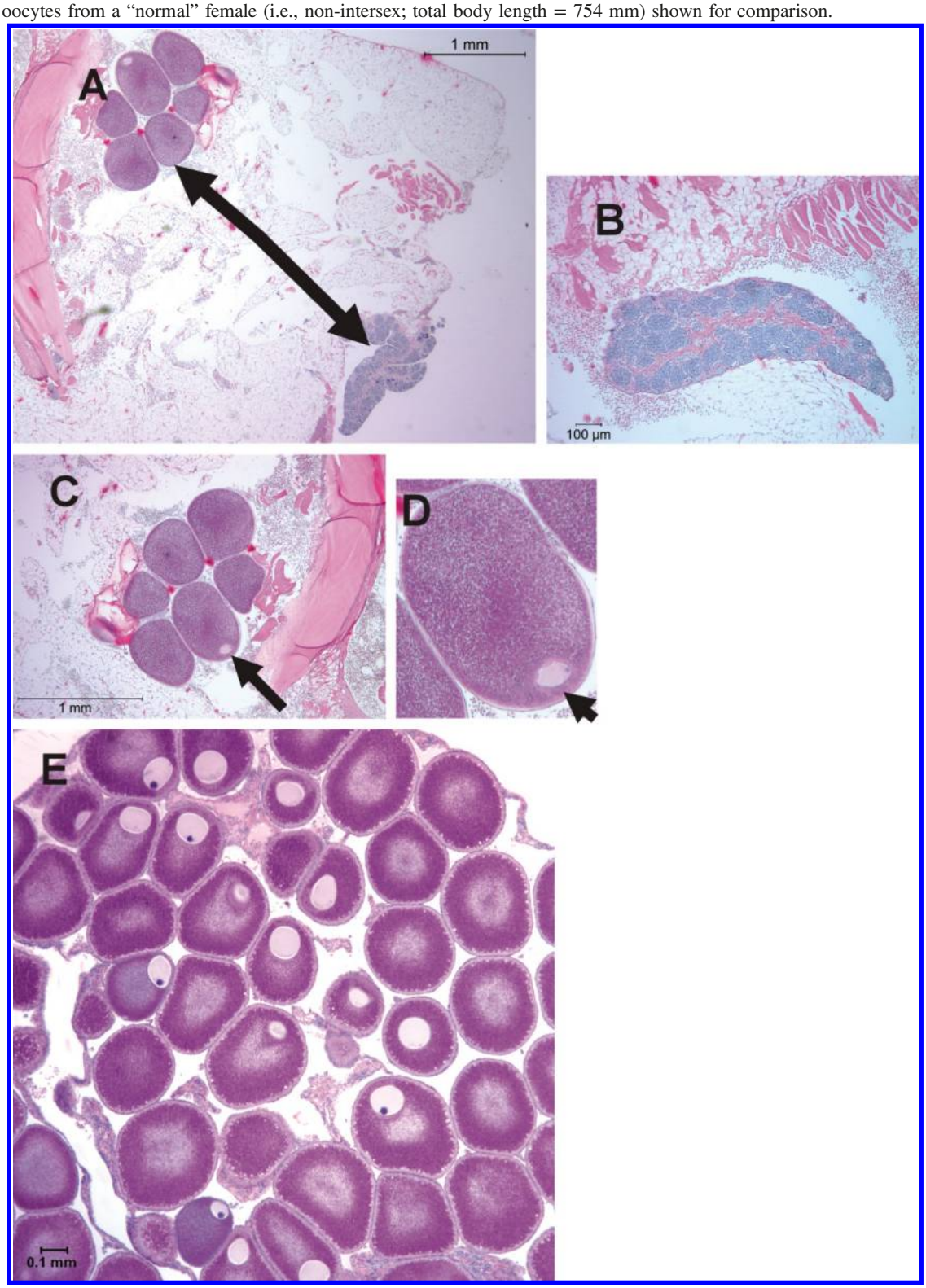
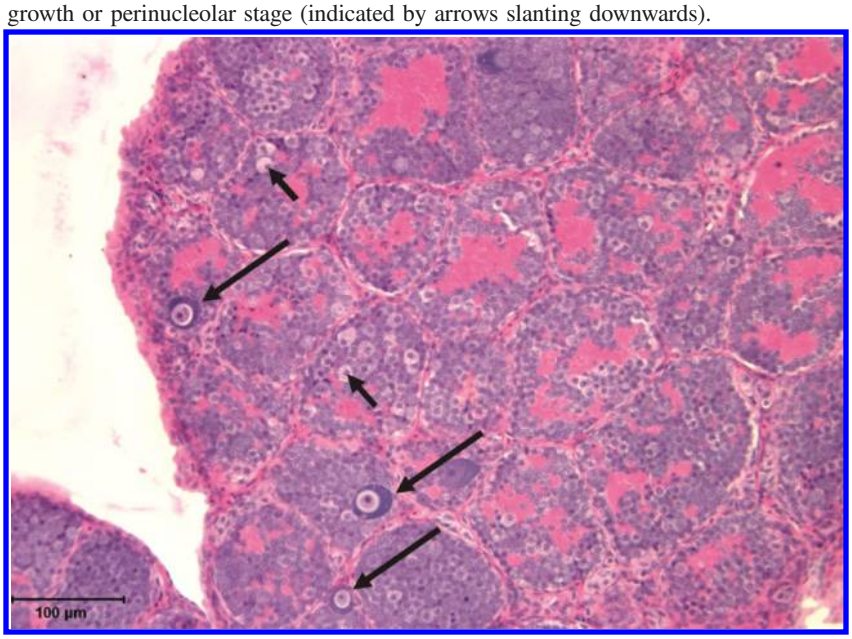


Fig. 2. Gonad histology of a male intersex adult Pacific lamprey (*Entosphenus tridentatus*) (total body length = 601 mm) collected from Willamette Falls, Oregon, USA, during August of 2008. Immature testes with spermatogonia (examples indicated by arrows slanting upwards) and oocytes in the primary growth or perinucleolar stage (indicated by arrows slanting downwards).



Discussion

In lampreys, the gonad in both sexes is unpaired and median, and is suspended from the dorsal wall of the body cavity. Lampreys are among the few vertebrates including teleost fish that do not have intraperitoneal genital ducts (Hardisty 1971). In the early larval stage, and after hatching, for periods varying from 6 months to over 2 years, the undifferentiated gonad shows comparatively little development (Hardisty 1971). Following this period, the germ cells tend to remain together to form cysts. This is followed by oogenesis that occurs in all larval lamprey gonads that leads to a gonad containing only oocytes in the cytoplasmic growth phase (Hardisty 1971). In females, it is only after metamorphosis that the oocyte enters the final period of growth and vitellogenesis. Prior or during metamorphosis, in males, the oocytes undergo atresia and the remaining germ cells differentiate into nests of primary spermatogonia (Hardisty 1971). More explicitly, Hardisty (1971) stated that "*…in the differentiation of the male gonads, a large proportion of the germ cells undergo degeneration in the earlier stages of the meiotic prophase, oocytes which survive to the cytoplasmic growth phase are eventually eliminated by atresia. Because of this extensive degeneration of germ cells, the testis is reduced in size and contains only small numbers of residual germ cells. It is from these undifferentiated elements that nests of primary spermatogonia are developed by renewed mitotic divisions either shortly before, or during metamorphosis.*" Therefore, in lampreys, there is a relatively extended "intersexual" stage followed by a period of gonadal sex differentiation; the timing of these periods depend upon the species of lamprey (Hardisty 1971).

Brook lamprey (*Eudontomyzon mariae* (Berg, 1931)) were reported to have 6% of collected "adults" (2 out of 33 fish) as hermaphrodites (Holcik and Delic 2000). However, it is not clear that the "adult" fish had metamorphosed, as the descriptions in the paper are vague, and the reports of two fish having eyes covered by skin suggests that at least some of the fish had not fully metamorphosed. Larval lamprey are sexually labile and indeterminate prior to metamorphosis (Wicks et al. 1998; Barker and Beamish 2000; Beamish and Barker 2002).

Older literature from the early 1800s to the early 1900s provide further tantalizing reports of "hermaphroditism" in various other lamprey species (reviewed in Breder and Rosen 1966), yet the interpretation of these claims is problematic for several reasons. For example, the anatomical descriptions and observations are neither clear, nor scientific, nor accurate (e.g., Home 1815). It was later noted "*That Sir Edward Home was mistaken in supposing the Lamprey to be a hermaphrodite animal has long been well known.*" (Ewart 1876). Others have made extraordinary claims that have not been independently verified, including the hypothesis of parthenogenesis in lampreys, based on the supposed observation of egg development to the morula stage without fertilization (Gage 1928, cited in Breder and Rosen 1966). Still others reported on hermaphroditism in premetamorphic and metamorphosizing fish (e.g., Okkelberg 1921), which seems reasonable because of the aforementioned lability and indeterminacy prior to metamorphosis.

We refer to our fish as "male intersex" because of the predominance of testicular tissue compared with the few oocytes, and because lampreys are gonochoristic, not hermaphroditic. It seems highly unlikely that the adult male intersex fish that we discovered would be able to self-fertilize or that they would be able to spawn viable eggs. We do not know whether these fish would be able to fertilize eggs from females. One of the two male intersex Pacific lamprey showed signs of testicular atresia, like the "normal" male lamprey collected at the same location (Willamette Falls) and during the same time period (late summer) (Clemens 2011).

Because premetamorphic lamprey can possess both female and male gonial cells (Wicks et al. 1998; Barker and Beam-

ish 2000; Beamish and Barker 2002), we hypothesize that the presence of male intersex fish is a remnant larval trait, and that these intersex fish failed to fully develop as males retaining remnant oocytes during sex differentiation prior to metamorphosis. Another, perhaps not mutually exclusive hypothesis, is that hermaphroditism may be the ancestral trait common to the paraphyletic cyclostomes. That hermaphroditism (we suggest "intersex" is more accurate and appropriate) has been reported, albeit not clearly or thoroughly from other lampreys, could suggest an infrequent tendency for this trait to occur across species of lampreys. Intersex in adult lampreys might be more prevalent than indicated by the literature, and it could be more widely reported if more researchers examined the gonad histology from adult lampreys.

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