Anatomical, hormonal and histological descriptions of captive Russian sturgeon (Acipenser gueldenstaedtii) with intersex gonads

Karen Jackson b,c, Avshalom Hurvitz a,b, Svetlana Yom Din a,b, Doron Goldberg b, Oren Pearlson b,c, Gad Degani b,c, Berta Levavi-Sivan a,*

a Faculty of Agricultural, Food and Environmental Quality Sciences, Department of Animal Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel
b MIGAL—Galilee Technology Center, P.O. Box 831, Kiryat Shmona 10200, Israel
c School of Science and Technology, Tel-Hai Academic College, Galilee, Israel

Received 2 August 2005; revised 16 April 2006; accepted 18 April 2006
Available online 5 June 2006

Abstract

Sturgeons are known throughout the world as the source of black caviar. Their declining populations in their native habitats, mainly the Caspian Sea, due to over-fishing for meat and caviar production, destruction of their spawning grounds and water pollution, have led to their introduction into aquaculture in areas with suitable conditions, including Israel. Recently, we noticed an unusual phenomenon in these normally gonochoristic species. Several 5-year-old female sturgeons were found to have one or more testicular sections in each of their two gonads, forming an intersexual gender. Further examination of other fish from the same age group revealed 14% fish with intersex gonads among a population of 5000 fish that had been pre-selected as females. This phenomenon has not been found however in other age groups of Russian sturgeons, cultured at the same facility. Sturgeons are a generally gonochoristic species, and hermaphroditism is only very infrequently observed under natural or normal breeding conditions. Moreover, these rare cases have all been from polluted habitats. The present work is the first description of fish containing intersex gonads in Russian sturgeon (Acipenser gueldenstaedtii). We describe the phenomenon anatomically and histologically, and examine plasma steroid levels and pituitary gonadotropin gene expression by comparing fish with intersex gonads with normal females and males of the same age group. Intersex gonads were typical female ovaries with one or more white testicular components embedded in each. The testis components were not uniform in size or location among the two gonads of each fish or among different fish, and they showed marked differences in distribution. The ovarian component of the intersex gonad was at the pre-vitellogenic stage as in normal females, and the testis component contained spermatids and mature spermatozoa as in normal males of the same age. However, in terms of estradiol and 11-ketotestosterone plasma levels, as well as of pituitary gonadotropin (βLH and βFSH) gene-expression levels, the fish with intersex gonads were more similar to the normal males than to the normal females, even though the testis part of the intersex gonad was smaller than the ovarian part. To examine the possibility that the fish containing intersex gonads were hybrids, phylogenetic trees were constructed from the consensus sequences of Cytochrome b and control region (D-loop) genes. Results indicated no differences between the fish with intersex gonads and normal males or females of the same age group. However, statistically significant differences were found between different age groups of Russian sturgeon, as well as of white sturgeons (A. transmontanus), grown under the same culture conditions.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Sturgeon; Estradiol; Intersex; Gonadotropins; LH; FSH; Gene expression

1. Introduction

Sturgeons are the world-renown source of black caviar. Their declining populations in their native habitats, mainly the Caspian Sea, due to over-fishing for meat and caviar...
production, destruction of their spawning grounds and pollution of the water, have led to their introduction into aquaculture in areas with suitable conditions. Due to its high production rates and caviar quality, Russian sturgeon (*Acipenser gueldenstaedtii*) was chosen to enrich the variety of commercial fish species in Israel (Hurvitz et al., 2005).

In aquaculture, sturgeon fish are usually separated by sex at the age of 3 years (size 3–4 kg). Most males are sold for meat, whereas the females are grown to maturity, expected at the age of 7 or 8 years under aquaculture conditions (Doroshov et al., 1997).

The Russian sturgeon, like other Acipenseriformes, is a tetraploid species with 250 ± 8 chromosomes (Fontana, 1994). There is no external sexual dimorphism, and sex chromosomes have not been identified. However, it is known that all sturgeons are gonochoristic and evidence for female heterogametic genetic sex determination has been found in the white sturgeon (*A. transmontanus*) (Van Eenennaam et al., 1999) and in shortnose sturgeon (*A. brevirostrum* Lesuere) (Flynn et al., 2006).

Intersexuality (ovotestis) is a condition whereby an individual possesses oocytes or different stages of spermatogonia, at varying degrees of development, within the normal gonad of the opposite gender (i.e. spermatocytes in the ovaries or oocytes in the testis). Very little is known about the physiological mechanisms of gonadal differentiation in spontaneous intersexual individuals of gonochoristic species. Intersexuality in sturgeon is not a normal situation, and the few cases of hermaphroditism that have been reported in wild sturgeons have all been from polluted habitats (Chapman et al., 1996; Harshbarger et al., 2000; Van Eenennaam and Doroshov, 1998).

Chirkina (1957) reported ovotestis in artificially produced besters (hybrids between *Huso huso* × *A. ruthenus*) and recently, Williot et al. (2005) found hermaphroditism in sterlet sturgeon (*A. ruthenus*).

Genetic factors may also be a cause for the instability of gonadal differentiation in gonochoristic fish. This is particularly true for sturgeons which have many sets of chromosomes (2n = 258 ± 8 in *A. gueldenstaedtii*) and can form hybrids of diploid, triploid or tetraploid chromosome number with adverse consequences on their fertility (reviewed by Fontana et al., 2001). In the common carp, male sex reversal in gynogenetic females was induced by a recessive mutation in the genetic sex-determining gene (*Komen et al., 1992*).

In rainbow trout, a mutation in the genetic sex-determination system, the *mal* mutation, was described, associated with the development of testicular tissue in the gonads of expected female individuals (Quillet et al., 2002).

Molecular analysis has been applied to determine phylogenetic relationships between sturgeon species and to genetically characterize some subspecies. Such analyses have been conducted in sturgeon mainly for conservation purposes and for forensic identification (Fontana et al., 2001). As in other animal taxa, most molecular markers currently employed for genetic characterization in sturgeons are based on mitochondrial DNA (mtDNA). In fish, some mtDNA regions exhibit a high degree of variability, and as such represent a very useful tool for taxonomic analysis (Avise et al., 1987; Kocher et al., 1989; Meyer et al., 1990).

Control regions are employed to discriminate closely related species or populations belonging to the same species, and they are the most analyzed mtDNA region for intraspecific studies because of their high variability (Campton et al., 2000; Ludwig et al., 2000). Cytochrome *b* (Cyt *b*) is used for species identification and phylogenetic analysis (Jenneckens et al., 2000; Birstein and DeSalle, 1998; Briolay et al., 1998), as well as for forensic identification of sturgeon products.

The present work describes intersexuality in Russian sturgeon. We describe the phenomenon anatomically and histologically, as well as in terms of plasma steroid and pituitary gonadotropin gene-expression levels, by comparing the fish having intersex gonads with normal females and males. To investigate the possibility that the intersex fish were hybrids, we used both mtDNA markers: Cyt *b* and the control region. A comparison was also made between the sequences of these genes from other age groups of sturgeons reared in the same culture system.

2. Materials and Methods

2.1. Fish and sampling procedure

Russian sturgeons (*A. gueldenstaedtii*), originated in the Azove Sea, were brought from Russia as fertilized eggs and reared at “Dan Fish Farms” (Upper Galilee, Israel; 31°30'N, 34°45'E) under the aquaculture conditions described in Hurvitz et al. (2005). The parent fish were caught in the wild (Azove Sea) and kept indoors at a breeding facility in Krasnodar, Russia, for several years. Fish reproduction was performed in Russia and the fertilized eggs were incubated for 5 days before they were sent to Israel for hatch out, 2 days after that. Larvae were fed for 2 weeks with *artemia* nauplii and then with the trout starter feed that is routinely used at the farm.

At the age of 3 years (3–4 kg BW), fish were sexed by gonadal endoscopy. Most males were sold for meat while the females were left to grow. At the age of 5 years, fish were examined again by endoscopy to determine their oocyte diameter; 50 fish, randomly selected from the 5000 fish in this particular age group, i.e. that in which fish with intersex gonads were found, were sampled for endocrinological, genetic and histological analyses. Sampling was performed in the spring, when water temperature was stable at 22 °C. Each fish was anesthetized in a clove oil bath (0.25 mg/l) and blood was taken from the caudal vasculature into heparinized syringes. After centrifugation, the plasma was stored at −20 °C until processing. The pituitary gland was removed and stored in RNA Later (Ambion, Inc., Austin, TX) for total RNA extraction. The gonads were removed, examined externally and a sample was taken for histology. From the fish with intersex gonads (*n = 7*), samples were taken from the ovarian part, testicular part and the interface between them.

For the genetic study, five to six fish were randomly sampled from each of the following groups: (1) 5-year-olds: six normal females, five normal males and five fish with intersex gonads; (2) 3-year-olds: five females; (3) 1-year-old fingerlings: five fish; (4) 7-year-olds (*A. transmontanus*): five females. The fish were briefly anesthetized by clove oil bath and a 1-cm² piece of the tail fin was cut and stored frozen (−20 °C) until processing.

In order to check whether intersex gonads are also found in fish of other age groups, 500 fish, representing a 10% random sampling from a population of 5000 fish originating from three separate eggs batches, were each examined by endoscopy for gonad abnormalities.
2.2. Histological analysis

The gonad samples were fixed in Bouin’s fluid and were subsequently processed for light microscopy. Paraffin sections of 4–7 μm were stained with hematoxylin and eosin. The terminology used for the sturgeon hybrid, the bester (Amiri et al., 1996a,b), was adopted as described by Hurvitz et al. (2005). Mean oocyte diameter was calculated for each fish after measuring five of the largest oocytes present in a histological section.

2.3. Real-time PCR

To compare the mRNA levels of the β-subunits of sturgeon (st) FSH and LH, the relative abundance of their mRNA was normalized to the amount of an endogenous reference, the 18S subunit of rRNA, by the comparative threshold cycle (Cₚ) method, according to Levavi-Sivan et al. (2004) and Hurvitz et al. (2005). The relative amount of each β-subunit’s mRNA was calculated by the formula 2⁻^ΔΔCₚ, where ΔCₚ corresponds to the difference between the Cₚ measured for stFSH or stLH and that measured for 18S rRNA. To validate this method, serial dilutions were prepared from a pituitary cDNA sample (0.5, 0.1, 0.02, 0.01 and 0.005), and the efficiencies of each β-subunit and 18S ribosomal RNA amplification were compared by plotting ACₚ versus log dilution (template), according to the method of Muller et al. (2002). Linear regressions of plots emerging from ACₚ versus log (serial dilutions of cDNA samples) were prepared as described above.

Total RNA and cDNA were prepared according to Hurvitz et al. (2005). Gene-specific primers used for the real-time PCR were designed using the Primer3 Software (Perkin-Elmer, Foster City, CA). The primers were sequenced in each case. 18S rRNA cDNAs was amplified from a pituitary cDNA sample (0.5, 0.1, 0.02, 0.01 and 0.005), and the efficiencies of each β-subunit and 18S ribosomal RNA amplification were compared by plotting ACₚ versus log (serial dilutions of cDNA samples) were prepared as described above. The following R² values and slopes, respectively: 0.976 and –3.00 for 18S rRNA; 0.999 and –3.096 for stFSH; and 0.992 and –2.80 for stLH.

Total RNA and cDNA were prepared and used for Hurvitz et al. (2005). Gene-specific primers used for the real-time PCR were designed using the Primer3 Software (Perkin-Elmer, Foster City, CA). The primers used for stFSH were 5'-GGTCGCGGAACCTGTTGATC3' (forward) and 5'-CCACGGGATTGGTGATGAAAA3' (reverse) (GenBank Accession No. AY519657). Primers for stLH were 5'-AGAGAGAGACGGC TTCGTGAG3' (forward) and 5'-GATGAGAGACCTTTGGCACC3' (reverse) (GenBank Accession No. AY333426). Primers for 18S rRNA were 5'-CCACAGGAGATGGACAATTAAA3' (forward) and 5'-GCTGATGACCCGACACTTACT3' (reverse) (GenBank Accession No. AF188400). The PCR mixture and conditions were as described in Hurvitz et al. (2005). Amplification of stFSH, stLH and 18S rRNA cDNAs was performed simultaneously in separate tubes and in duplicate, and the results were analyzed with Q-Gene software (BioTechniques Software Library at: www.BioTechniques.com). Dissociation-curve analysis was run after each real-time experiment to ensure that there was only one product. To control for false-positives, a reverse transcriptase negative control was run for each template and primer pair.

2.4. ELISA for steroids

Plasma estradiol-17β (E2) and 11-ketotestosterone (11-KT) were determined by enzyme-linked immunosorbent assay (ELISA) according to Cuisset et al. (1994) and Levavi-Sivan et al. (2004), using acetylated steroids as a label. The anti-11-KT was donated by Dr. D.E. Kime (Sheffield, UK) and was previously described in Cuisset et al. (1994) and Levavi-Sivan et al. (2004), using acetylcholinesterase as a label. The anti-11-KT was donated by Dr. D.E. Kime (Sheffield, UK) and was previously described in Cuisset et al. (1994) and Levavi-Sivan et al. (2004).

2.5. DNA extraction and PCR

To prepare the DNA samples for PCR, the gonads were digested with proteinase K (2 mg/ml). The primers were 5'-cttattgatagatgaaac-3' and 5'-atggtggttactacta-3' (Table 1; GenBank Accession No. AF238762) were used to amplify the mitochondrial conserved region Cytochrome b (Cyt b). The primers Hetro1, Hetro2 and REVA (Table 1; GenBank Accession No. AF238762) were used to amplify the mitochondrial variable control region (D-loop).

PCR amplification was performed in a 50-µl solution containing 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 1 mM of each dNTP, 0.5 µM of each primer, 10–500 ng genomic DNA and 2.5 units of Taq DNA polymerase (Promega, Madison, WI). PCR parameters were initial denaturation for 3 min at 94 °C, and then 32 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 54 °C and extension for 1 min at 72 °C. Amplification was ended with a final elongation step at 72 °C for 10 min. After the amplification, the PCR products were separated by electrophoresis in a 1.3% agarose gel and stained with ethidium bromide. The DNA bands were excised from the gel, and the DNA was extracted using the Jet quick Gel Extracting Spin Kit (Genomed, Löhne, Germany) and suspended in DDW. The DNA was sequenced at the Center for Genomic Technologies of the Hebrew University. At least three independent clones were sequenced in each case.

2.6. DNA sequence analysis

Sturgeons can form hybrids with a diploid, triploid or tetraploid number of chromosomes with adverse consequences on their fertility (reviewed by Fontana et al., 2001). The phylogenetic analysis was designed to test the possibility that the fish containing intersex gonads came from a hybrid between two different species of sturgeons. Multiple partial sequences of Cyt b and the D-loop were aligned and the phylogenetic tree was constructed using the MegAlign program of the DNASTAR software package (Burland, 2000; Clewley, 1995).

2.7. Statistical analysis

Data are presented as means ± SEM. The significance of the differences between group means of hormone or mRNA levels was determined by one-way analysis of variance (ANOVA) followed by Students–Newman–Keul’s (SNK) test using the Graph-Pad Prism software (GraphPad, San Diego, CA) with the level of significance set at p < 0.05.

3. Results

Out of 50 females from the 5-year-old group pre-selected for gender at the age of 3 years, seven fish (14%) were found to have intersex gonads. Twenty-one 5-year-old sturgeons (seven normal females, seven normal males and seven fish with intersex gonads) were sampled in May 2004 when water temperature was stable at 22 °C.

Among the 500 fish representing the 10% random sampling from another population of 5000 fish originated from three separate egg batches, not even one case of fish with intersex gonads was found by endoscopy.

3.1. Anatomy and histology of the gonads

Three types of gonads could be discerned macroscopically: testis, ovary and intersex. The testes appeared as a
pair of white–pink longitudinal bodies extending along the body cavity. Five-year-old males are already mature, so there is very little adipose tissue in the gonad, which is composed mostly of the germinal portion, contributing to the whitish color of the testis (Fig. 1A). Female gonads were also longitudinal, extending along the body cavity, but were yellowish in color with dark spots (Fig. 1B). The female’s gonads are largely composed of adipose tissue, in addition to a narrow zone of germinal tissue (Fig. 1B). Intersex gonads were typical ovaries with one or more testicular white components embedded in each. The testis components were not uniform in size or in location among the two gonads of the same fish, or among fishes, showing marked differences in their distribution patterns (Figs. 1C and D).

### 3.1.1. Ovarian histology

Histological analysis showed that all females were at the pre-vitellogenic stage, according to the classification of Van Eenennaam and Doroshov (1998) for Atlantic sturgeon and that of Amiri et al. (1996a, b) for hybrid sturgeon. Oocytes were located at the periphery of the ovarian lamellae, either isolated or forming cysts. A single layer of follicle cells surrounded the oocytes. Even the most developed oocytes (180–400 μm in diameter) were still pre-vitellogenic. The ooplasm showed strong basophilia and the nucleus contained many prominent nucleoli next to the nuclear membrane and several cortical alveoli next to the oocyte membrane. Vitellogenic granules were not detected (Figs. 2A and B).

### 3.1.2. Testicular histology

The testis was organized into lobules oriented towards the central lumen. Each lobule contained numerous seminiferous tubules that were irregularly arranged and formed a network that converged into a deferent duct at the level of the gonad wall. Spermatogenic cells, at different stages of spermatogenesis, occurred within the lobules. They formed cysts in the seminiferous lobules. Each cyst was bounded by a thin layer of connective tissue and contained cells at the same stage of differentiation: primary and secondary spermatogonia, spermatocytes and spermatids. Mature spermatozoa were found in the lumen of the lobule (Figs. 2C and D).

### 3.1.3. Intersex histology

The female gonadal portion of the fish with intersex gonads was similar to the normal sturgeon ovary. All observed oocytes were at the pre-vitellogenic stage with diameter of 180–400 μm. The testicular portions were randomly dispersed along the ovary in designated sections and were formed by lobules containing cysts of cells at different stages of spermatogenesis and spermiogenesis. Some free mature spermatozoa were found within the lumen of the lobules. Ovarian follicles and testicular lobules were in close proximity at the interface between these tissues (Figs. 2E and F).

### 3.2. Expression of stFSHβ and stLHβ

Real-time quantitative PCR, which enables the specific and sensitive detection of transcripts, was used to study the expression of the GtH β-subunits in males, females and fish with intersex gonads. mRNA levels of stFSHβ in females were significantly higher than in both normal males and fish with intersex gonads. The levels of stLHβ were significantly higher than those of stFSHβ in males and fish with intersex gonads but were not significantly different when compared amongst the three different groups (Fig. 3).

### 3.3. Plasma steroid levels

Plasma estradiol (E2) level in fish with intersex gonads was somewhat higher than in normal females but the difference was not statistically significant. 11-Ketotestosterone (11-KT) level was significantly higher in fish with intersex gonads than in females, and as high as in normal males (Fig. 4).

### 3.4. Genetic analysis

Natural introgressive hybridization between species has been described in all major groups of organisms (Barton and Hewitt, 1989). This phenomenon seems relatively widespread and is of important evolutionary significance for adaptation and speciation (Barton, 2001). With regard to the other groups of vertebrates, teleosts (mainly freshwater species) show a higher aptitude to hybridize (Smith, 1992). Furthermore, viable hybrids are often fertile and gene introgression could frequently occur after natural or man-induced secondary contacts (Billington and Hebert, 1991). Sturgeons can form hybrids with a diploid, triploid or tetraploid number of chromosomes with adverse consequences on their fertility (reviewed by Fontana et al., 2001). The phylogenetic analysis was designed to test the possibility that the fish containing intersex gonads came from a hybrid between two different species of sturgeons. The phylogenetic trees constructed from the consensus sequences of the Cyt b (Fig. 5) and control region (Fig. 6) genes showed no differences between the fish with intersex gonads and normal males or females of the same age group. However, statistically significant differences were found between the different age groups of the Russian sturgeon, as well as between Russian and white sturgeons.

### 4. Discussion

In our previous work (Hurvitz et al., 2005), we showed that male and female Russian sturgeons have distinctly different rates of sexual maturation. While females at the age of 3 and 4 years exhibit gonads at the pre-vitellogenic stage, with small oocytes and very low levels of estradiol (<1 ng/ml), males at the same age have mature testes containing spermatids and spermatozoa, as well as a high level of 11-KT. In the present work, normal females at the age of
Fig. 1. Macroscopic view of 5-year-old *A. gueldenstaedtii* gonads. (A) Male. (B) Female. (C) Intersex. (D) Excised intersex gonad. Scale bar: 20 mm. TS, testis; OV, ovary; Fat, fat pad.

Fig. 2. Histological sections of a 5-year-old sturgeon ovary, testis and intersex gonad. (A) Normal female gonad contains clusters of oogonia (black arrows) and oocytes of various sizes. (B) Detail of pre-vitellogenic oocyte. Note the many nucleoli next to the nuclear membrane. (C) Gonads of normal male showing testicular lobes with cells at different stages of development. (D) Magnification showing types of testicular cells. (E) Section of gonads of fish containing intersex gonads *A. gueldenstaedtii* showing a pre-vitellogenic oocyte close to testicular lobules containing spermatozoa at the interface between ovary and testis. (F) Magnification showing the close proximity between oocyte and spermatozoa. Hematoxylin and eosin staining. Scale bar: 100 μM. ac, adipocyte; oc, oocyte; nl, nucleoli; gv, germinal vesicle; ca, cortical alveoli; gc, granulosa cells; sg, spermatogonium; sd, spermatides; sz, spermatozoa.
Means marked by different letters differ significantly ($p < 0.05$).

5 years had higher levels of E2 (1.68 ± 0.6 ng/ml) than those aged 3–4 years, but were still in the pre-vitellogenic stage (Fig. 2). Normal males at the age of 5 years had mature testes with spermatids and spermatozoa, and high levels of 11-KT and E2. Interestingly, the plasma steroid profile of the fish with intersex gonads was found to be similar to that of the males. Although they contained both male and female gonad parts, the ovarian part of the gonad was still young and pre-vitellogenic, whereas the male part contained mature testicular tissue. Barannikova et al. (2004), as well as Semenkova et al. (2002), found low levels of E2 in pre-vitellogenic females captured in the Caspian Sea, which increased during vitellogenesis. Our results show somewhat higher levels of E2 in females, males, and fish containing intersex gonads, as is often the case in fish grown under culture conditions (Amiri et al., 1996b; Pelissero et al., 1991; Webb et al., 1999, 2002). It should be noted that a wide range of steroid levels have been reported for sturgeons under natural conditions (Semenkova et al., 2002) and in culture (Amiri et al., 1996b; Pelissero et al., 1991; Webb et al., 1999, 2002).

11-KT levels found in this study in males and fish with intersex gonads were similar to those of Barannikova et al. (2004) for male Russian sturgeons with stage II gonads. However, they were lower than in the more advanced stages. These differences reflect natural vs. culture conditions, stage of maturity, feed composition and species specificity (Barannikova et al., 2004), and probably also differences in the assays.

In the present study, βLH mRNA levels were higher than βFSH mRNA levels in 5-year-old Russian sturgeon males and fish containing intersex gonads. This is in accordance with our previous work (Hurvitz et al., 2005). However, βFSH mRNA levels in the fish with intersex gonads were significantly lower than those of normal females and as low as in the males (Fig. 3). Although the females contained pre-vitellogenic follicles, their levels of βFSH mRNA were higher than those of males that exhibited mature testes. Taking both the plasma steroid levels and the expression of pituitary gonadotropins together, the fish with intersex gonads appear to be more similar to the normal males than to the normal females, although anatomically and histologically, the male part is smaller than the female part in the fish with intersex gonads. This could be due to the fact that the male part contains mature testis components, whereas the female part is premature.

The situation described in this study could stem from one or more of the following situations: (1) the fish were exposed to an endocrine-disruptive substance during a certain stage of their life; (2) the fish with intersex gonads have a different genetic background; (3) this specific group...
was exposed to a certain “shock” (such as temperature) which resulted in gonads with intersex parts.

Sex determination and differentiation are diverse and labile mechanisms in fish. A range of types of gonadal differentiation have been described, including gonochoristic species possessing purely ovarian or testicular tissue, as well as hermaphroditic species that can initially mature as either males (protandrous) or females (protogynous) (Devlin and Nagahama, 2002). The flexibility of sex-determination systems makes the fish sensitive to external influences, such as environmental or hormonal factors, such that even the sexual phenotype in gonochoristic fish can be modified (Devlin and Nagahama, 2002). Exogenous sex steroids administered at the time of sex determination can strongly influence the course of sex differentiation (Pferrer, 2001). For example, producing all-male tilapia by exposing tilapia larvae to 17α-methyltestosterone or other androgens is a common practice in aquaculture (Cruz and Mair, 1994; Desprez et al., 2003; Hiott and Phelps, 1993). Omoto et al. (2002) have shown incomplete feminization of a hybrid sturgeon, the Bester (H. huso x A. ruthenus), by feeding E2 at 10 μg/g diet to fish from 14 to 31 months of age, and complete feminization and masculinization by feeding E2 and MT, respectively, at only 1 μg/g diet from 3 to 18 months of age.

Endocrine disruptors such as DDT and PCBs are known to mimic the female sex hormone estrogen and can cause the feminization of male fish in both freshwater and marine environments. In northern England, wild populations of the flounder Platichthys flesus have been found to have high levels of vitellogenin in the plasma of males, as well as testicular abnormalities due to exposure to sewage effluent (Lye et al., 1997). In the Mediterranean Sea, evidence of the presence of fish containing intersex gonads in a wild population of Mediterranean swordfish (Xiphias gladius L.) has been recently reported. Forty of 162 specimens (25%) macroscopically classified as males showed the presence of female germ cells within the testes (De Metrio et al., 2003). In Italy, barbel (Barbus plebejus, Cyprinidae) captured in the Po River downstream of the confluence of a polluted tributary, the Lambro River, were found to have intersex gonads due to endocrine-disrupting chemicals (Vigano et al., 2001). The presence of specimens with intersex gonads is well documented in wild populations of gonochoristic teleosts (Allen et al., 1999; Nolan et al., 2001; Vigano et al., 2001), as well as in reptiles (Guillette et al., 1994) living in polluted environments.

Sturgeons are gonochoristic species maturing as either male or female; intersex (both types of gonads existing at the same time in one fish) is rare and unusual (Van Eenennaam and Doroshov, 1998). However, in the Mississippi River south of Saint Louis, Missouri, in the US, 29% of male shovelnose sturgeons (Scaphirhynchus platyrynchos) were found to contain intersex gonads, with testes containing ovigerous lamellae in addition to mature sperm. This phenomenon was caused by the organochlorine chemicals polluting the river water and accumulating in the fish’s tissues (Harshbarger et al., 2000).

Histological evidence for hermaphroditism in juveniles has been found in Odontesthes bonariensis, where a few oocytes at the diplotene stage were identified in the testis (Struussmann et al., 1996), and an intersex condition has also been identified in the roach, Rutilus rutilus: intersexuels were identified at very low (0.02) frequency in wild populations (Schultz, 1996). Similarly, oocytes (termed testis-ova) are apparent in the testis of Tilapia zillii (Yoshikawa and Oguri, 1978), Channa punctatus (Srivastava and Singh, 1989) and Dicentrarchus labrax (Roblin and Brusle, 1983), but these are usually smaller than the oocytes found within normal ovaries.

Since the fish with intersex gonads described in this study were females that contained small male gonad portions, estrogenic compounds or aromatase inhibitors are unlikely causes. Rather, androgenic compounds or some other factors related to the intensive aquaculture conditions may have caused the unusual intersex condition found here. This possibility, albeit viable, is unlikely since none of the other groups of sturgeons (5000 sturgeons from three different batches of eggs) that were raised at the same farm during the same time period, fed with the same trout feed and exposed to the same conditions, developed any signs of intersex gonads. Moreover, there was no intentional practice of any kind to reverse fish sex at this farm and fish with intersex gonads had never been found there until the described sturgeon case. Nevertheless, one cannot rule out the possibility that either the parent fish of the particular lot or the eggs, during their first 5 days of incubation in Russia, were exposed to some endocrine-disrupting chemicals or other environmental factors that may have caused the sexual abnormality.

Slowly evolving regions, such as those of rDNA, are employed for high-level taxonomic characterizations; on the other hand, fast-evolving ones, such as control regions, are employed to discriminate closely related species or populations belonging to the same species. The fact that in the phylogenetic analyses of both D-loop and Cyt b, the female fish, male fish and fish with intersex gonads clustered together, while other age-group clusters in other groups suggested that the fish with intersex gonads and those of both genders were, genetically from the same origin, eliminates the possibility of genetic variation between groups. In other words, the fish containing intersex gonads do not come from a hybrid between two species of sturgeon. Nevertheless, the genetic background of fish in the same population may differ in other than the D-loop and Cyt b, e.g., in the various genes controlling sex (such as DMY, DMRT1, aromatase, SOX9), resulting in a population containing individuals that respond differently to external factors. The possibility of a mutation in one or more of these genes cannot be ruled out.

Sex determination in fish is a flexible process with respect to evolutionary patterns observed among genera and families, and within individuals it is subject to
modification by external factors. These influences can affect the fate of both somatic and germ cells within the primordial gonad, and include the action of genetic, environmental (e.g., temperature, pH, day length, radiation, water quality, crowding, fertilization timing), behavioral and physiological factors (Chan and Yeung, 1983; Devlin and Nagahama, 2002). A change in one or more of these factors, at the first stages of sex determination in a specific group of sturgeons, could explain the emergence of intersex gonads. The biochemical and endocrine systems which have been found to influence sex differentiation in fish still do not provide complete insight into the initial events that eventually lead a germ cell to become a spermatogonium, an oogonium or a mixture of the two. Steroid and gonadotropin profiles, as well as the histological analyses describing intersex gonads in sturgeon, can shed some light on these processes.

Acknowledgments

This study is supported by a research grant from the Israeli Ministry of Science, Culture and Sport, Regional R&D, No. 01-18-00372. We thank Prof. David Kime, Sheffield, for providing the detailed ELISA protocol, as well as the 11-KT antiserum. The technical help of Ms. Rima Vaiman is acknowledged with appreciation.

References


Amiri, B.M., Maebayashi, M., Adachi, S., Yamauchi, K., 1996a. Testicular development and serum sex steroid profiles during the annual sexual cycle of the male sturgeon hybrid, the better. J. Fish Biol. 48, 1039–1050.


Schultz, H., 1996. Drastic decline of the proportion of males in the roach (Rutilus rutilus L.) population of Bautzen Reservoir (Saxony, Germany): results of direct and indirect effects of biomanipulation. Limnologica 26, 153–164.


