Husbandry and Dietary Effects on Sturgeon (Acipenser transmontanus) Farmed for Caviar

By

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Dedication

This work is dedicated to my parents.

Acknowledgment

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Abstract

A study was conducted at two sturgeon farms to test the hypotheses that husbandry and diets with different protein and lipid content affect the body weight, condition factor, caviar yield, egg texture and stage of development (egg size and germinal vesicle migration) at harvest and that vernalization affects the fish weight, condition factor, the concentrations of plasma sex steroids (testosterone, T and estradiol-17β, E_2), and the incidence of the ovarian follicular atresia. Ninety-two individually marked females, originated from the same brood fish were fed two different diets for 6 months on each farm, followed by a 5 month vernalization at a communal coldwater facility, where the dietary treatments continued. Fish were sampled, before and after vernalization, for length, weight, plasma T and E_2, and for caviar yield, egg diameter, polarization index (PI), and egg texture at the roe harvest. A two-way analysis of variance (ANOVA) did not reveal significant effects of farm, diet and their interaction on body weight, condition factor, caviar yield, egg texture and stage of development at harvest ($P > 0.05$). A mixed model with repeated measure revealed a significant decrease of fish weight, condition factor, and plasma T concentration during vernalization ($P < 0.0001$), as well as an effect of farm on plasma T concentration ($P < 0.0001$) and an interactive effect of farm × sampling time ($P = 0.0086$) on plasma E_2 concentration. Fishes maintained normal ovarian follicles and the plasma E_2 concentrations during vernalization period. Histological examination revealed ovarian follicular atresia in only one female at harvest.

Keywords: famed white sturgeon, temperature, commercial diet, caviar yield, plasma steroids.

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Introduction

Since the first commercial harvest of caviar from farmed white sturgeon (*Acipenser transmontanus*) began in 1995, caviar production on sturgeon farms of California has gradually increased to about 15,000 kg/year (Van Eenennaam et al., 2004; Peter Struffenegger, pers. communication). The white sturgeon caviar sells for $62 to $88 per ounce (28.35 g) depending on the grade (Sterling Caviar, 2010). However, during the past few years, more than 20% brood fish have produced low-grade eggs classified as "soft" (Peter Struffenegger, pers. communication). The caviar yield at harvest was also very variable in farmed population. The inferior caviar quality may affect consumer perception and may potentially lead to a significant loss of farm revenue. It was speculated that the softness of the sturgeon egg may be caused by the environmental conditions (such as high rearing temperature during the vitellogenic phase of ovarian development), management stress, and changes in commercial diet composition.

Although the vitellogenin synthesis and oocyte growth are stimulated by higher temperature (20-24 °C) in sturgeon (Doroshov et al., 1997), the late phase of vitellogenesis is sensitive to elevated temperature. It has been noted that holding sturgeon in cold water (12-14 °C) during late vitellogenesis and before final ovarian maturation prevents ovarian follicular atresia and allows fish to maintain elevated plasma concentration of sex steroids required for maintenance of the ovarian follicles (Webb et al., 2001; Linares-Casenave et al., 2002). The vernalization is currently used on farms to prevent follicular atresia. Gravid females are selected from the stock reared in warm water facility (20-24 °C) and transported in the fall to another, cold water facility (12-14 °C) where they are held until harvest in the spring. Handling and simulated transport

of the adult white sturgeon induced pronounced cortisol response (Belanger et al., 2001) that may potentially affect secretion of sex steroids and normal ovarian development. The dietary protein, lipid, and micronutrients are important determinants of the egg quality. Farmed white sturgeon are currently fed two different commercial diets based on the ingredients in salmon and trout diets. There is substantial literature describing nutritional requirements and dietary composition of sturgeon (Hung, 1991; Hung et al., 1997; Garcia-Gallego et al., 2009). But little is known about the effect of dietary ingredients on the differentiation and growth of eggs harvested for caviar.

We hypothesize that rearing temperature, diets and the management stress associated with the transfer of fish to the cold water facility affect the body weight, condition factor, caviar yield, egg quality, and plasma concentrations of sex steroids. The study was conducted at two commercial sturgeon farms, with each farm using two commercial diets. Our specific objectives were to:

1) determine effects of farm and diet on the body weight, condition factor, caviar yield, egg texture and stage of development (egg size and polarization index) at harvest;

2) determine effects of holding fish in coldwater facility (vernalization) on the body weight, condition factor, plasma concentrations of testosterone and estradiol-17β, and the incidence of the ovarian follicular atresia.

Literature review

General biology of sturgeon

Order Acipenseriformes represents the ancient ray-finned fish, sturgeon and paddlefish. Their fossils are known from the lower Jurassic (Bemis et al., 1997). The family of sturgeons, Acipenseridae, includes four genera (*Acipenser, Huso,* P*seudoscaphirhynchus* and *Scaphirhynchus*) and 25 extant species (Froese and Pauly, 2010). Eight species are found in North America, including five species of genus *Acipenser*: white sturgeon (*A. transmontanus*), green sturgeon (*A. medirostris*), Atlantic sturgeon (*A. oxyrinchus*), with the sub-species Gulf sturgeon (*A. oxyrinchus desotoi*), lake sturgeon (*A. fulvescens*) and shortnose sturgeon (*A. brevirostrum*); and three species of *Scaphirhynchus*: the shovelnose sturgeon (*S. platorynchus*), pallid sturgeon (*S. albus*), and the very rare Alabama sturgeon (*S. suttkusi*) (Froese and Pauly, 2010).

Sturgeons have polyploid origin and are subdivided by chromosome number into three groups of species, diploid, tetraploid, and hexaploid (Fontana et al., 2008). Sturgeon ancestor is hypothesized to have 2n = 60 chromosomes, and the diploid species have duplicated genome (2n = 120). Tetraploid and hexaploid species are believed to evolve from hybridization and genome duplication events (Fontana et al., 2008). Among the species in North America, only shortnose sturgeon is known to have hexaploid genome, with 2n = 372 chromosomes. Both Pacific species, white and green sturgeon, are tetraploid, with 2n = 271 and 249 chromosomes, respectively (Van Eenennaam et al., 1998; Van Eenennaam et al., 1999).

Sturgeon are longlived fish that have heavy, roughly cylindrical bodies and mostly cartilaginous skeleton (Doroshov, 1985; Conte et al., 1988). The life history pattern varies among the species. The shovelnose, pallid, Alabama, and lake sturgeon are potamodromous, living in freshwater during their entire life. Green, Atlantic, and Gulf sturgeon are anadromous, living in the saltwater and spawning in freshwater (Schultz, 2004). White and shortnose sturgeon are semi-anadromous, residing mainly in the brackish water estuaries but ascending rivers for spawning (Boreman, 1997; Schultz, 2004).

Sturgeon are iteroparous, but do not spawn annually in the wild. Spawning intervals are estimated to be 1-6 years for males and 2–11 years for females (Billard and Lecointre, 2001). The age at first sexual maturation varies among the species as well as male and female. In wild Atlantic sturgeon, the age and body size at first maturity are 12-14 years and 117-118 cm for males, and 14-18 years and 173-198 cm for females (Van Eenennaam and Doroshov, 1998). In wild white sturgeon, males sexually mature at age 10-12 years and females within the age interval 15-32 years.

Development of sturgeon aquaculture

The economic value of sturgeon was recognized in the 1860s. Since then, sturgeons were extensively fished, mainly for caviar (Wilson and Mckinley, 2004). Harvest of the Atlantic, lake, and white sturgeon reached peaks of 3,350; 3,000; and 2,494 metric tons, respectively, during 1890-1895 (Van Eenennaam et al., 2004). Overfishing and environmental impact on the sturgeon spawning habitat caused a rapid decline of wild populations in Europe and North America, stimulating the first studies on the artificial reproduction of sturgeon during late 1880s and early 1900s (Post, 1890; Ryder, 1890;

Stone, 1900; Carter, 1904, quoted by Van Eenennaam et al., 2004). Later, the ex-USSR established large-scale hatchery stocking program for sturgeon in the Caspian and Azov Seas, spawning wild broodstock, releasing juveniles, and harvesting mature females for caviar after their return to rivers for spawning (Marti, 1979, quoted by Doroshov, 1985). The history of the sturgeon hatchery propagation is a century old, but the reliable methods of artificial spawning and culture of the offspring have been established for Atlantic, shortnose, white and lake sturgeon relatively recently (Smith et al., 1980, 1981; Buckely and Kynard, 1981; Doroshov et al., 1983; Czeskleba et al., 1985, quoted by Van Eenennaam et al, 2004). Most recently, hatchery reproduction has been established for the Gulf (Parauka et al., 1991), shovelnose (Keenlyne, 1993), pallid (Burton, 2000), and green (Van Eenennaam et al., 2001) sturgeons.

Among eight native sturgeon species of North America, only white sturgeon has been bred in captivity for several generations in California and northern Italy. Shortnose sturgeon (listed as endangered in the U. S.) has been developed as an aquaculture species in Canada (Van Eenennaam et al, 2004). Four European species, Siberian (*A. baerii*), beluga (*H. huso*), stellate (*A. stellatus*), and Russian (*A. gueldenstaedtii*) sturgeons, are cultured in Florida (Tzankova, 2007).

White sturgeon dominates production of cultured species. Initially, white sturgeon aquaculture focused on the production of meat. Later, a decline of commercial sturgeon fisheries and growing market demand for caviar stimulated development of caviar production (Logan et al., 1995). Currently, farmed white sturgeon is marketed for both meat and caviar in North America and Western Europe (Van Eenennaam et al., 2004).

Production cycle of farmed white sturgeon

Farmed white sturgeon populations originated from the wild brood fish caught in the San Francisco Bay, Sacramento and Columbia Rivers during 1980-1995 (Conte et al., 1988; Doroshov et al., 1999). The brood fish were caught in different locations using trammel nets, baited hooks or snagging during the winter or spring and then transported to the hatcheries for spawning (Conte et al., 1988). Since the stage of gonad maturity varied at capture, some brood fish were immediately induced to spermiate and ovulate, while the others were held for several months in the hatchery until they reached full maturation (Van Eenennaam et al., 2004). Conte et al. (1988) provided comprehensive overview of the methods and techniques used in sturgeon hatcheries. In white sturgeon aquaculture, the domestically raised broodstock generations gradually replaced the wild-caught fish since 1990 and the use of wild brood fish ceased in 1995. In California, white sturgeon has been bred on farms over four generations since late 1980s.

The current caviar production system is based on rearing and management of multi-age cohorts (Sanders et al., 2003). Commercial culture cycle begins at a farm with spawning induction of domesticated broodstock. The gamete maturation is stimulated by the injection of 10-20 μg/kg gonadotropin releasing hormone agonist GnRHa. The milt is collected by catheter and the ovulated eggs by caesarian section (Van Eenennaam et al., 2004). The eggs are fertilized, using semi-dry method, and incubated in the MacDonald jars at temperature 15-18 °C (Conte et al., 1988; Dettlaff et al., 1993). The hatched larvae swim up and out of the incubator into the larval receiving tanks. The larvae are transferred to cultivation tanks and reared to large juveniles using artificial diets. External feeding begins in 8-10 days after hatch (Van Eenennaam et al., 2004). The juveniles are

reared in outdoor tanks to subadults (2-3 years) that have sexually differentiated gonads. Three-year old fish are sexed by making a small mid-ventral incision anterior to the pelvic fins or by using portable ultrasound equipment (Doroshov et al., 1983; Colombo, 2004; Wildhaber and Bryan, 2006). The males are selected for the brookstock and the rest is sold to the meat market. The females are reared for another 3-4 years until they reach full sexual maturity (7-8 years), and are harvested for caviar or selected for breeding (Van Eenennaam et al., 2004).

Reproductive Physiology of sturgeon

In farmed white sturgeon, males reach first sexual maturity at 3-4 years and the body weight of 7-14 kg. The majority of females mature at age 7-8 years (Doroshov et al., 1997). Both sexes reach first sexual maturity at early age compared to the wild fish as many other species used in modern aquaculture (Taranger et al., 2010). Cultured males exhibit an annual testicular cycle, with meiosis starting in the fall and spermiogenesis completed in February - June. Females have biennial ovarian cycle, with vitellogenesis beginning in the fall and a final ovarian maturation completed in March - June of the second year (Doroshov et al., 1997). Compared to spermatogenesis, the oogenesis is long and more asynchronous in population. The ovarian cycle includes differentiation of the ovarian follicle (age 2-3 years), the primary growth of the oocyte (age 3-4 years), vitellogenic growth of the oocyte (age 5-6 years), and a final ovarian maturation, resumption of meiosis, and ovulation (age 7-8 years) (Doroshov et al., 1997). During vitellogenesis, the oocyte increases in size due to uptake of the yolk precursor protein, vitellogenin synthesized in liver (Linares-Casenave et al., 2003). At the end of vitellogenic growth phase, the oocyte undergoes polarization), with a nucleus (germinal

vitellogenic growth phase, the oocyte undergoes polarization), with a nucleus (germinal vesicle) migrating to the animal pole and the yolk platelets and oil droplets to the vegetal hemisphere. After ovulation, the ovary contains post-ovulatory follicles and a new generation of oocytes in previtellogenic phase of development (Doroshov et al., 1997).

As in teleosts, the reproductive cycle of sturgeon is regulated by hypothalamus-pituitary-gonad reproductive axis and by environmental cues (Dettlaff et al., 1993; Doroshov et al, 1997). The brain peptide GnRH1 controls synthesis and release of two pituitary gonadotropins, follicle-stimulating hormone FSH and luteinizing hormone LH (Lescheid et al., 1995; Moberg et al., 1995; Querat et al., 2000). In males, FSH and LH modulate secretion of sex steroids controlling spermatogenesis and spermiation in males. In females, FSH is secreted mainly at the pre-vitellogenic and vitellogenic ovarian stages and regulates differentiation and growth of ovarian follicle during vitellogenesis, while the LH regulates final ovarian maturation and spawning (Moberg et al., 1995). The pituitary gonadotropins regulate synthesis and secretion of gonadal steroids, testosterone (T), 11-ketotestosterone (11-KT) and estradiol-17β (E_2). Testosterone stimulates synthesis and accumulation of the FSH and LH in pituitary gland (Pavlick and Moberg, 1997) and provides substrate for synthesis of E_2 in granulosa cells. The 11-KT is present in circulation of sturgeon female but its function is not known. The E_2 stimulates secretion of vitellogenin in liver and has important role in maintenance of the ovarian follicle until ovulation. The final ovarian maturation is controlled by the LH and mediated by the maturation-inducing steroid, 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P, Webb et al., 2002a). The endogenous regulation of the reproductive cycle in sturgeon is generally similar to that of teleosts (Moberg and Doroshov, 1996).

Effect of environmental factors on reproduction

During the growout phase, warm water (18-24 °C) is used on farm to achieve optimal growth and early puberty in sturgeon. However, the colder water (10-12 °C) is required for normal egg maturation and spawning. Thermal environment during late phase of vitellogenesis is an important environmental factor in reproductive cycle of sturgeon (Doroshov et al., 1997). Wild sturgeon enter Sacramento River in the late fall and spend several months at low temperatures (5-10 °C) before migration upstream and spawning in the spring at temperatures 14-16 °C (Kohlhorst, 1976). Farmed sturgeon must have a several month vernalization period (holding in the cold water from November to March or April) to successfully complete vitellogenesis and gamete maturation (Webb et al., 1999; Hochleithner and Gessner, 1999; Webb et al., 2001). The over winter exposure to cold water (10-12 °C) appears to be mandatory for normal germinal vesicle migration (GVM) in the oocyte, normal egg maturation (GVBD), and ovulation, as well as for the production of fertile eggs (Webb et al., 1999). When females are held in cold water, they exhibit a slow decrease in plasma E_2 from approximately 10 ng/ml at the peak of vitellogenesis to 1-2 ng/ml at maturation (Moberg et al., 1995). However, they maintain high plasma testosterone concentration (50-100 ng/ml) until spawning. Plasma concentrations of gonadotropins and sex steroids decrease when the fish in late stage of vitellogenesis are exposed to temperature 18 °C, resulting in the follicular atresia and regression of the ovary (Moberg and Doroshov, 1996; Webb et al., 1999; Webb et al., 2001; Linares-Casenave et al., 2002).

Photoperiodicity in sturgeon reproduction is largely based on timing of spawning season in natural populations (Dettlaff et al., 1993). Moberg et al. (1995) reported that the

seasonal day length modulates release of the FSH and LH from sturgeon pituitary. Most sturgeon farms in California and Idaho grow fish in the outdoor facilities exposed to natural photoperiod. We found no studies describing effect of manipulated photoperiod on reproductive cycle of sturgeon.

The salmonid formula based feeds are provided for the growout phase through the maturation of sturgeon (Hung, 1991). In commercial facilities, automatic (12 or 24 hour) and demand feeders are commonly used, and the sturgeon are usually consume 60-70% of the daily ration from the late afternoon through the night (Van Eenennaam et al., 2004). Currently, commercial diets with a variable amount of protein content from 30% to 50% and lipid content from 8% to 25% are used in the sturgeon aquaculture (Van Eenennaam et al., 2004). There is little information on the optimal feeding rates and feed conversion in sturgeon broodstock. High energy salmon diets promote growth and early maturity in female white sturgeon, but they also promote accumulation of fat and proliferation of adipose tissue in the ovary (Barrows et al., 2002). Caprino et al. (2008) reported lipid composition of caviar from farmed white sturgeon fed the squid oil (SQ) and soybean oil (SB) enriched diets. The total lipid content was similar for two diets but the SQ diet resulted in the higher n-3 polyunsaturated fatty acid (PUFA) content in the sturgeon eggs and in an increased egg yield.

Materials and methods

Study design

The study was designed as a 2 × 2 factorial experiment, with two farms (1 and 2) and two commercial diets (A and B) used at each farm. The two farms had similar ambient photoperiod but different temperature regimes. Tank emperature was recorded every 4 hours by temperature data loggers (Maxim Integrated Products, Inc. Sunnyvale CA, U.S.A.) at each farm from April to the end of September, 2008, and then at the coldwater site. Fish were transferred to this site at the end of September, 2008. The two commercial sturgeon diets were based on salmonid-type pelleted feed and were modified according to nutritional requirements of sturgeon (Hung, 2000). Fishmeal and fish oil were present in both diets. Diet A contained 42% protein and 18% fat, while Diet B contained 45% protein and 14% fat. The other ingredients included taste enhancers in Diet A and krill in Diet B.

The females used in this study originated from two separate spawning (March and August 2001) and were 8 years old at the time of caviar harvest. At both farms, fish were reared in outdoor flow-through tanks, with a diameter of 9.1 m and a depth of 1.5 m, filled with 89 m^3 water. In April 2008, farmers started feeding two randomly chosen production tanks on each farm, one with Diet A and the another with Diet B. The fish were fed to satiation, using demand feeders during the entire study period. On September 23rd - October 2nd 2008, fish were transported (40 min) in oxygenated tanks to a common coldwater facility for vernalization. Before transport, twenty three randomly chosen females from each production tank on each farm were individually tagged, measured for length (± 1 cm) and live weight (± 0.1 kg), and a blood was collected from the caudal

vasculature for steroid hormones analysis. At the cold water facility, all fish on Diet A from both farms were placed in a communal tank and fed Diet A until harvest. Similarly, the Diet B fish from both farms were placed in one tank and continued to receive Diet B. During March 2009, all individually tagged fish were sampled for fork length, body weight and blood plasma, and were immediately transported to the caviar plant. At harvest, fish were euthanized by concussion to the head and re-weighed (± 0.1 kg). Ovaries were removed and weighed, and the eggs were separated from the ovaries using a stainless steel screen. Separated eggs were weighed (± 1 g), salted, packed in variable capacity tins (30-1800 g), and re-weighed in the tins after packing, to account for some loss of water after salting. Condition factor was calculated as $K = 100 \times$ (body weight, g / fork length^3, cm)

Caviar yield, egg texture and stage of development

At harvest, screened egg weight (SEW) for each fish was recorded as a total weight of eggs separated from the ovarian tissue before salting. Tinned egg weight (TEW) was a total weight of salted and packed eggs from each fish. The egg yield was calculated as a percent body weight (TEW%).

Fifteen eggs from each female fixed in 10% formalin were measured for diameter (± 0.001 mm) and then bisected with a razor blade along the animal–vegetal axis. PI was calculated as the distance of the egg nucleus from the inner border of the chorion divided by the egg diameter (Chapman and Van Eenennaam, 2007). All measurements were made using a dissecting scope with camera lucida and a digital image analyzing tablet. Egg texture analysis was conducted to determine the firmness of the eggs, one of the discriminative sensory characteristics of caviar. Random samples of freshly separated

eggs were kept in sturgeon Ringer solution at temperatures 7.7-8.0 °C during analysis. Thirty individual eggs from each female were measured for texture with a TA-XTPlus Texture Analyzer (Texture Technologies Corp. Scarsdale NY, U.S.A.). The firmness was defined as the peak force in Newton required to burst the egg at the first compression of the egg to 60% of its original height and was calculated using Texture Technologies Exponent Software Version 4.010.0 (Texture Technologies Corp. Scarsdale NY, U.S.A.).

Plasma sex steroids

Approximately 20 ml of blood was collected from each fish. Plasma was separated by centrifugation at 3400 rpm for 5 minutes, and stored at -80 °C until analyzed. Plasma T and E_2 were measured by radioimmunoassay following method of Fitzpatrick et al. (1986) modified by Webb et al. (2002a). 100 µl of plasma was extracted twice with 2 ml of diethyl ether (Webb et al., 2002a). The recovery was 96% for T and 86% for E_2. The intra- and inter-assay variations were less than 5% and 10%, respectively. The assay detection limit was 1.0 ng/ml for T and 0.2 ng/ml for E_2.

Histology of ovarian follicles

Histological analysis was conducted to examine structural changes in the egg chorion, which are sensitive indicators of the early stage of ovarian atresia (Linares-Casenave et al., 2002). Formalin-fixed eggs from each female were dehydrated, cleared and infiltrated with paraffin in a Tissue-Tek VIP 5 vacuum infiltration processor (Sakura Finetek USA, Inc. Torrance CA, U.S.A.). These dehydrated eggs were embedded into paraffin blocks in a Tissue-Tek TEC embedding center (Sakura Finetek USA, Inc. Torrance CA, U.S.A.). Successive 5 µm thick sections from each block were made using LKB historange microtome (LKB, Bromma, Sweden) and stained by hematoxylin and

Eosin (H&E) and periodic acid Schiff (PAS) (Lana, 1968; Presnell and Shchreibman, 1997). Schiff's reagent was purchased from Fisher Scientific Co. Fair Lawn NJ, U.S.A. and tested for activity with 37% formalin before each staining session. Control for the PAS staining was an omission of the oxidation reaction with periodic acid (Fausto et al., 2004). All slides were examined under Olympus BH-2 microscope with 4×, 10×, and 20× objectives and digital imaging software.

Statistical analysis

Owing to some mortality that occurred after transport of the fish to the cold water facility, data were unbalanced. In addition, one fish in the treatment Diet A × Farm 1 was excluded from analysis because it was in a late stage of ovarian follicular atresia. Data from the remaining 20 fish fed Diet A on Farm 1, 20 fish fed Diet B on Farm 1, 21 fish fed Diet A on Farm 2, and 15 fish fed Diet B on Farm 2 were used for analysis.

The measurements of TEW and TEW% at caviar harvest were analyzed by two-way analysis of variance (ANOVA), according to the linear fixed model:

$$Y_{ijk} = \mu + Farm_i + Diet_j + Farm \times Diet_{ij} + e_{ijk} \ (i = 1, 2; j = A, B; k = 1, 2,..., n_{ij})$$

where Y_{ijk} is the k^{th} observation from a fish fed the j^{th} diet from the i^{th} farm, μ is a constant, $Farm_i$ is the effect of the i^{th} farm, $Diet_j$ is the effect of the j^{th} diet, $Farm \times Diet_{ij}$ is the interaction effect of the i^{th} farm and the j^{th} diet and e_{ijk} is the random residual for the ijk^{th} observation with null mean and variance σ_e^2.

The measurements of egg diameter and PI had fifteen observations and that of egg texture at harvest had thirty observations on individual fish, and therefore a term for the

random contribution of the observed fish was included in the model. Accordingly, the mixed linear model was applied:

$Y_{ijkl} = \mu + Farm_i + Diet_j + Farm \times Diet_{ij} + Fish_{ijk} + e_{ijkl}$ ($i = 1, 2$; $j = A, B$; $k = 1, 2, 3,..., n_{ij}$; $n_{ij} = 1, 2,...,15$ for egg diameter and PI and $n_{ij} = 1, 2,..., 30$ for egg texture)

where Y_{ijkl} is the l^{th} observation from an egg in the k^{th} fish fed the j^{th} diet from the i^{th} farm, $Fish_{ijk}$ is the random effect (with null mean and variance σ_f^2) of the k^{th} fish nested in the ij^{th} farm by diet subclass, and e_{ijkl} is the random residual (with null mean and variance σ_e^2) for the $ijkl^{th}$ observation.

The measurements of body weight, K, and plasma steroids (T and E_2) were taken more than once on each fish. The analysis of these repeated measurements were based on the mixed linear model:

$Y_{ijkl} = \mu + Farm_i + Diet_j + Time_l + Farm \times Diet_{ij} + Farm\times Time_{il} + Diet\times Time_{jl} + Fish_{ijk} + e_{ijkl}$ ($i = 1, 2$; $j = A, B$; $k = 1, 2, 3,..., n_{ij}$; $l = 1, 2$)

where Y_{ijkl} is the l^{th} observation of the k^{th} fish fed the j^{th} diet from the i^{th} farm and $time_l$ is the effect of the l^{th} time of measurements. The effect of fish was considered a random effect, as above, whereas all the other elements of the model were evaluated as fixed effects. Differences were considered significant at $P \leq 0.05$. Computations for all analyses were done with Statistical Analysis System (SAS) software version 9.2.

Results

Temperature regime

Minimum – maximum tank temperatures on Farm 1 were 18.0-23.5 °C, while Farm 2 temperatures ranged 16.5-28.0 °C. Average tank temperature on Farm 1 during the growout phase (June - August 2008) was 2.5 °C lower compared to Farm 2 (Fig. 1). At the coldwater vernalization facility, the average temperature from October 2008 to March 2009 was 12 ± 3 °C (mean ± SD).

Farm and diet effects on the body weight, condition factor, caviar yield and egg quality

Data collected at harvest are summarized in Table 1. Only TEW and TEW % are presented since they are more relevant to commercial production of caviar. Screened egg weight (SEW and SEW %) showed the same results in statistical analysis ($P > 0.05$), and TEW had significant linear relationship with SEW (TEW = 0.9945 SEW - 36.4885 (g); $R^2 = 0.994$; $P = 3.69 \times 10^{-83}$). The effects of farm, diet, and farm × diet interaction on fish weight, condition factor, caviar yield, and all the egg characteristics at harvest were not significant (Table 2).

Egg diameter in pooled treatments ranged 3.13-3.30 mm and the individual female oocyte polarization index (PI) ranged 0.08-0.16 (25-75% quartiles). The eggs in 63% females (N = 76) did not reach definitive size associated with the oocyte, $PI \leq 0.100$, at the time of harvest (Chapman and Van Eenennaam, 2007).

The effects of holding in coldwater facility

The individual fish body weight and condition factor significantly decreased between the first (Oct 2008) and second (Mar 2009) sampling ($P < 0.0001$, Table 3). On

an average, fish lost around 3 kg (6%) body weight, and condition factor decreased from 1.14 to 1.06 (Fig. 2). The effects of farm × diet, farm × time, and diet × time interactions were not significant (Table 3).

Plasma T concentrations were significantly affected by the farm origin ($P < 0.0001$) and sampling time ($P < 0.0001$, Table 3). However, there was no significant effect of diet or interaction effects (Table 3). Mean plasma T concentration significantly decreased from 101.13 ng/ml to 67.64 ng/ml at Farm 1 and 136.49 ng/ml to 115.04 ng/ml at Farm 2 after the 5-month vernalization period ($P < 0.0001$, Fig. 3).

Plasma E_2 concentrations were significantly affected by the interaction between the farm origin and sampling time ($P = 0.0086$, Table 3). In the Farm 1 fish plasma E_2 did not increase greatly (3.44 ng/ml and 3.71 ng/ml at first and second sampling, respectively), while in the Farm 2 fish E_2 exhibited greater increase from 2.25 ng/ml to 3.64 ng/ml between two samplings (Fig. 3).

Histological analysis

Seventy six females of a total 77 sampled at caviar harvest did not exhibit signs of ovarian atresia. The follicular atresia in advanced stage was found in a single female from the treatment Diet A × Farm 1. Plasma E_2 concentration in this fish was below detection limit (< 0.2 ng/ml) and plasma T concentration was 2.64 ng/ml.

Typical section of the egg envelope (zona radiata, or chorion) in normal, non-atretic female shows three striated layers (L_1 - L_3, Fig. 4A). All three layers of chorion are stained PAS-positive (magenta color) and don't show signs of degeneration. Positive PAS staining indicates presence of carbohydrates in all three layers. The cells of

layer are evenly spaced and don't exhibit hypertrophis changes, characteristic for follicular atresia (Fig. 4A) (Linares-Casenave et al., 2002). The basal lamina positioned between granulosa and thecal cell layer is also PAS-positive (Fig. 4A). Cortical granules in the cytoplasm cortex are PAS-positive and appear to be very close to the oolemma. The PAS control slide does not exhibit staining of chorion, basal lamina, and cortical granules (Fig. 4B).

In a single fish with follicular atresia, the chorion, granulosa layer, cortical alveoli, melanin granules, and yolk platelets are degraded. The dark pigment (aging pigment, product of lipid peroxidation) aggregates in the ovular space (Fig. 4C, D). The thecal layer is heavily vascularized and increases in thickness. The PAS-positive basal lamina is still present at this advanced stage of atresia (Fig. 4D).

Discussion

Farm and diet effects on harvest

Although the factorial analysis of variance at harvest in this study did not reveal any significant effects of farm and diet on caviar yield, the limited number of fish used in this study could have affected statistical result. Some trends suggest potential dietary effect, as sturgeon fed Diet B had tendency to produce more caviar than Diet A, on both farms (Table 1). This is in agreement with previous report that the diet lower in lipid resulted in higher caviar yield as a percent of ovary weight due to decrease of the fat contents in the ovaries (Barrows et al., 2002).

The PI is a measure of the stage of egg maturation, and the egg diameter is a measure of the egg growth. The statistical analysis did not reveal significant effects of farm and diet on the egg diameter and PI, indicating that the farm and diet did not affect the egg maturation or egg growth. The high ratio of the fish variance to the total variance of the egg diameter and PI (0.43 and 0.82, respectively, Table 2) indicates that this study was limited by the small number of sampled fish. The trend that sturgeon fed Diet B had slightly larger eggs and a relatively lower P value on both farms (Table 1) suggests a potential dietary effect. The tendency for the larger eggs in fish fed the higher protein, such as in Diet B, is in agreement with increased egg diameter in trout fed diets with increased levels of protein (Smith et al., 1979).

The PI and egg diameter have an inverse relationship. In addition, the definitive egg size may depend on the fish size and individual fecundity, a total number of grown eggs. Generally, the definitive egg size is reached in sturgeon when the PI value is less than

0.100 and the oocytes cease vitellogenic growth and approach maturation (Doroshov et al., 1997). In this study, the eggs of 37% females had PI's less than 0.100 and thus had reached definitive size at caviar harvest. The high percentage of fish with the eggs in a less advanced stage (PI > 0.100) occurred in all treatments. This indicates that the large proportion of fish harvested in March did not fully complete oocyte growth and the caviar yield could increase if these fish are harvested later in the season. Transferring the fish to a cold water facility later in the fall and/or harvesting later in the spring could potentially increase caviar yield.

Egg texture was not affected by the rearing temperature at two farms in this study, as there was no significant effect of the farm origin (Table 1 and 2). Diet had no significant effect on egg texture, but the eggs from Diet A treatment were consistently firmer on both farms, compared to Diet B (Table 1). This potential dietary effect should be studied further. Similar to the egg diameter and PI data, the low number of sampled fish, particularly in Farm 2 × Diet B treatment, resulted in the high ratio of the fish variance to the total variance reaching 0.56 (Table 2). The ratio of residual variance to the total variance (0.44) indicated that the correlation of observation on the eggs from the same fish was high.

Vernalization effects on body weight and plasma sex steroids

Fish in all four treatments lost weight and their condition factors decreased during the vernalization period. In many species, somatic growth decreases before the spawning due to either reduction or cessation of feed intake because gamete production and reproductive behavior affect the timing and magnitude of the somatic growth (Taranger et al., 2010). However, the loss in fish weight observed in this study appears to be

somewhat excessive. An alternative explanation could be the stress associated with fish transport and the subsequent environmental conditions at the vernalization site, such as potential effect of hypoxia. It may be hypothesized that the weight loss could lead to ovarian follicular atresia, but this was not the case in this study, as only 1 of the 77 females exhibited follicular atresia, whereas the remaining females had intact follicles and physiologically normal plasma sex steroid concentrations for normal oogenesis, compared to fish undergoing follicular atresia. Previous observations indicate that ovarian regression occurs in sturgeon when the plasma E_2 concentrations decrease below 0.5 ng/ml and the plasma T below 30 ng/ml (Webb et al., 2001, Linares-Casenave et al., 2002, Talbot et al., 2010).

The testosterone concentration was higher in Farm 2 fish before transport to coldwater facility but decreased proportionally to their initial concentrations in fish of both farm origins during vernalization. The difference in initial concentrations is difficult to explain but it may be related to the differences in the temperature regimes of tanks in two farms, and potentially in tank stocking densities. Interestingly, the Farm 2 fish maintained higher plasma T concentrations even after several months of holding in the communal tanks of coldwater facility. It should be noted that the fish from both farms (except for the one that had follicular atresia) had plasma testosterone above 30 ng/ml before harvest.

Our data on plasma E_2 concentrations (Fig. 3) indicate that plasma E_2 slightly increased and remained at a level required for the normal late oogenesis in sturgeon during vernalization. The majority of fish in this study had physiologically normal plasma concentration of E_2 before harvest, concordant with the very low (one fish)

incidence of the follicular atresia (Webb et al., 1999, Webb et al., 2001, Linares-Casenave et al., 2002). The increase of plasma E_2 concentration in Farm 2 fish may relate to the stocking densities in the communal tanks since the individual fish could absorb by gills waterborne sex steroids released by other fish in water (Scott and Ellis, 2007).

Ovarian follicle and follicular atresia

In the ovarian follicle of white sturgeon, three layers of chorion, basal lamina, and cortical granules were stained by PAS but not reacted in the control. This indicates glycoprotein nature of all three layers of sturgeon chorion (Fig. 4A, B). The study did not reveal early stage of ovarian atresia in sampled fish. The ovarian atresia in sturgeon is initiated by the transformed granulosa cells exhibiting phagocytic activity, including lysosomal digestion of the chorion. The phagocytic cells continue invade ovular space in the intermediate stage of atresia and digest the oocyte content (Linares-Casenave et al., 2002). Interestingly, the basal lamina remained intact in the advanced stage of atresia observed in one fish in this study, in agreement with the observation of Linares-Casenave (2002) (Fig. 4D). In the advanced phase of atresia, the melanin granules were absorbed and the dark pigments appeared (Fig. 4C, D).

A single female that was found in this study in advanced stage ovarian follicular atresia had plasma T concentration 2.64 ng/ml and the E_2 below detection limit. Other females had elevated plasma sex steroids. This is in agreement with the previous studies that suggested that a certain plasma concentrations of sex steroids, above 30 ng/ml for T and above 0.5 ng/ml for E_2, are required for normal maintenance of ovarian follicles in the final stages of oogenesis (Webb et al., 1999, Webb et al., 2001, Linares-Casenave et al., 2002, Talbot et al., 2010).

Conclusions

The study did not reveal significant effects of farm and diet on the body weight, condition factor, caviar yield and egg quality at harvest. Vernalization of fish in the cold water facility led to the loss of body weight and decrease in condition factor. The plasma concentrations of testosterone decreased at both farms, but the plasma concentrations of estradiol-17β were maintained at the same level or slightly increased. Farm origin had effects on plasma sex steroid concentrations, but the fish from both farms had a normal physiological levels of sex steroids in the late phase of oogenesis and follicular atresia was observed in only one fish.

Table 1. Summary of caviar harvest data (LSM ± SE). BW: Body Weight; K: Condition Factor; TEW: Tinned Egg Weight; TEW%: TEW as a percent of BW; T: Testosterone; E_2: Estradiol-17β; ED: Egg Diameter; PI: Polarization Index. Fifteen eggs were measured for ED and PI for each fish, and thirty eggs for texture.

	Farm 1		Farm 2	
	Diet A	Diet B	Diet A	Diet B
BW(kg)	41.18 ± 1.82	38.49 ± 1.82	41.39 ± 1.77	38.94 ± 2.10
K	1.024 ± 0.025	1.070 ± 0.025	1.100 ± 0.025	1.074 ± 0.029
TEW (g)	3462 ± 244	3617 ± 244	3476 ± 238	3552 ± 282
TEW (%)	8.523 ± 0.477	9.348 ± 0.477	8.401 ± 0.466	9.034 ± 0.551
ED (mm)	3.201±0.031	3.233±0.031	3.195±0.030	3.284±0.036
PI	0.150±0.011	0.110±0.011	0.124±0.011	0.124±0.013
Texture (N)	0.422±0.017	0.408±0.017	0.424±0.016	0.406±0.020

Table 2. Summary of P values and variance for variables at caviar harvest. For the fixed linear model, variance is MSE. For the mixed linear model, variance is composed of variance from fish and residual.

	P value			Variance		
	Farm	Diet	Farm × Diet	Total	Fish	Residual
BW (kg)	0.8593	0.1769	0.9495	66.29		
K	0.1272	0.7097	0.1748	0.0127		
TEW (g)	0.9194	0.6478	0.8754	1.19×10^{6}		
TEW (%)	0.6603	0.1448	0.8467	4.555		
ED (mm)	0.4913	0.0667	0.3768	0.0332	0.0185	0.0147
PI	0.6285	0.0998	0.0965	3.139×10^{-3}	2.588×10^{-3}	5.51×10^{-4}
Texture (N)	0.9982	0.3681	0.9252	1.254×10^{-2}	5.466×10^{-3}	7.073×10^{-3}

Table 3. Summary of P values and variance for variables between treatments. Variance is composed of variance from fish and residual. F: Farm; D: Diet; T: Time. F×D: Farm × Diet interaction. F×T: Farm × Time interaction. D×T: Diet × Time interaction.

	P value						Variance		
	Farm	Diet	Time	Farm × Diet	Farm × Time	Diet × Time	Total	Fish	Residual
BW (kg)	0.9748	0.2460	**<0.0001**	0.7335	0.9270	0.4640	68.60	66.52	2.08
K	0.1472	0.5597	**<0.0001**	0.0503	0.2220	0.8940	0.0141	0.0126	0.0015
T (ng/ml)	**<0.0001**	0.2935	**<0.0001**	0.5619	0.1837	0.3225	1074.95	319.02	755.93
E_2 (ng/ml)	0.2338	0.2760	**<0.0002**	0.1107	**0.0086**	0.3224	5.990	4.364	1.626

Figure legends

Figure 1. Tank thermographs, recorded every four hours. Solid and dashed lines represent Farm 1 and 2, respectively.

Figure 2. Body weight and condition factor (K) (LSM ± SE) before transport to the coldwater facility (Oct 08) and at caviar harvest (Mar 09). A: Body weight. Means are significantly different ($P < 0.0001$). B: condition factor (K). Means are significantly different ($P < 0.0001$).

Figure 3. Plasma sex steroid concentration (LSM ± SE) for fish at each farm before transport to the coldwater facility (Oct 08) and at caviar harvest (Mar 09). A: Plasma concentration of testosterone (T). Means are significantly different between Farm 1 (white bar) and Farm 2 (black bar), and between sampling times, $P<0.001$. B: Plasma concentration of estradiol-17β (E_2). Solid and dashed lines represent Farm 1 and 2, respectively. The effect of farm × sampling time interaction was significant ($P = 0.0086$).

Figure 4. Photomicrographs of histology of ovarian follicles. A: PAS staining of the ovarian follicle. B: PAS control staining. C: H&E staining of late follicular ovarian atresia. D: PAS staining of late follicular ovarian atresia. BL: basal lamina; CG: cortical granules; EV: egg envelope; GL: granulosa cell layer; MG: mlanin granules; OG: oil globules; PG: pigment granules; TL: thecal cell layer; YG: yolk globules.

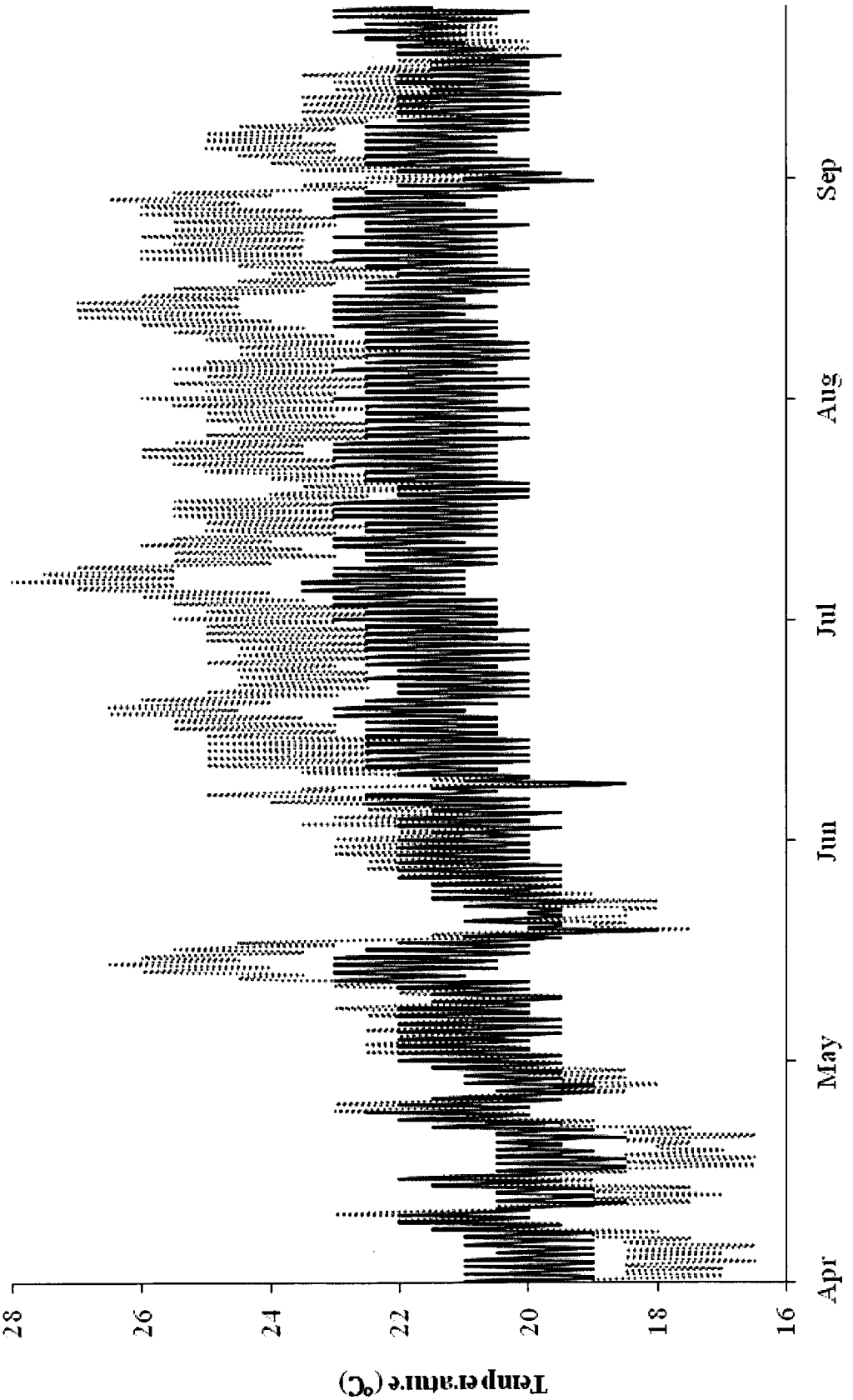


Figure 1

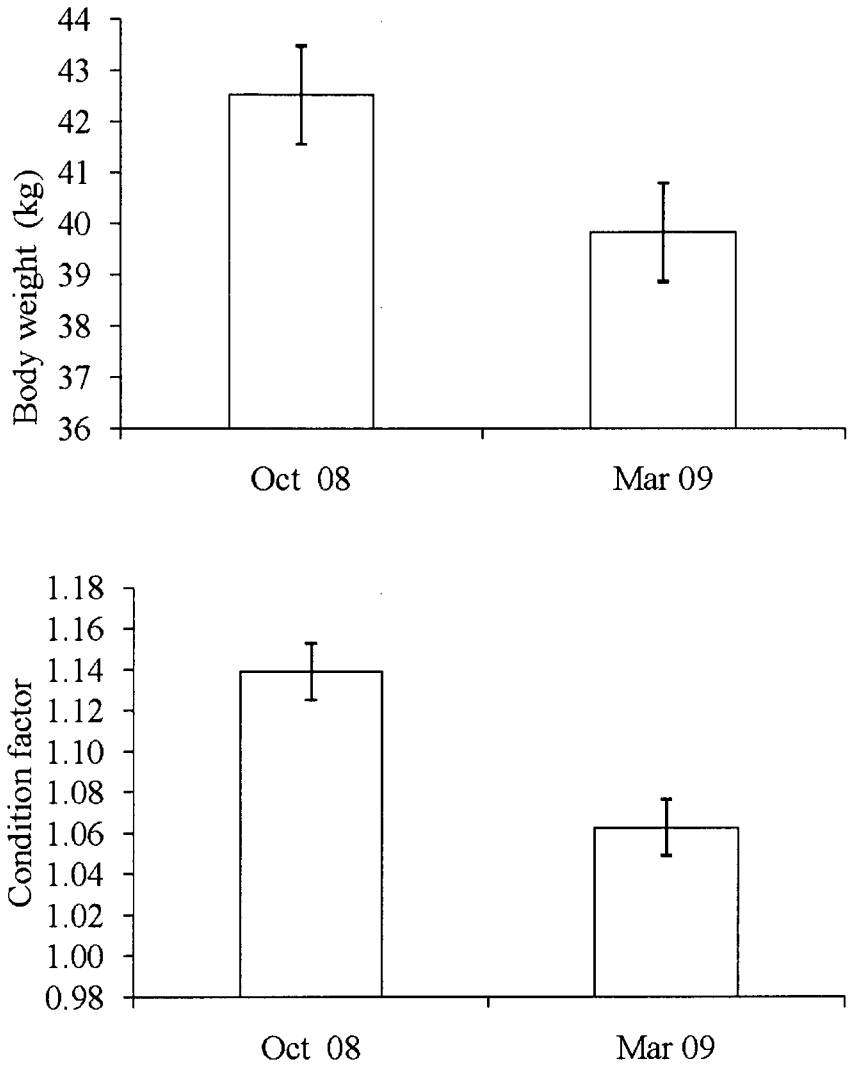

Body weight (kg)
44
43
42
41
40
39
38
37
36
Oct 08
Mar 09
Condition factor
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
Oct 08
Mar 09

Figure 2

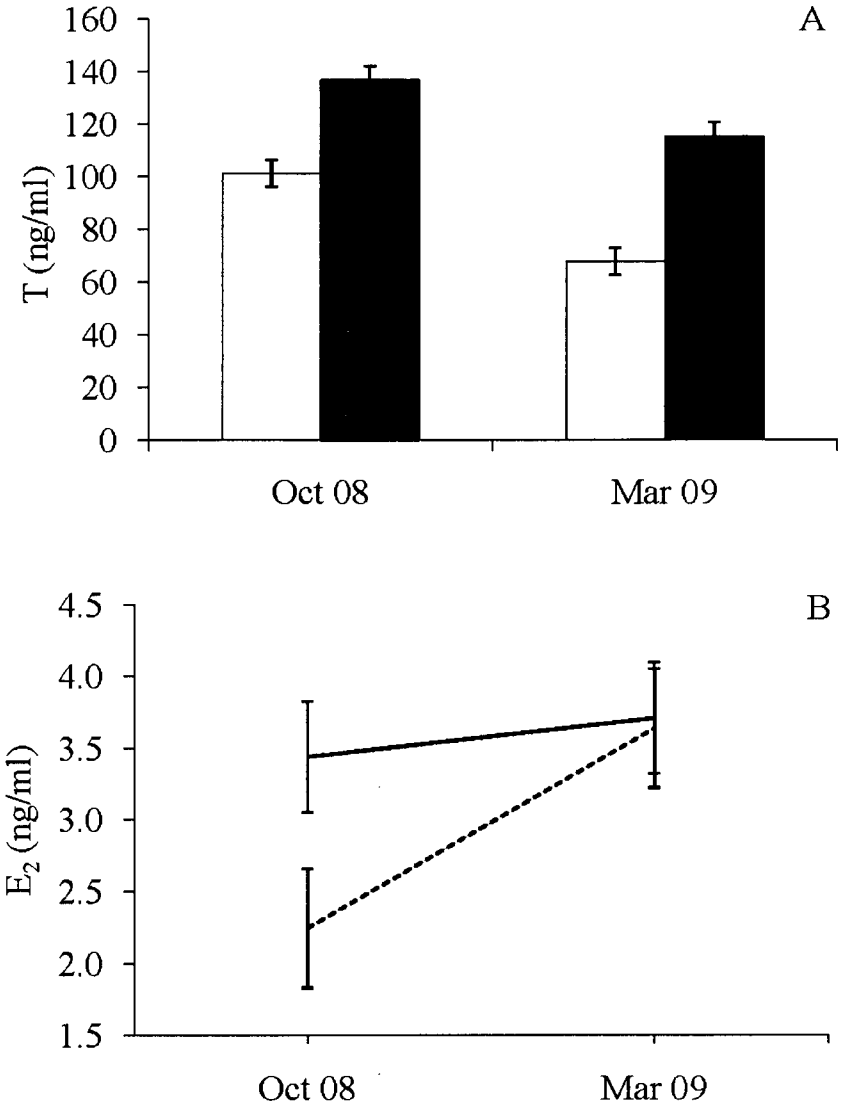
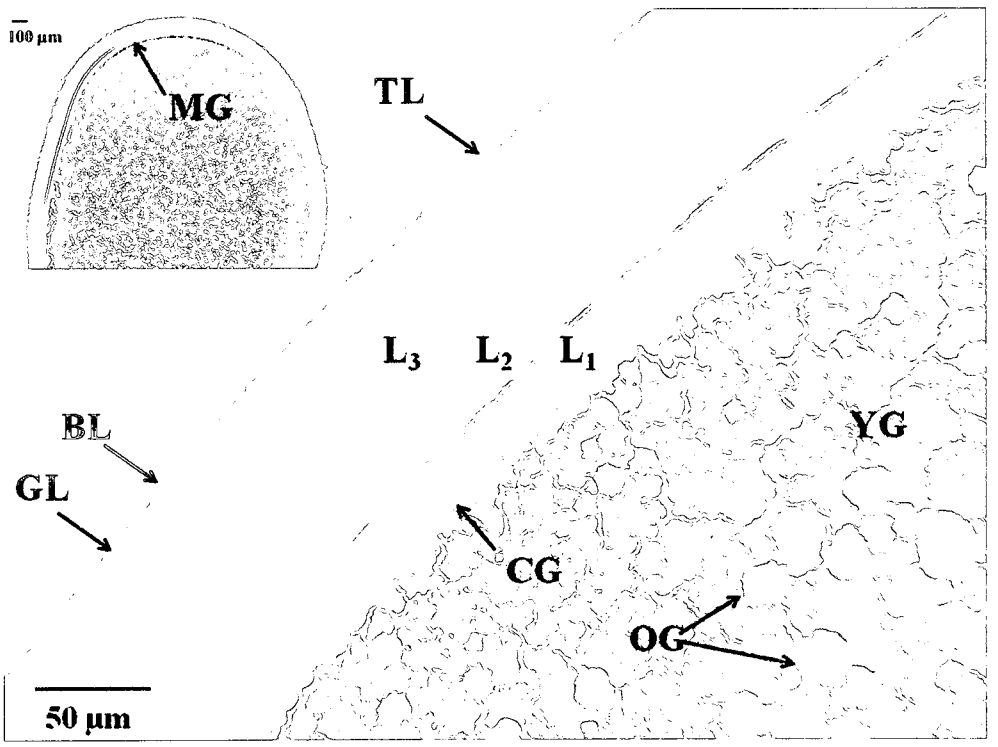

A
160
140
120
100
80
60
40
20
0
T (ng/ml)
Oct 08
Mar 09
B
4.5
4.0
3.5
3.0
2.5
2.0
1.5
E2 (ng/ml)
Oct 08
Mar 09

Figure 3

A


100 µm
MG
TL
L3 L2 L1
BL
GL
YG
CG
OG
50 µm

B

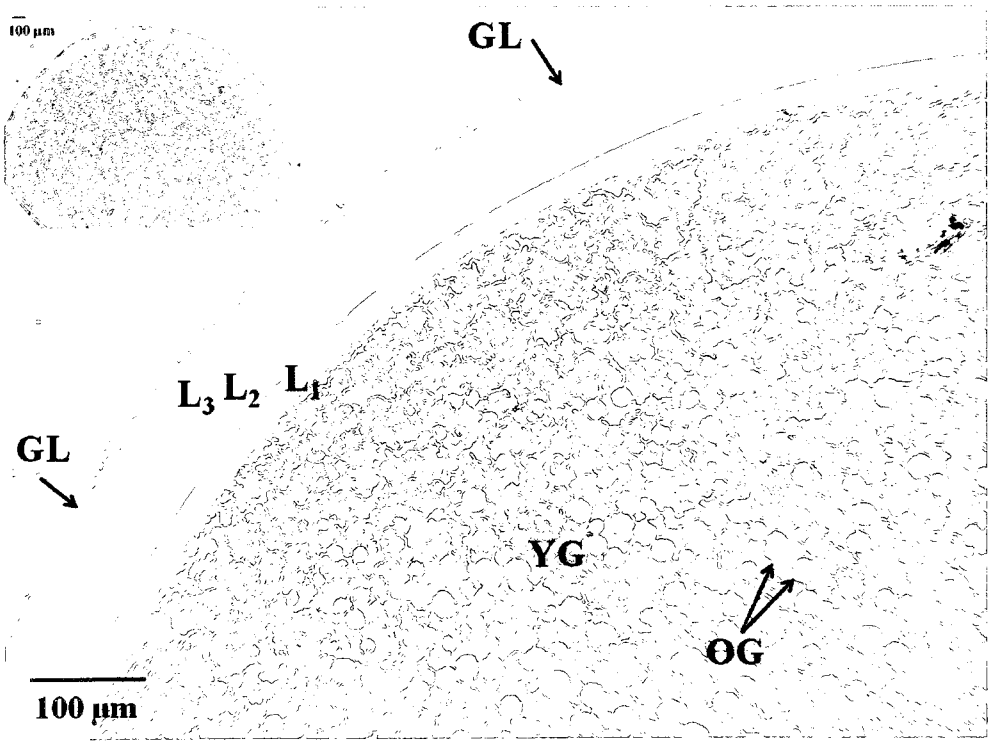
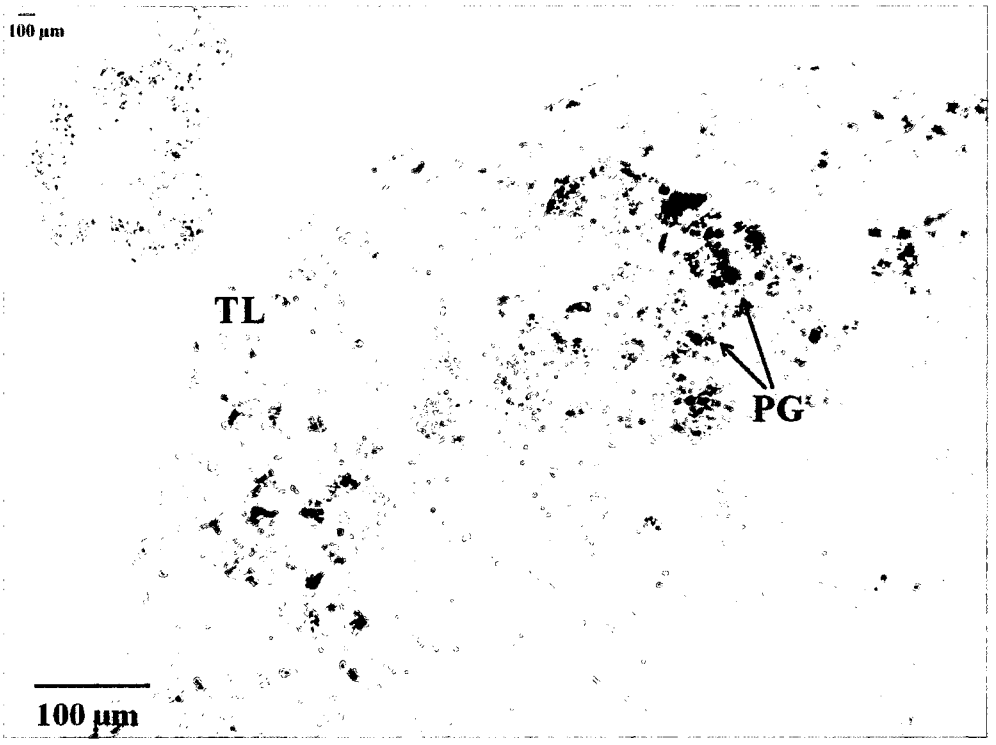

100 µm
GL
L3 L2 L1
GL
YG
OG
100 µm

Figure 4

C



D

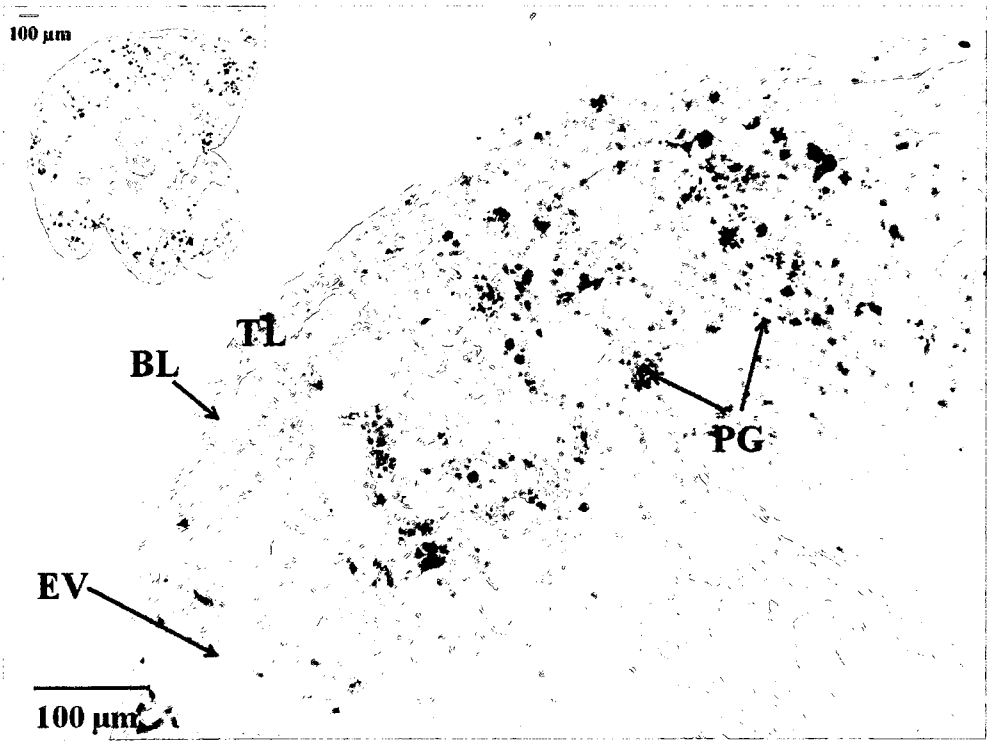


Figure 4

References

Barrows, F.T., Van Eenennaam, J.P., Doroshov, S.I. 2002. The effect of phase feeding energy on growth, maturation and caviar production of white sturgeon, *Acipenser transmontanus.* In: Rosenthal, H., Battenfeld, C., Bruch, R.M., and Binkowski, F.P. (Eds). Technical compendium to the proceedings of the 4th International Symposium on Sturgeon. Extended Abstract (No 180). University of Wisconsin, Sea Grant Institute.

Belanger, J. M., Son, J. H., Laugero, K. D., Moberg, G. P., Doroshov, S. I., Lankford, S. E., and Cech, J. J. Jr. 2001. Effects of short-term management stress and ACTH injections on plasma cortisol levels in cultured white sturgeon, *Acipenser transmontanus*. Aquaculture. 203, 165-176.

Bemis, W. E., Findeis, E. K., and Grande, L. 1997. An overview of Acipenseriformes. Environ. Biol. Fish. 48, 25-71.

Billard, R. and Lecointre, G. 2001. Biology and conservation of sturgeon and paddlefish. Rev. Fish. Biol. Fish. 10, 355-392.

Boreman, J. 1997. Sensitivity of North American sturgeons and paddlefishes to fishing mortality. Environ. Biol. Fish. 48, 399-405.

Buckley, J. and Kynard, B. 1981. Spawning and rearing shortnose sturgeon from the Connecticut River. Prog. Fish-Cult. 43, 74-76.

Burton, K. 2000. New hope for the pallid sturgeon. Endangered Species Bulletin. Vol. XXV, No. 2, 4-5.

Caprino, F., Moretti, V. M., Bellagamba, F., Turchini, G. M., Busetto, M. L., Giani, I., Paleari, M. A., and Pazzaglia, M. 2008. Fatty acid composition and volatile

compounds of caviar from farmed white sturgeon (*Acipenser transmontanus*). Anal. Chim. Acta. 617, 139-147.

Carter, E. N. 1904. Notes on sturgeon culture in Vermont. Trans. Am. Fish. Soc. 33, 60-75.

Chapman, F. A. and Van Eenennaam, J. P. 2007. Sturgeon aquaculture - specialized techniques: Determining the stage of sexual maturity in female sturgeon for artificial spawning: the egg polarization index or PI. Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Colombo, R. E., Wills, P. S., and Garvey, J. E. 2004. Use of ultrasound imaging to determine sex of shovelnose sturgeon. N. Am. J. Fish. Manage. 24, 322-326.

Conte, F. S., Doroshov, S. I., Lutes, P. B., and Strange, E. M. 1988. Hatchery manual for the white sturgeon (*Acipenser transmontanus* Richardson) with application to other North American Acipenseridae. Division of Agriculture and Natural resources, University of California, Oakland, California. Publ. 3322.

Ćzeskleba, D.G., AveLallemant, S., and Thuemler, T.F. 1985. Artificial spawning and rearing of lake sturgeon, *Acipenser fulvescens*, in Wild Rose State Fish Hatchery, Wisconsin, 1982-83. In: F.P. Binkowski, F. P. and Doroshov, S. I. (Eds.) North American Sturgeons: Biology and Aquaculture Potential. Dr. W. Junk Publishers, Dordrecht, pp. 79-86.

Dettlaff, T. A., Ginsburg, A. S., and Schmalhausen, O. I. 1993. Sturgeon fishes: developmental biology and aquaculture. Springer-Verlag, New York.

Doroshov, S. I. 1985. Biology and culture of sturgeon, Acipenseriformes. In: Muir, J. F. and Roberts, R. J. (Eds.) Recent Advances in Aquaculture. Westview Press, Boulder, Colorado, pp. 251-274.

Doroshov, S. I., Clark, W. H. Jr., Lutes, P. B., Swallow, R. L., Beer, K. E., McGuire, A. B., and Cochran, M. D. 1983. Artificial propagation of the white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture. 32, 93-104.

Doroshov, S. I., Moberg, G. P., and Van Eenennaam, J. P. 1997. Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. Environ. Biol. Fishes. 48, 265-278.

Doroshov, S. I., Van Eenennaam, J. P., and Moberg, G. P. 1999. Development of white sturgeon broodstock. J. Appl. Ichthyol. 15, 326-327.

Fausto, A. M., Picchietti, S., Taddei, A. R., Zeni, C., Scapigliati, G., Mazzini, M., and Abelli, L. 2004. Fomation of the egg envelope of a teleost, *Dicentrarchus labrax* (L.): immunochemical and cytochemical detection of multiple components. Anat. Embryol. 208, 43-53.

Fitzpatrick, M. S., Van Der Kraak, G., and Schreck, C. B. 1986. Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final ovarian maturation. Gen. Comp. Endocrinol. 62, 437-451.

Froese, R. and D. Pauly. Editors. 2010. FishBase.World Wide Web electronic publication. www.fishbase.org, version 01/2010.

Fontana, F., Congiu, L., Mudrak, V. A., Quattro, J. M., Smith, T. I. J., Ware, K., and Doroshov, S. I. 2008. Evidence of hexaploid karyotype in shortnose sturgeon. Genome. 51, 113-119.

Garcia-Gallego, M., Domezane, A., de la Higuera, M., Hidalgo, M.C., Furné, M., Morales, A.E., and Sanz, A. 2009. On nutrition and feeding studies as the basis for the culture of different sturgeon species. In: Carmona, R. et al. (eds.) Biology, Conservation and Sustainable Development of Sturgeons. Springer Science. pp. 215-233.

Hochleithner, M. and Gessner, J. 1999. The sturgeon and paddlefish (Acipenseriforms) of the world. Biology and Aquaculture. Aquatech Publ., Kitzbuehel, Austria.

Hung, S.S.O. 1991. Nutrition and feeding of hatchery-produced juvenile white sturgeon (*Acipenser transmontanus*): an overview. In: Williot. P. (Ed.) Acipenser Actes du premier colloque international su l'esturgeon. Bordeaux, France, pp. 65-77.

Hung, S. S. O., Liu, W., Li, H., Storebakken, T., Cui, Y. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. Aquaculture. 151, 357-363.

Hung, S.S.O. 2000. Feeds and feeding of sturgeon. Int. Aquafeed. 4, 24-27.

Keenlyne, K.D. 1993. Recent North American studies on pallid sturgeon, *Scaphirhynchus albus* (Forbes and Richardson). In: Gershanovich A. D. and Smith. T. I. J. (Eds.) The 2nd International Symposium on Sturgeons. VNIRO Publishing, Moscow, pp. 225-234.

Kohlhorst, D.W. 1976. Sturgeon spawning in the Sacramento River in 1973, as determined by distribution of larvae. Calif. Fish Game. 62, 32-40.

Lana, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Third Edition. American Registry of Pathology. MaGraw-Hill. Inc. New York, NY.

Lescheid, D. W., Powell, J. F., Fischer, W. H., Park, M. Craig, A., Bukovskaya, O., Barannikova, I. A., and Sherwood, N. M. 1995. Mammalian gonadotropin-releasing hormone (GnRH) identified by primary structure in Russian sturgeon, *Acipenser gueldenstaedti*. Regul. Pept. 55, 299-309.

Linares-Casenave, J., Kroll, K. J., Van Eenennaam, J. P., and Doroshov, S. I. 2003. Effect of ovarian stage on plasma vitellogenin and calcium in cultured white sturgeon. Aquaculture. 221, 645-656.

Linares-Casenave, J., Van Eenennaam, J. P., and Doroshov, S. I. 2002. Ultrastructural and histological observations on temperature-induced follicular ovarian atresia in the white sturgeon. J. Appl. Ichthyol. 18, 382-390.

Logan, S. H., Johnston, W. E., and Doroshov, S. I. 1995. Economics of joint production of sturgeon (*Acipenser transmontanus* Richardson) and roe for caviar. Aquaculture. 130, 299-316.

Marti, Y. Y. 1979. Development of sturgeon management in the southern seas of the USSR. In: Biological Resources of Inland Waters of the USSR, Moscow, Publ. House "Nauka", pp. 73-85.

Moberg, G. P., Watson, J. G., Doroshov, S. I., Papkoff, H., and Pavlick, R. J. Jr. 1995. Physiological evidence for two sturgeon gonadotropins in *Acipenser transmontanus*. Aquaculture. 135, 27-39.

Moberg, G. P. and Doroshov, S. I. 1996. Probing the endocrine control of sturgeon reproduction. In: Doroshov, S., Binkowski, F., Thuemler, T., and Mackinlay, D. (Eds.) Culture and Management of Sturgeon and Paddlefish Symposium Proceedings. San Francisco, CA. Am. Fish. Soc. pp. 105-111.

Parauka, F.M., Troxel, W.J., Chapman, F.A., and McBay, L.G. 1991. Hormone-induced ovulation and artificial spawning of Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotoi*). Prog. Fish-Cult. 53, 113-117.

Pavlick R. J. Jr. and Moberg, G. P. 1997. The effect of chronic testosterone administration on sturgeon gonadotropins in juvenile and pre-vitellogenic white sturgeon (*Acipenser transmontanus*). Gen. Comp. Endocrinol. 105, 218-227.

Post, H. 1890. The sturgeon, some experiments in hatching. Trans. Am. Fish. Soc. 19, 36-40.

Presnell, J. K. and M. F. Shchreibman. 1997. Animal Tissue Techniques. 5th Edition. Johns Hopkins University Press, Baltimore, Maryland.

Quérat, B., Sellouk, A., and Salmon, C. 2002. Phylogenetic Analysis of the vertebrate glycoprotein hormone family including new techniques of sturgeon (*Acipenser baeri*) β subunits of the two gonadotropins and the thyroid-stimulating hormone. Biol. Reprod. 63, 222-238.

Ryder, J. A. 1890. The sturgeon and sturgeon industries of the eastern coast of the United States, with an account of experiments bearing upon sturgeon culture. U.S. Fish. Comm. Bull. 8, 231-238.

Sanders, B. J., Fadel, J. G., and Wade, E. M. 2003. Economic optimization modeling of white sturgeon (*Acipenser transmontanus*) caviar and meat production under different management conditions. Aquaculture. 217, 409-430.

Schultz , K. 2004. Ken Schultz's Field guide to fresh water fish. John Wlley and Sons, Inc, Hoboken, NJ.

Smith, C. E., Osbom, M. D., Piper, R. G., and Dwyer, W. P. 1979. Effect of diet composition on performance of rainbow trout broodstock during three year period. Prog. Fish-Cult. 41, 185-188.

Smith, T. I. J., Dingley, E. K., and Marchette, D. E. 1980. Induced spawning and culture of Atlantic sturgeon. Prog. Fish-Cult. 42, 147-151.

Smith, T. I. J., Dingley, E. K., and Marchette, D. E. 1981. Culture trials with Atlantic sturgeon, *Acipenser oxyrinchus*, in the USA. J. World Maricult. Soc. 12, 78-87.

Scott, A. P. and Ellis, T. 2007. Measurement of fish steroids in water- a review. Gen. Comp. Endocrinol. 153, 392-400.

Sterling Caviar. http://www.sterlingcaviar.com

Stone, L. 1900. The spawning habits of the lake sturgeon (*Acipenser rubicundus*). Trans. Am. Fish. Soc. 29, 118-128.

Talbott, M. J. 2010. Determining morphological and biochemical parameters associated with ovarian follicular atresia and quality and yield of caviar in white sturgeon (Acipenser transmontanus) females. M. S. Thesis. Montana State University, Bozeman, Montana.

Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F-A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E., and Hansen, T. 2010. Control of puberty in farmed fish. Gen. Comp. Endocrinol. 165, 483-515.

Tzankova, Z. 2007. U.S. Farmed Sturgeon. Sea food report. Monterey Bay Aquarium. CA.

Van Eenennaam, A. L., Murray, J. D., and Medrano, J. F. 1998. Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson

(Pisces, Acipenseridae), a fish with a very high chromosome number. Genome. 41, 51-61.

Van Eenennaam, A. L., Murray, J. D., and Medrano, J. F. 1999. Karyotype of the American green sturgeon. Trans. Am. Fish. Soc. 128, 175-177.

Van Eenennaam, J. P., Chapman, F. A., and Jarvis, P. L. 2004. Aquaculture. In: LeBreton, G. T. O., Beamish, F.W. H., and McKinley, R. S. (Eds.) Sturgeons and Paddlefish of North America. Dordrecht, the Netherlands, pp. 277-311.

Van Eenennaam, J. P. and Doroshov, S. I. 1998. Effects of age and body size on gonadal development of Atlantic sturgeon. J. Fish Bio. 53, 624-637.

Van Eenennaam, J. P., Webb, M. A. H., Deng, X., Doroshov, S. I., Mayfield, R. B., Cech, J. J. Jr., Hillemeier, D. C., and Willson, T. E. 2001. Artificial spawning and larval rearing of Klamath River green sturgeon. Trans. Am. Fish. Soc. 130, 159-165.

Webb, M.A.H., Feist, G. W., Schreck, C. B., Foster E. P., and Fitzpatrick, M. S. 2002a. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. Trans. Am. Fish. Soc. 131, 132-142.

Webb, M. A. H., Feist, G. W., Trant, J. M., Van Eenennaam, J. P., Fitzpatrick, M. S., Schreck, C. B., and Doroshov, S. I. 2002b. Ovarian steroidogenesis in white sturgeon (*Acipenser transmontanus*) during oocyte maturation and induced ovulation. Gen. Comp. Endocrinol. 129, 27-38.

Webb, M. A. H., Van Eenennaam, J. P., Doroshov, S. I., and Moberg, G. P. 1999. Preliminary observation on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture. 176, 315-329.

Webb, M. A. H., Van Eenennaam, J. P., Feist, G., Linares-Casenave, J., Fitzpatrick, M., Schreck, C. B., and Doroshov, S. I. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon. Aquaculture. 201, 137-151.

Wildhaber, M. L. and Bryan, J. L. 2006. Ultrasonic and endoscopic examination of lower Missouri and lower Mississippi pallid sturgeon to determine sex and 2006 broodstock potential. US Geological Survey, Columbia, Missouri.

Wilson, J. A. and Mckinley R. S. 2004. Distribution, habitat, and movements. In: LeBreton, G. T. O., Beamish, F. W. H., and McKinley, R. S. (Eds.) sturgeon and paddlefish of North America. Dordrecht, the Netherlands, pp. 40-72.