Final Report

Using Ovaprim™ as a conservation tool for lake sturgeon, *Acipenser fulvescens*: The short and long term effects of endocrine manipulation during the reproductive cycle.

Dr. W. Gary Anderson, Dr. Janet Genz and Craig McDougall

Report compiled for:
Environmental Licensing and Protection Department,
Manitoba Hydro,
360 Portage Avenue,
Winnipeg,
MB, Canada,
R3C 0G8

Dr. W. Gary Anderson
Department of Biological Sciences,
University of Manitoba,
Winnipeg,
MB, Canada,
R3T2N2
e-mail: Gary.Anderson@ad.umanitoba.ca
Phone: (204) 474-7496
Fax: (204) 474-7588

Lay Summary

The present study was initiated to determine the effectiveness of Ovaprim® as a conservation tool in spawning lake sturgeon. The active ingredient in Ovaprim is salmon gonadotropin releasing hormone or salmon GnRH for short. So we also examined the effectiveness of sturgeon GnRH as a conservation tool. Both substances were extremely useful in stimulating maturation of eggs in female fish and release of those eggs by the female fish following injection into the muscle. It was expected that sturgeon GnRH would be more effective in the sturgeon because this is the natural hormone for these fish. Indeed following injection of sturgeon GnRH it appeared that female fish were freely producing eggs as soon as 12 hours after injection. Following injection of Ovaprim it took as much as 24 hours before females produced eggs. GnRH is found in all animals and works by stimulating the production of sex steroids that stimulate maturation and production of eggs and sperm. Therefore measurement of estradiol (the female sex steroid) and testosterone (the male sex steroid) in the blood and muscle of fish after injection with GnRH will provide an indication of how long the injected GnRH is active. If the injected GnRH remained active one would expect high levels of both estradiol in the blood and muscle of the fish. In this study blood and muscle levels of both testosterone and estradiol did not increase significantly after injection of either Ovaprim or sturgeon GnRH. Indeed following injection of sturgeon GnRH the concentrations of blood estradiol and testosterone in male and female fish were significantly lower than capture levels within 48h of injection suggesting that the actions of the GnRH were extremely rapid and it was no longer effective even after that short time period. Given that the injected GnRH did not influence the reproductive hormones in lake sturgeon within 48 h following injection it is highly unlikely that eating a fish 48 hours after injection with GnRH would result in the consumption of bioactive amounts of GnRH. In support of this the half-life of GnRH in human blood is between 2-4 minutes. This means that every 2-4 minutes half of the GnRH is destroyed. As a peptide hormone this is not unusual and there is no reason to expect a significantly longer half-life of sturgeon GnRH when administered to the sturgeon. Therfore, it is highly likely that with 48 hours of injection the level of GNRH in the blood would be undetectable. The main differences between the sturgeon GnRH and Ovaprim used in this study were that 1) Ovaprim contains a dopamine inhibitor to promote the actions of the salmon GnRH in Ovaprim. The actions and persistence of the dopamine inhibitor were not assessed in this study but may have a longer lasting effect. 2) Ovaprim contains salmon GnRH,

this is a peptide hormone that has a slightly different structure than sturgeon GnRH and therefore is not recognised as quickly by the enzymes responsible for breaking down the peptide in sturgeon. As a consequence the half-life of salmon GnRH in the sturgeon is likely to be slightly longer and therefore will persist in the blood for a longer period of time. It is recommended that sturgeon GnRH be used in the future to stimulate egg and sperm production from wild caught lake sturgeon for conservation purposes.

Abstract

This research aimed to assess hormonal profiles in blood and muscle of spawning male and female adult lake sturgeon, *Acipenser fulvescens* following administration of Ovaprim® or synthetic homologous sturgeon gonadotrophic releasing hormone (GnRHa). The present report presents data from two years of study. In the first year (spring 2011) Ovaprim was administered to seven female and five male fish and in the second year of study (spring 2012) GnRHa was administered to five female and five male fish in addition to sturgeon Ringers solution being administered to four male and four female fish as controls. Serial blood samples were collected for up to 27 days post capture and administration of the hormones in both years, and samples were assessed for circulating concentrations of the sex steroids, estradiol and testosterone in addition to the glucocorticoid, cortisol in male and female fish. Following Ovaprim administration plasma levels of estradiol in males and females were not significantly different from capture levels. However, in GnRH treated and control fish circulating levels of estradiol in males and females were lower than capture levels within 24h of administration. Circulating levels of testosterone in Ovaprim treated male and female fish were significantly higher at capture compared to all other timepoints whereas circulating levels of testosterone in GnRH treated male and female fish were significantly lower than capture levels between 4 (female) and 15 days post administration. Circulating levels of testosterone did not differ from capture levels in male and female control fish. Circulating levels of cortisol in control fish were highest at capture in male control fish compared to all other timepoints and were significantly lower than capture levels four days post administration in female control fish. In GnRH treated male fish circulating levels of cortisol were significantly lower from capture levels within 5 days post administration in male fish but did not differ from capture levels in GnRH treated female fish throughout the study period. Ovaprim treated males had significantly lower circulating cortisol

within 15 days post administration and circulating cortisol concentrations did not differ from capture levels in Ovaprim treated females throughout the study period.
Testosterone concentration in muscle tissue did not differ between sampling timepoints in Ovaprim and control male fish and was significantly lower than capture levels 19 days post administration in GnRH treated male fish. In Ovaprim treated female fish estradiol levels in the muscle did not differ from capture levels throughout the study period and estradiol levels in the muscle tissue for the control and GnRH treated fish were below the detectable limit of the assay at all timepoints.
Plasma osmolality followed similar profiles in both male and female fish in all treatments with values typically being highest at capture. Plasma pH values for male and female fish were pooled and profiles were again similar across treatments with pH being lowest at capture then typically increasing within 24h of capture. Similarly plasma total CO_2 values were pooled between the sexes, however, in Ovaprim treated fish plasma total CO_2 was lowest at 1 day post administration then increased, but not significantly so for the remaining timepoints. In GnRH and control fish plasma total CO_2 tended to be highest at capture. Plasma ion concentration for 2011 are presented and varied across the experimental time frame for both male and female fish, however, significant differences in ion concentration, particularly calcium were more prevalent in female fish. Data on egg size, sperm motility, fertilisation and hatching success are presented for both years of the study, in addition to biochemical measurements of sperm, eggs, seminal fluid and ovarian fluid.

Introduction

Hormonal manipulation of the endocrine reproductive axis has been used in both the aquarium trade and aquaculture industry for a number of years and studies have shown that administration of gonadotropin releasing hormone (GnRH) in fish will significantly influence profiles of sex steroids in the blood in addition to enhancing gamete development leading to ovulation and spermiation in female and male fish respectively. In brief, GnRH plays a central role in the coordination of the reproductive endocrine axis in all vertebrates examined to date (Kah et al., 2007). Released from the hypothalamus in the brain GnRH targets the pituitary gland (also in the brain) in all vertebrates to stimulate the production and release of follicle stimulating hormone and leutinising hormone in mammals or the equivalent of these peptide hormones in

fishes. These two peptides then target the gonads in females and males to stimulate the production of the sex steroids which are predominantly the estrogens in females and the androgens in males. The actions of GnRH are in a large part under negative feedback control by the sex steroids. However, as ovarian follicles mature in females increasing amounts of estrogens are produced, in particular estradiol, this then triggers a positive feedback loop and stimulates the production of GnRH from the hypothalamus until the eggs are ovulated when there is a rapid decrease in estrogens in the blood. Therefore, the developmental stage of gametes is critical to the timing and release of GnRH from the hypothalamus or indeed administration of exogenous GnRH to stimulate final maturation and ovulation.

GnRH has been has been identified in sturgeon and indeed the predominant form of GnRH in those sturgeon species examined is a human form of the peptide (Sherwood et al., 1991; Sherwood et al., 1993). As stated, the release of GnRH from the hypothalamus targets the pituitary to stimulate the release of sex hormones that ultimately regulate gamete activity and the production of sex steroids (androgens and estrogens) by the developing gametes (Counis et al., 2005). In mammals, the sex hormones are luteinising and follicle stimulating hormone (LH and FSH) and the equivalent in sturgeons have been identified as gonadotrophic hormone I and II (GTH-I and II) (Moberg et al., 1995). GTH-I has been shown to regulate follicular development and vitellogenesis whereas GTH-II appears to be more closely involved with the final stages of egg maturation and ovulation (Moberg et al., 1995). Sex steroids are also produced and released by the developing gametes and these androgens and estrogens play a vital role in feedback regulation of the sex hormone production by the gametes and the reproductive cycle.

Administration of GnRH analogues has successfully been used in a number of sturgeon species, including the white sturgeon, *Acipenser transmontanous* (Doroshov et al., 1997) the Siberian sturgeon, *Acipenser baerii* (Williot et al., 2002) and the stellate sturgeon, *Acipenser stellatus* (Bayunova et al., 2006). In each of these species aspects of fertility and short term hormonal profiles have been reported over periods up to 7 days post administration. However, longer term effects are not know for any sturgeon species and resultant fertilization success when compared to naturally ovulating animals is also not well understood.

The present study aimed to examine the effects of Ovaprim and homologous GnRH administration on fertilisation success. Furthermore, circulating and muscle tissue levels of estradiol (the ‘female sex’ steroid) and testosterone (the ‘male’ sex steroid) were assessed before,

during and after Ovaprim, GnRH and vehicle (sturgeon Ringers) administration. Circulating levels of cortisol, ion, pH and plasma osmolality were also assessed to examine the effects of capture, handling and containment on the stress axis of the adult fish and to provide an indication of the general health status of those fish. Finally measures of sperm and egg quality have also been examined. This is the final report of a two year study conducted in the spring of 2011 and 2012.

Methods

Chemicals

There were three chemicals administered to Lake Sturgeon during the study, they were Ovaprim, homologus GnRH and Lake Sturgeon Ringers. Ovaprim is an indexed drug by the United States Food and Drug Administration for injection into ornamental finfish only. It contains salmon GnRHa (<0.1%), domperidone (1%) and propylene glycol (>95%). Both GnRH and domperidone are active ingredients and propylene glycol acts as the vehicle for administration. The actions of GnRH on the reproductive endocrine axis are described above and domperidone acts as an antagonist to the naturally occurring neurotransmitter, dopamine. The addition of the domperidone suppresses the inhibitory actions of dopamine on GnRH release from the hypothalamus and gonadotrophic hormones (LH and FSH) from the pituitary gland thus enhancing the actions of the exogenously administered salmonGnRH. The second main chemical used in the present study was a human analogue of GnRH also known as leutinising hormone releasing hormone (LHRH). As described previously this is also the principle form of GnRH identified in sturgeons. This sturgeon GnRH was dissolved in Lake sturgeon Ringers which was the final solution injected into lake sturgeon and acted as a vehicle for sturgeon GnRH administration and therefore acted as a control. The recipe for Sturgeon Ringers was as follows: in mM: 108.0 NaCl, 1.8 KCl, 2.1 Na_2HPO_4, 0.2 KH_2PO_4, 0.8 $MgSO_4 \cdot 7H_2O$, 1.6 $CaSO_4$ and 8.0 $NaHCO_3$). There are no components of this recipe that are bioactive on the endocrine reproductive axis.

Collection and holding of experimental animals

Lake Sturgeon were captured by gill net downstream from the spillway at the Pointe du Bois generating station on the Winnipeg River (est. 1909, 50º17’52N, 95º32’51W). In 2011 7 female

and 5 male fish were collected and in 2012 9 female and 9 male fish were collected. Fish were then transported in oxygenated river water to the University of Manitoba, Winnipeg where fish were transferred from transport tanks to 600 gallon (2275 litre) holding tanks, with a maximum density of 3 fish per tank, where they were allowed to recover for 24 h.

Sampling timeline and procedures

Blood and tissue samples were taken over the course of the 4 week experimental period. In 2011 after a 24 h recovery period following transport, sturgeon were injected with 0.5 ml/kg of Ovaprim, in two doses, the first dose being 0.125 ml/kg and a second injection 20 hours later of 0.375 ml/kg as per the manufacturers' recommendation (Syndel Labs, Vancouver, BC). Spawning for male fish involved lifting the fish out of the water and gently massaging the underbelly. This readily led to collection of ejaculate from the male. For females, fish were anaesthetised in MS-222 (100 ppm) and the under belly was massaged in an effort to extrude eggs from the vent. If eggs were not readily available by this route a small incision (approximately 3 cm) was made on the ventral surface of the fish to access the abdominal cavity and eggs were retrieved by this route. Females were typically "stripped" for 10 minutes until a reasonable quantity of eggs was obtained. In 2012 a similar approach was adopted with the exception that 5 male and 5 female fish were administered with two doses of GnRH (H-4070 LHRH acetate, Bachem, Torrance, CA, USA); 10 $\mu g.kg^{-1}$ followed by20 $\mu g.kg^{-1}$ separated by 20 h. The sampling protocol is illustrated in figure 1 and was identical in both years with two exceptions. In 2012 an extra blood sample was taken 12h following administration of GnRH and vehicle and the 3 day muscle sample was omitted.

Blood was sampled via the caudal sinus using a 5 ml syringe fitted with a 22 or 23 gauge needle, rinsed with Ringer's solution containing 100 U/ml ammonia heparin. Blood samples were then spun at 13xG for 5 min at room temperature, the plasma was transferred to a new sample tube, measured for pH (Accumet, Fisher Scientific, Ottawa, On. Canada) and placed on ice. Total CO_2 (tCO_2), as a measure of bicarbonate concentration (Corning 950 CO_2 analyser, Olympic Analytical, UK), and osmolality (Wescor, Logan, UT, USA) of plasma samples was determined within 2 h of sampling and the samples subsequently stored at -80 °C for further analysis. A 1 cm^3 muscle biopsy was taken from near the dorsal scutes on the right hand side approximately half way down the length of each fish and flash-frozen in liquid nitrogen.

Sample analysis

Plasma samples were processed to extract cortisol, estradiol, and testosterone. Similarly, muscle biopsies were processed via solid-phase extraction and measured for estradiol and testosterone in females and males, respectively. The isolated hormones were measured via competitive binding radioimmunoassy, using ^{3}H-labeled compounds and a specific antibody for each hormone. In addition, plasma samples were measured for composition of free ions (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, F^{-}, Cl^{-}, PO_4^{3-}, SO_4^{2-}) using ion chromatography (Metrohm, Mississauga, On. Canada).

Egg and sperm quality

Egg and sperm quality were assessed by two basic physical parameters in the first instance. Egg diameter was taken from at least 10 eggs from each female administered with Ovaprim, GnRH and vehicle. Sperm motility for Ovaprim treated males was assessed over a 30 second time period following activation and is expressed as a percent reduction of motile sperm. Spermacrit was assessed in GnRH treated males as a percent of sperm per unit volume of semen. Fertilisation success was assessed as an approximate proportion of fertile eggs that remained in hatching jars following daily removal of unfertilised eggs throughout development, until hatch. Hatching success was determined as the percentage of fish that survived through larval development to the first feeding stage. In addition to the above parameters triglyceride content, as a measure of lipids, (B-Bridge International, Cupertino, CA, USA) glucose content as a measure of carbohydrates, (Wako, Richmond, VA, USA) and total protein (Bio-Rad, Mississauga, ON, Canada) was measured in sperm, eggs, seminal fluid and ovarian fluid as biochemical indicators of egg and sperm quality. Finally, where possible, pH, osmolality and total CO_2 was measured in the ovarianfluid, semen (sperm + seminal fluid) and seminal fluid.

Histology

In 2012 approximately 50 mg of testicular and ovarian tissue was fixed from each fish for examination of gonad development stage. Following biopsy tissue was immediately fixed in ice cold 4% paraformaldehyde (Allen et al. 2009). After 48 hours in 4% paraformaldehyde, tissues were dehydrated in an increasing gradient of ethanol of 50%, 70%, 80%, 95%, 100% (3 times) for 30 minutes each. Tissues were then stored in 100% ethanol at 4°C for approximately 1 month

prior to rehydration in 50% alcohol and embedding in paraffin wax blocks. Tissues were then sectioned using a R. Jung A.G. Heidelberg microtome (serial ID 27445) in 8µm sections and fixed to slides with formalin (1g calcium chloride, 1g cadmium chloride, 10mg formalin concentrated in 100ml distilled water). Following procedures outlined in Humason (1979), slides were allowed to dry on a hot plate then placed in slide bright solution 3 times for 2-3 minutes to remove wax, then rehydrated in 100%, 95%, 80% and 70% ethanol for 30 seconds at each alcohol concentration, rinsed in water and stained with haematoxylin and eosin. For the hematoxylin stain, slides were placed in Harris modified hematoxylin without acetic acid (aluminium sulfate 4.4% W/V, ethylene glycol 25% W/V, hematoxylin (partially oxidized) 0.5% W/V and a wetting agent) for 5 minutes, rinsed with water, put in acid alcohol (2-3 drops concentrated HCl with 60 ml 70% ethanol) for 10 seconds, rinsed with water, put in Scott's solution (1g sodium bicarbonate, 10g magnesium sulphate and a pinch of thymol in 500ml distilled water) for 3 minutes, rinsed with water, and 70% alcohol for 10 seconds. For the eosin stain, the slides were placed in eosin (1g eosin Y, 1000ml 70% ethyl alcohol and 5ml glacial acetic acid diluted 1:1 with 70% ethanol and 2-3 drops of acetic acid) for 2-3 minutes. After the stains were applied, the sections on the slides were dehydrated from water to 100% ethanol with concentrations of ethanol in the opposite order as above for 30 seconds in each concentration below 100% ethanol and for >1 minute at 100% ethanol, moved to slide bright (for 2-3 minutes, 3 times), placed in xylene for >2 minutes then mounted and dried prior to examination using light microscopy.

Tracking

Prior to release, four females (fork length range: 807 – 1060 mm) and two males (627, 890 mm) were implanted with Vemco acoustic transmitters, following the methods of McDougall et al. (2013). Two types of V16 transmitters were used, which measured 16 mm by 68 mm and had a weight in air of 24g. The tags were programmed to emit their uniquely coded acoustic signal every 180-210 seconds, while the V16-4L tags were programmed to emit their uniquely coded acoustic signal every 900 – 1100 seconds. Acoustic tags applied were a maximum of 1.7% of body mass. Following implantation on July 1st 2011, the lake sturgeon were transported to Eight Foot Falls boat launch approximately 2 km downstream of Pointe du Bois.

Movements of acoustically tagged fish were monitored using ten Vemco VR2W receivers strategically deployed throughout the Slave Falls Reservoir following the methods of McDougall et al. (2013). Receivers were deployed on July 5, 2011 and downloaded on three occasions prior to conclusion of the study on October 10, 2012. Using Garmin Mapsource, each receiver was assigned an appropriate river kilometer distance from the Slave Falls GS. As interim results suggested that one of our tagged fish may have left the Slave Falls Reservoir, we utilized a Vemco VR100 manual tracker to search for tags downstream of the Slave Falls GS. We moved incremental distances of ~500 m along the primary flow axis (~50% of the tags maximum efficient detection range), shut off the boat motor, and deployed the hydrophone for ~10 minutes. Given the tag in question was one that pinged every 180-210 seconds, we deemed this interval more than adequate. Detected tag IDs were recorded.

Detection data recorded on VR2Ws were imported into Microsoft Excel 2010 for processing. To account for potential false detections, a unique tag was required to be detected twice within a 30 minute interval in order to be deemed valid, as suggested by the manufacturer (Pincock 2012). As the purpose of monitoring acoustically tagged lake sturgeon was simply to assess long-term survival following the use of Ovaprim, we aimed to quantify if tagged fish were moving at their own volition (and therefore alive) several months after release. For presentation purposes, we present movement extent data in terms of linear river km, separated by season.

Data analysis

Initial analyses indicated significant differences between sexes and significant interaction between time and gender for plasma and muscle hormones, and plasma osmolality. Males and females were therefore analyzed separately for these parameters. Differences due to hormonal treatment were analyzed via one-way ANOVA. Differences with respect to time were analyzed using repeated measures one-way ANOVA. Specific differences between time points and treatments were determined using the Holm-Sidak post-hoc test. Data that were not normally distributed were analyzed using one-way ANOVA on ranks followed by Dunn's post-hoc test. Means were accepted as significantly different when $p<0.05$.

Results

Male and female Ovaprim treated fish had an average body mass of 4.45±0.94 and 7.11±0.87 kg respectively. Male and female GnRH treated fish had an average body mass of 8.05±1.13 and 14.36±1.10 kg respectively. Male and female fish administered with sturgeon Ringer as a vehicle for control had an average body mass of 7.39 ± 1.12 and 14.32 ± 1.95 kg respectively.

Plasma ion, pH, total CO_2 and osmolality measurement

Two-way ANOVA indicated that plasma osmolality was significantly affected by time, and also was significantly different between the sexes 1.5 and 2 days post-injection, with an extremely significant interaction between these two factors. Based on this information, each gender was analyzed independently using a repeated measures ANOVA (Fig. 2). In males, regardless of treatment, osmotic pressure of the plasma typically decreased post capture and returned to capture levels within 15 days post administration. In females plasma osmotic pressure remained significantly lower than capture levels in GnRH and control fish throughout the study period but did not differ from capture levels in Ovaprim treated fish

Plasma pH and tCO_2 did not differ between male and female fish so values were pooled. Repeated measures one way ANOVA demonstrated that plasma pH was lowest at capture in all treatments throughout the study period (Fig. 3). Total CO_2 was lowest in Ovaprim treated fish following administration of Ovaprim and significantly increased within 3 days. There was no difference in plasma total CO_2 in the GnRH treated fish throughout the study period and in control fish plasma total CO_2 was significantly lower than capture levels within 11 days post capture (Fig. 3).

Measured values of the ions sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}) in the plasma of adult lake sturgeon are shown in Figure 4 and are available only for Ovaprim treated fish. All ions were significantly different with respect to time as assessed by nonparametric one-way ANOVA, although only a few differences were identified at specific time points against measurements of fish in the field via Dunn's post-hoc test. In particular plasma Ca^{2+} in female fish was significantly elevated from capture levels at 2 and 7 days post Ovaprim administration and plasma levels of K^+ in female fish was significantly reduced with respect to capture values at 2.5,

5 and 7 days post Ovaprim administration. In addition plasma levels of Na^+ were reduced at 5 days post administration in females and plasma SO_4^{2-} was elevated from day 5 through 19 post Ovaprim administration in females. Interestingly the ion balance in males was not as disturbed as there was only a single significant difference at day 19 post Ovaprim administration in males where SO_4^{2-} was elevated (Fig. 4).

Plasma and muscle steroid analysis

Plasma levels of cortisol in control male fish were significantly the highest at capture compared to the remaining timepoints in the study period. Ovaprim treated male fish were not different from capture levels throughout the study period with the exception of 15 d post-injection and the final sampling timepoint and plasma cortisol levels in GnRH treated male fish were significantly lower than capture levels within 5 days following GnRH administration (Fig. 5). In female sturgeon, plasma levels of cortisol in Ovaprim treated fish did not differ from capture levels throughout the study period. Following GnRH administration, cortisol levels were significantly lower ($p<0.006$), but the observed increase at 12 and 24h was not significant according to post-hoc tests. Plasma cortisol levels in control fish were significantly lower than capture levels from 4 to 11days after vehicle administration (Fig. 5).

The sex steroids estradiol and testosterone were measured in the plasma of both male and female fish. As expected, in males plasma estradiol was lower than females throughout the sampling period regardless of treatment and did not differ from capture levels regardless of treatment. In females, plasma estradiol varied considerably following Ovaprim administration but did not differ ($p<0.064$) throughout the study period (Fig. 6). Following GnRH administration plasma levels of estradiol in females was lower within 24 h of administration. Plasma levels of estradiol at capture were lowest in the control fish, and both Ovaprim and GnRH treated fish had significantly higher estradiol concentrations. Estradiol concentrations in control fish were significantly lower from capture values within 24 hours of capture and remained so throughout the study period (Fig. 6).

Plasma levels of testosterone were significantly higher in male fish at capture compared to all other sampling timepoints in the Ovaprim treated fish (Fig. 7). In GnRH treated males plasma testosterone was significantly lower than capture levels within 15 days of GnRH administration until the end of the sampling period, and in control fish plasma levels of

testosterone did not differ throughout the study period. In females plasma levels of testosterone followed the same profiles as male fish with the exception that testosterone levels were significantly lower than capture levels within 4 days post administration of GnRH (Fig. 7).

Due to concern over available material and that the predominant circulating sex steroid in females was estradiol and in males was testosterone the muscle tissue was only assessed for estradiol in female fish and testosterone in male fish. Interestingly, in male fish the highest levels of testosterone were recorded for GnRH treated fish at capture. Muscle testosterone in males was significantly lower than capture levels in GnRH treated males 19 days post GnRH administration. There was no difference throughout the study period in muscle testosterone for Ovaprim treated and control male fish (Fig. 8). Muscle estradiol was only detectable in Ovaprim treated females and while values are not significant concentrations were lower than capture levels within 2.5 days post administration of Ovaprim (Fig. 8).

Egg and sperm analysis

Average egg diameter was significantly lower in Ovaprim treated fish (Table 1), sperm motility and spermacrit was only measured in Ovaprim and GnRH treated fish respecitively. No values are available for sperm in control fish as despite these fish freely providing sperm at capture, no sperm was recovered in this treatment group when fish were held in the aquarium at the University of Manitoba. Fertilisation success was consistent between Ovaprim and GnRH treated fish; control fish had lower fertilisation success, but this did not differ significantly from the hormone-treated fish when assessed via one-way ANOVA ($p<0.099$). Hatching success was highest in Ovaprim treated fish and lowest in control fish (NB: sperm from GnRH treated males was used to fertilise eggs obtained from control females), but this also fell short of significance ($p<0.057$), due to greater variation in replicates at this stage of larval development (Table 1).

Protein was the predominant biomolecule measured in sperm, eggs, seminal fluid and ovarian fluid followed by triglycerides then finally glucose (Fig. 9). However the profiles for each fluid or gamete were markedly different between treatments. Why these fluids differed between treatments is unclear at this stage. Further, ovarian fluid sampled from control fish had the lowest pH and total CO_2 and the highest osmolality (Figs 10 & 11). However, there was no difference in pH, total CO_2 or osmolality of eggs, sperm seminal fluid, ovarian fluid or semen

between GnRH and Ovaprim treated fish with the exception that total CO_2 of semen was significantly lower in GnRH treated fish compared to Ovaprim treated fish (Figs 10 & 11).

Histology

Light microscopy of gonad samples from all 2012 sampled fish showed 8 males had mature gonads (Fig. 12) and a single male in the control treatment was found to be immature with gonial cells enclosed in cysts (Fig. 13). All females were at the level of development of post-spawning with post-ovulatory follicles and atretic bodies present (Fig. 14)

Movement data

Following release, the six acoustically tagged lake sturgeon were detected a median of 52,108 times (range: 3,415 – 103,729). Data indicated that five of six acoustically tagged fish were moving, essentially up until the conclusion of the study. Therefore, we concluded that all of these fish had survived the Ovaprim injection and handling associated with gamete extraction. Mean movement extent for the five fish tracked for the duration of movement monitoring was 6.5 rkm (SD: 2.0, range: 8.6 – 3.1; Figure 15).

The remaining fish (#51079) made downstream and upstream movements spanning rkm 2.5 – 7.5 between July 5 and July 19 2011, prior to moving downstream and last being detected at a receiver deployed at rkm 1.4 on July 25, 2011. Given that this was the lowermost monitoring location in the Slave Falls Reservoir, it was unclear if mortality had occurred or if the tag became active prematurely. Alternatively, the fish may have moved downstream out of the reservoir, as passage through the Slave Falls GS is not uncommon (McDougall et al. 2013).

Manual tracking conducted in the 10 km stretch of river downstream of Slave Falls detected many acoustic tags deployed as part of other research projects (McDougall et al. 2013; S. Peake, University of New Brunswick, pers. comm.), but the fish in question (#51079) was not located. As such, the long term fate of this fish could not be ascertained.

Discussion

The aim of this study was to examine the endocrine and physiological effects of Ovaprim and GnRH administration in spawning lake sturgeon. The observed decreases in plasma

osmolality following Ovaprim, GnRH and vehicle administration are likely the result of capture, handling and transport stresses. Exposure to such stress in fish has been shown to induce a reduction in gill permeability and branchial circulation as a result of the actions of catecholamines and the glucocorticoid, cortisol (Wendelaar Bonga, 1997). Interestingly in male fish this decrease in plasma osmolality was transient in nature returning to at or near capture values within 11 days post transport for control and GnRH treatments. However in female fish the decrease in plasma osmolality was sustained throughout the study period. Such a sex bias in this response has not previously been reported for sturgeons and was not expected particularly given that the circulating levels of cortisol did not differ appreciably between male and female fish in this study. Furthermore, this finding was contrary to recently observed transient increases in plasma osmolality following air exposure in juvenile lake sturgeon (Allen et al., 2009a). It is recognised that fish will respond to different stressors in different ways and that life stage may also influence the stress response (Barton et al., 2002). In the present study it was evident that Ovaprim administration induced the most variable cortisol response in both male and female fish whereas male and female fish administered with GnRH had circulating levels significantly lower than capture levels within 5 and 4 days respectively. Furthermore, peak levels in juvenile lake sturgeon were comparable to those in the present study (Allen et al., 2009a), however, baseline levels were not, particularly in the Ovaprim treated fish. Previous baseline levels as low as 2.4 $\pm$0.2ng.ml^{-1} have been reported for adult lake sturgeon (Baker et al., 2008) suggesting that the Ovaprim treated fish in this study were under chronic stress. Why Ovaprim induced fish exhibited such a variable response in regard to cortisol concentrations and tended to be higher than GnRH or control fish is unknown but may be related to the additional presence of a dopamine inhibitor in Ovaprim that was not present in either the GnRH or control treated fish. It would be interesting to determine what role this additional chemical may have on the upstream regulatory components of the endocrine stress axis such as corticotrophic releasing factor (CRF) or adrenocorticotrophic hormone (ACTH). The reduced plasma pH in all fish at time of capture is likely the result of the initial stress response at capture in addition to a reduced ability for ventilation in the gill during capture resulting in acid base disturbances and a decrease in pH (Barton et al., 2002; Wendelaar Bonga, 1997).

While circulating levels of testosterone in the male fish at capture are a little lower for the Ovaprim treated fish and a lot lower for the control and GnRH treated fish, circulating values for

estradiol in male and female fish and testosterone for female fish compare favourably with previous values reported in the literature for spawning Lake Sturgeon (Allen et al., 2009b). However, circulating levels of estradiol observed in the plasma of female Lake Sturgeon following Ovaprim and GnRH administration were not as clear cut as expected. Statistical analysis did not reveal any significant effect of either Ovaprim or GnRH on circulating concentrations of estradiol, while previous examination of hormonally induced spawning has resulted in significant increases in both testosterone and estradiol in the Siberian Sturgeon, *Acipenser stellatus* (Bayunova et al., 2006) although the time frame for collection in that study was shorter and values did return close to pre-manipulation levels between 12 and 24 hours post administration. It is possible therefore that the present study did not sample the female fish in the appropriate time frame to observe an increase in estradiol despite the addition of an extra sampling timepoint in the 2012 spawning season. Indeed hormonal manipulation of ovulation in the Siberian Sturgeon, *Acipenser stellatus* resulted in a sharp decline in estradiol just prior to ovulation (Semenkova et al., 2002). For male fish an increase in testosterone was observed following Ovaprim administration, and a similar increase was observed following GnRH administration in female fish, however, these increases were short lived and reached almost immeasurable levels in both sexes 3-4 days post administration.

To our knowledge tissue levels of sex steroids have not previously been reported in sturgeons and so this study provides the first indication of endocrine manipulation of sex steroids in tissue of any sturgeon species. It is evident that none of the treatments influenced muscle levels of sex steroids; indeed the highest levels were consistently found in the field samples shortly after the fish were retrieved from the net prior to any administration of hormones. It is also important to note that estradiol and testosterone are expressed as ng/g of muscle tissue, and are therefore 100-to-1000-fold less concentrated in the muscle than the measured levels in the plasma. Perhaps one of the more interesting results from the present study was the observed decline in muscle testosterone in male fish following GnRH administration but no change in control fish, in addition to the inability to detect estradiol in the muscle of female fish following administration of both GnRH and vehicle for control.

The observed fertilisation rates and hatching success in GnRH and Ovaprim treated fish were as anticipated. Interestingly the control fish had a reduced hatching success compared to the GnRH and Ovaprim treated fish and furthermore we were unable to collect sperm from control

males once at the University of Manitoba animal holding facility, despite clear spermiation by these fish at capture. We are not aware of published values for glucose, protein and triglycerides in the eggs, sperm, ovarian and seminal fluids and so are unable to compare these with other sturgeon species at this stage. Finally, in 2011 2 male and 4 female fish were implanted with acoustic transmitters to track movements of these fish in the Slave Falls reservoir. Movement data from these fish indicates that 5 of the 6 fish (4 females and one male) were actively moving throughout the reservoir for at least 18 months after release, suggesting that the injection of Ovaprim and subsequent handling and stripping of eggs did not effect the movement of these fish. Furthermore, the two males that were tagged were consistently located in the most upstream portion of the reservoir in the spring of 2012 where known spawning habitat is present.

In addition to the reported data there were a number of hormones that we proposed to measure in the original proposal namely 11-ketotestosterone, progesterone and GnRH. Unfortunately sample size precluded measurement of these additional hormones so the pragmatic decision was taken to concentrate on the three most recognised and reported hormones, namely cortisol, estradiol and testosterone. It is important to note that when injected in the muscle tissue the bioactive ingredients in Ovaprim and the homologous sturgeon GnRH would be rapidly taken up into the blood stream and target the pituitary gland for release of GTH I and II and subsequent stimulation of sex steroid production from the gonads. Therefore, measurement of sex steroid concentration is an excellent proxy for determining GnRH activity in the blood. Given that both estradiol and testosterone levels declined rapidly post administration in GnRH treated fish it is reasonable to assume that GnRH activity was rapidly diminished within 48 hours of administration. Furthermore, In humans the half-life of GnRH in the blood is typically minutes (Hayden, 2008). This is typical for peptide hormones that are native to the animal, therefore, it is not unreasonable to assume that the homologous GnRH administered in 2012 would have a similar half-life in lake sturgeon as this is the native hormone in sturgeons. However, half-life's of peptide hormones can be extended if they are non-native and the salmon GnRH administered in 2011 as Ovaprim would fall under this category, so it is again reasonable to assume that salmon GnRH would persist in the blood for a longer time, which may explain why values following Ovaprim administration were typically higher although not significantly so.

Conclusions

The reported values for cortisol, estradiol and testosterone for spawning adult lake sturgeon illustrate that in all treatments circulating levels of these three hormones never significantly exceeded the values at capture prior to endocrine manipulation of the reproductive axis. Furthermore, tissue levels of testosterone in males and estradiol in females are also consistently at the same levels or lower than values at capture. Estradiol in female muscle samples were undetectable in the GnRH and control treatments. Finally the significantly reduced circulating cortisol concentration in GnRH treated fish suggests that this treatment may have less of an impact on the cortisol stress response in these fish by an as yet un-described mechanism. These data alongside the reported fertilisation and hatching success in GnRH treated fish indicates that this hormone may be preferable for use as a conservation tool for spawning wild lake sturgeon.

Acknowledgements

This work would not have been possible without assistance in animal collection from employees of North/South Consultants Inc. and animal transport tanks borrowed from Grand Rapids fish hatchery. Thanks for assistance in animal care to the staff of the University of Manitoba Animal Holding Facility. Funding for this study was provided by Manitoba Hydro.

Figure Legends

Figure 1: Sampling protocol for blood and muscle tissue from adult male and female lake sturgeon. Red arrows indicate sampling of both blood and muscle, black arrows indicate sampling of blood only. *Ovaprim, GnRH and vehicle doses were separated by 20h. Fish were released close to the site of capture.

Figure 2. Osmotic pressure of plasma of adult lake sturgeon following injection of Ovaprim™ (0.5 ml kg^{-1}, n=5 male, 7 female), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (control, n=4 each male and female). Fish were stripped for gametes 2.5 d post-injection (dashed line). Initial t-test analyses indicated significant differences between sexes; males and females were therefore analyzed separately for the effect of time using repeated measures one-way ANOVA. An asterisk (*) indicates the observation at that time was significantly different ($p<0.05$) than day 0 within that treatment.

Figure 3. Adult lake sturgeon plasma pH (top panel), and total CO_2 (tCO_2, bottom panel) of blood plasma of adult lake sturgeon following injection of Ovaprim™ (0.5 ml kg^{-1}, n=12), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=10), or sham-injection of Ringer's solution (control, n=8). Fish were stripped for gametes 2.5 d post-injection (dashed line). An asterisk (*) indicates the observation at that time was significantly different ($p<0.05$) than at the time of capture ("Field") for pH, or from day 0 (day 1 for Ovaprim) for tCO_2, as assessed by one-way repeat measure ANOVA within each treatment.

Figure 4. Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, SO_4^{2-}, and PO_4^{3-} (mM) in the blood plasma of adult lake sturgeon following injection of Ovaprim^{TM} (0.5 ml kg^{-1}, n=12). An asterisk (*) indicates the concentration at that time was significantly different ($p<0.05$) than the values of samples taken at the time of capture (F).

Figure 5. Concentrations (ng ml^{-1}) of cortisol in the blood plasma of adult lake sturgeon following injection of Ovaprim^{TM} (0.5 ml kg^{-1}, n=5 male, 7 female), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (control, n=4 each male and female). An asterisk (*) indicates the concentration at that time was significantly different ($p<0.05$) than the values of samples taken at the time of capture ("Field") within that treatment. A hashmark (#) indicates the Field value is significantly different from all subsequent time points.

Figure 6. Concentrations (ng ml^{-1}) of estradiol in the blood plasma of adult lake sturgeon following injection of Ovaprim^{TM} (0.5 ml kg^{-1}, n=5 male, 7 female), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (control, n=4 each male and female). An asterisk (*) indicates the concentration at that time was significantly different ($p<0.05$) than the values of samples taken at the time of capture ("Field"). Samples below the detectable limit of the assay (0.039 ng ml^{-1}) were set a value of 0.

Figure 7. Concentrations (ng ml^{-1}) of testosterone in the blood plasma of adult lake sturgeon following injection of Ovaprim^{TM} (0.5 ml kg^{-1}, n=5 male, 7 female), mammalian LHRH

dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (control, n=4 each male and female). An asterisk (*) indicates the concentration at that time was significantly different ($p<0.05$) than the values of samples at time of capture (Field). A hashmark (#) indicates the Field value is significantly different from all subsequent time points. Samples below the detectable limit of the assay (0.078 ng ml^{-1}) were set a value of 0.

Figure 8. Concentrations (ng g muscle $tissue^{-1}$) of A) testosterone in muscle biopsies taken from male adult lake sturgeon following injection of Ovaprim™ (0.5 ml kg^{-1}, n=5), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5), or sham-injection of Ringer's solution (control, n=4), and B) estradiol in muscle biopsies taken from female adult lake sturgeon following injection of Ovaprim™ (0.5 ml kg^{-1}, n=7). Estradiol in muscle samples from female adult lake sturgeon injected with mammalian LHRH or sham-injected was below detectable limits of the assay. An asterisk (*) indicates the concentration at that time was significantly different ($p<0.05$) than the values of samples taken at the time of capture ("Field"). Samples below the detectable limit of the assay (0.039 ng ml^{-1} for estradiol 0.078 ng ml^{-1} for testosterone) were set a value of 0.

Figure 9. Glucose, triglyceride and protein concentrations in gametes and associated fluids collected from adult lake sturgeon 20 h after injection of Ovaprim™ (0.5 ml kg^{-1}, n=5 males, 7 females), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (control, n=4 each male and female). An

asterisk indicates significant difference ($p<0.05$) from control, or between treatments for sperm and seminal fluid, according to one-way ANOVA.

Figure 10. Osmolality of semen, seminal fluid, and ovarian fluid collected from adult lake sturgeon 20 h after injection of Ovaprim™ (white bars, 0.5 ml kg^{-1}, n=5 males, 7 females), mammalian LHRH dissolved in Ringer's solution (hatched bars, GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (black bars, control, n=4 each male and female).

Figure 11. pH (top panel) and total CO_2 (bottom panel, mEqv l^{-1}) of semen, seminal fluid, and perivitelline ovarian fluid collected from adult lake sturgeon 20 h after injection of Ovaprim™ (white bars, 0.5 ml kg^{-1}, n=5 males, 7 females), mammalian LHRH dissolved in Ringer's solution (hatched bars, GnRH, 30 µg kg^{-1}, n=5 each male and female), or sham-injection of Ringer's solution (black bars, control, n=4 each male and female). Milt could not be collected from sham-injected lake sturgeon. Different letters indicate significantly different means ($p<0.05$) between treatments as indicated by one-way ANOVA.

Figure 12. Mature male testicular tissue stained with H&E showing differentiated spermatozoa (SZ).

Figure 13. Immature male testicular tissue stained with H&E showing mitotically dividing gonial cells enclosed in cysts (GC).

Figure 14. Post-spawning female ovarian tissue stained with H&E showing post-ovulatory follicles (POF) and an atretic body (AB).

Figure 15. Seasonal movement extent for acoustic tagged lake sturgeon released back into the Slave Falls Reservoir. The winter season is defined as November 15, 2011 – March 31, 2012.

Table 1. Egg and sperm quality indices combined with fertilisation success and hatching success in eggs and sperm collected from female and male fish administered with Ovaprim™, mammalian LHRH, or sham-injection with Ringer's solution. Milt could not be collected from sham-injected lake sturgeon. Different letters indicate significant differences between treatments ($p<0.05$). Fertilisation success indicates the proportion of fertilized eggs surviving until hatch. Hatching success indicates the proportion of larval fish surviving through the prolarval stage.

	Control	Ovaprim	GnRH
Gamete Quality			
Average egg diameter (mm)	3.16 ± 0.027^{a}	2.49 ± 0.031^{b}	3.17 ± 0.038^{a}
Sperm motility (%)	--	44.5	--
Spermatocrit (%)	--	--	8.5
Developmental Success			
Fertilisation success (%)	64.68 ± 11.2	84.96 ± 2.5	84.01 ± 4.4
Hatching success (%)	1.19 ± 0.6	51.53 ± 4.06	34.02 ± 15.8

Figure 1

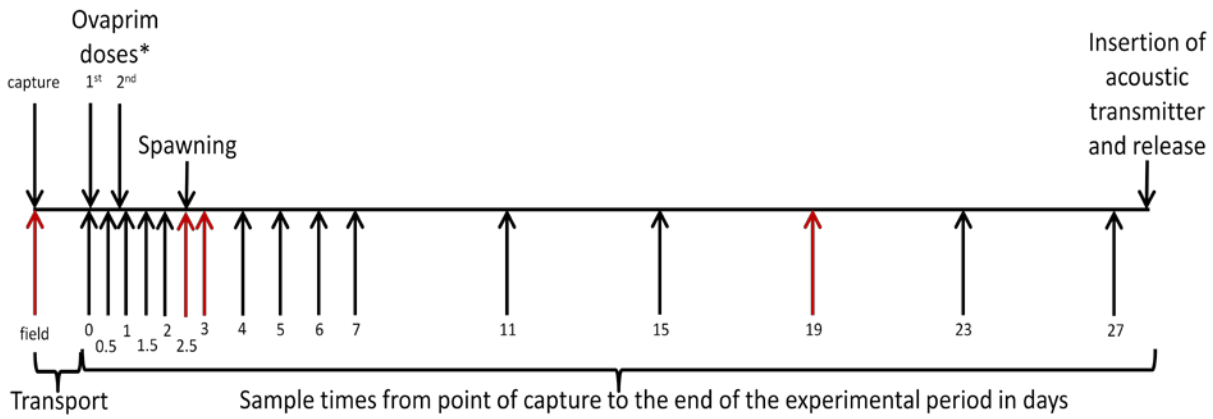
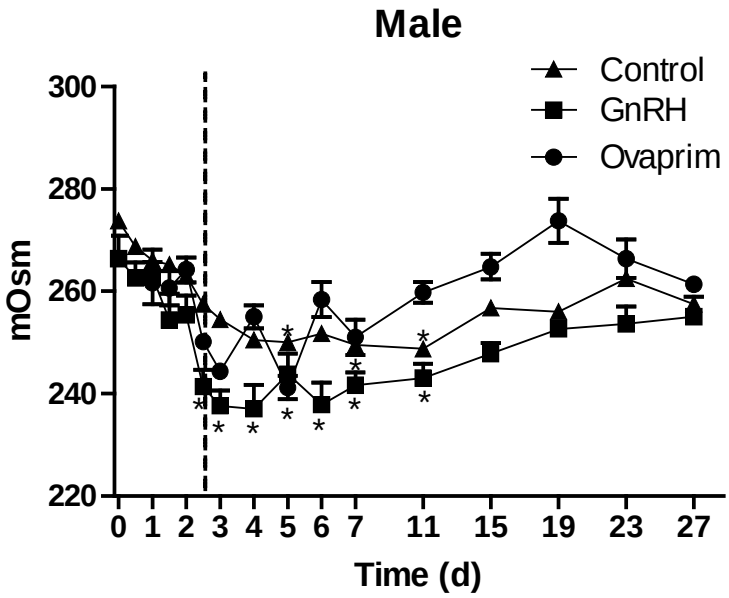

Ovaprim doses*
capture
1st
2nd
Spawning
Insertion of acoustic transmitter and release
field
0
0.5
1
1.5
2
2.5
3
4
5
6
7
11
15
19
23
27
Transport
Sample times from point of capture to the end of the experimental period in days

Figure 2


Male
Control
GnRH
Ovaprim
mOsm
300
280
260
240
220
0 1 2 3 4 5 6 7 11 15 19 23 27
Time (d)

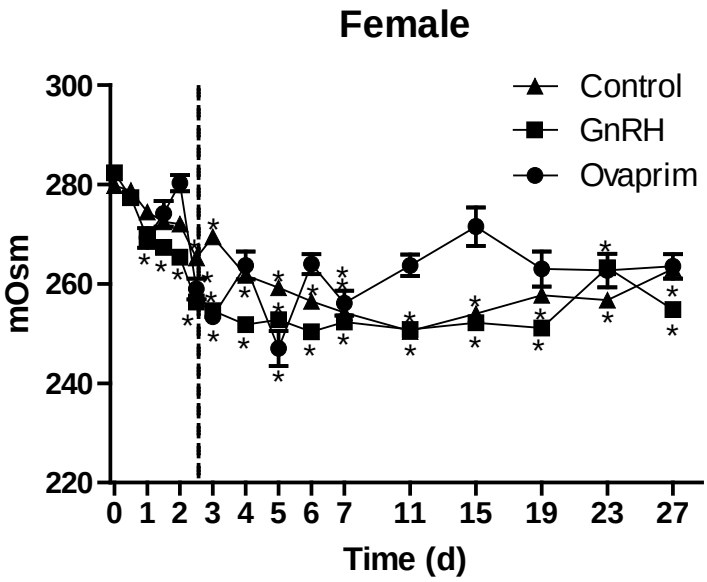
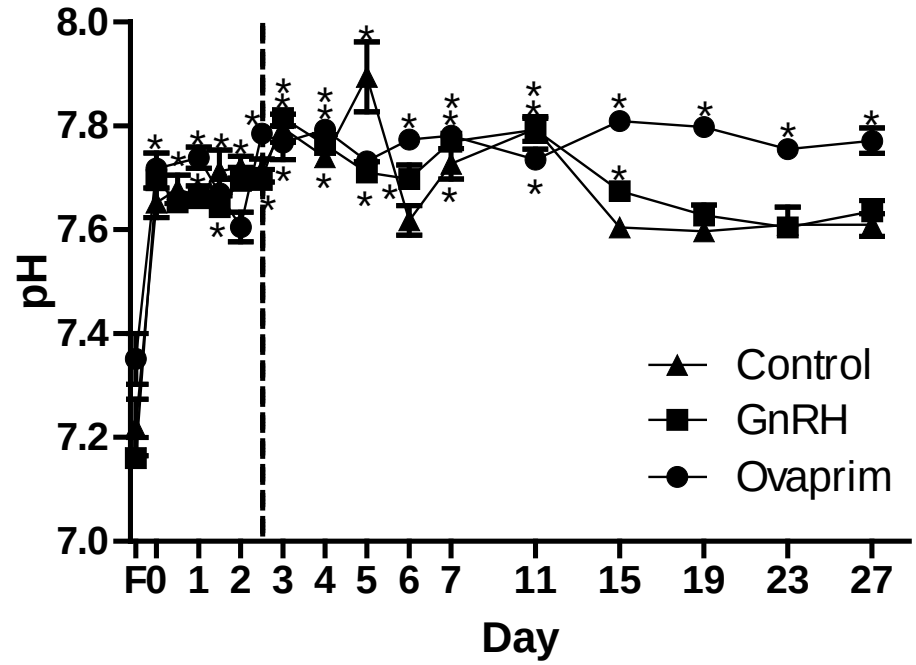

Female
Control
GnRH
Ovaprim
mOsm
300
280
260
240
220
0 1 2 3 4 5 6 7 11 15 19 23 27
Time (d)

Figure 3


8.0
7.8
7.6
7.4
7.2
7.0
pH
F0 1 2 3 4 5 6 7 11 15 19 23 27
Day
Control
GnRH
Ovaprim

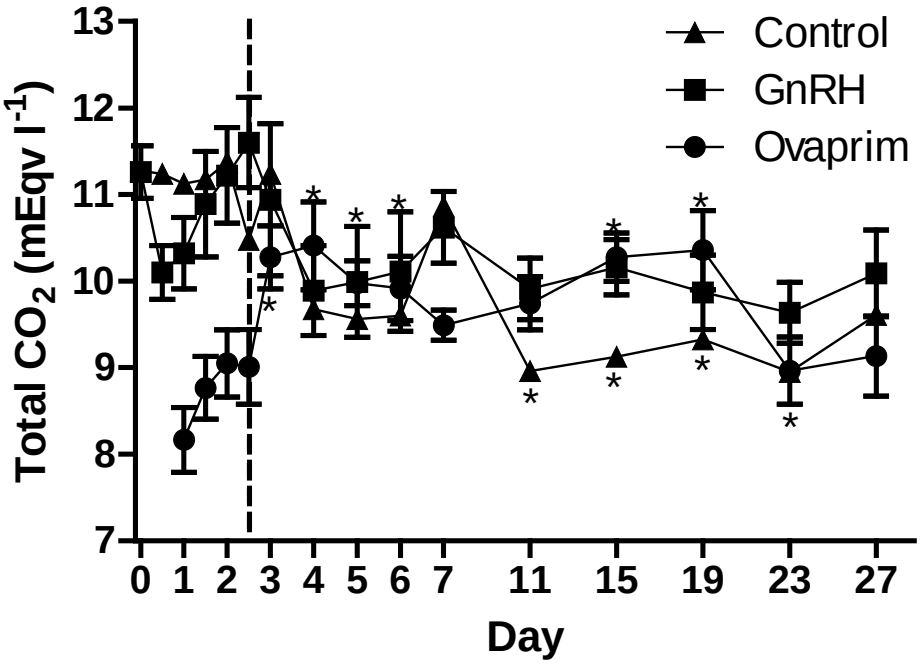

13
12
11
10
9
8
7
Total CO_2 (mEqv l^{-1})
0 1 2 3 4 5 6 7 11 15 19 23 27
Day
Control
GnRH
Ovaprim

Figure 4

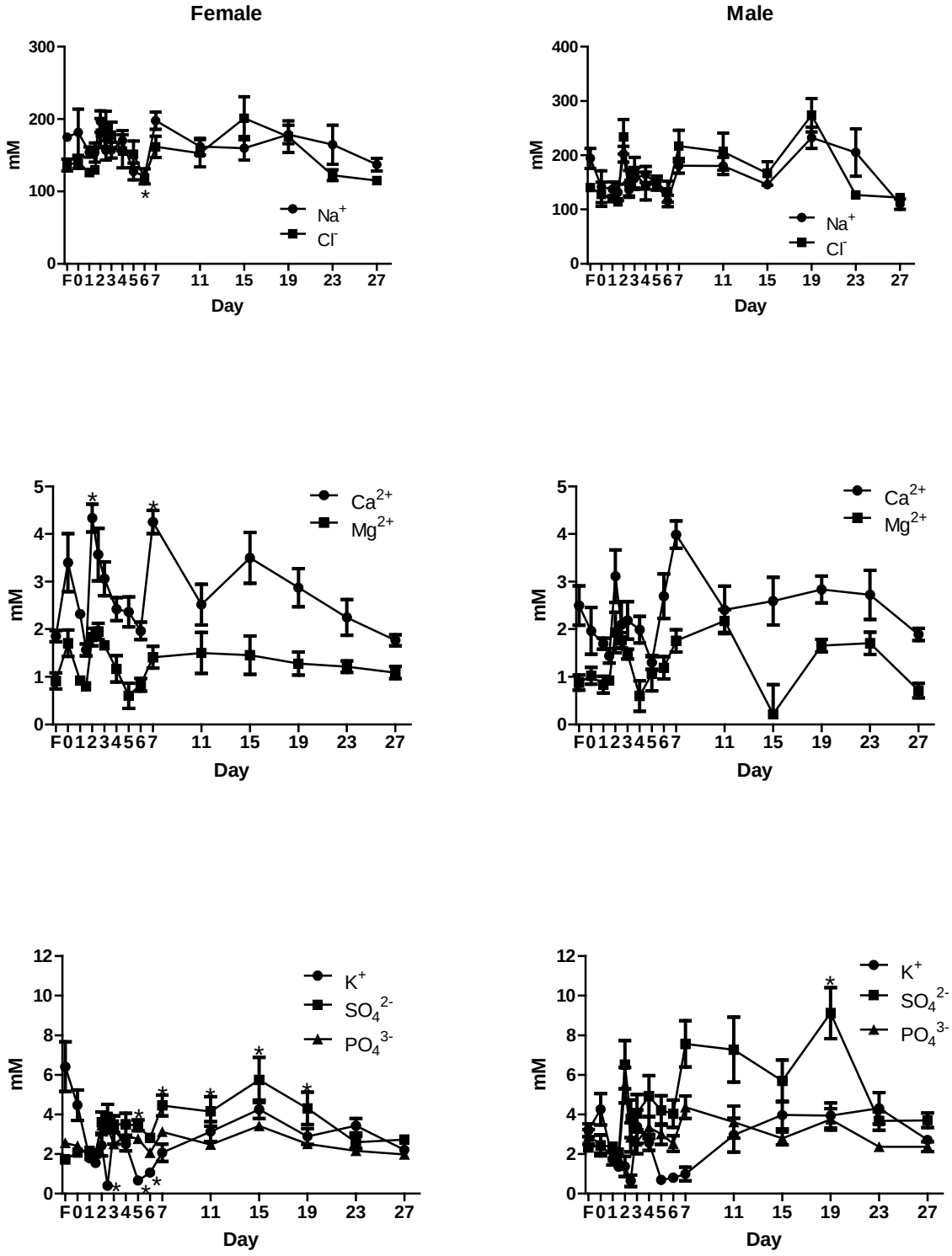
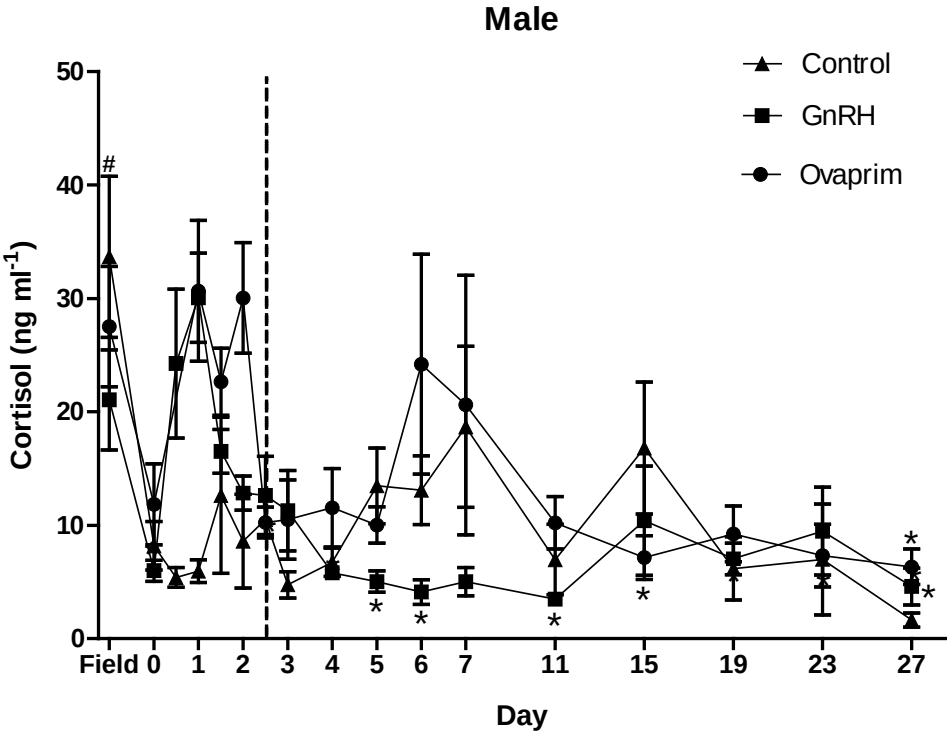

Female
Male
mM
Day
Na+
Cl-
Ca2+
Mg2+
K+
SO4 2-
PO4 3-
F 0 1 2 3 4 5 6 7 11 15 19 23 27

Figure 5


Male
Control
GnRH
Ovaprim
Cortisol (ng ml-1)
50
40
30
20
10
0
#
*
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day

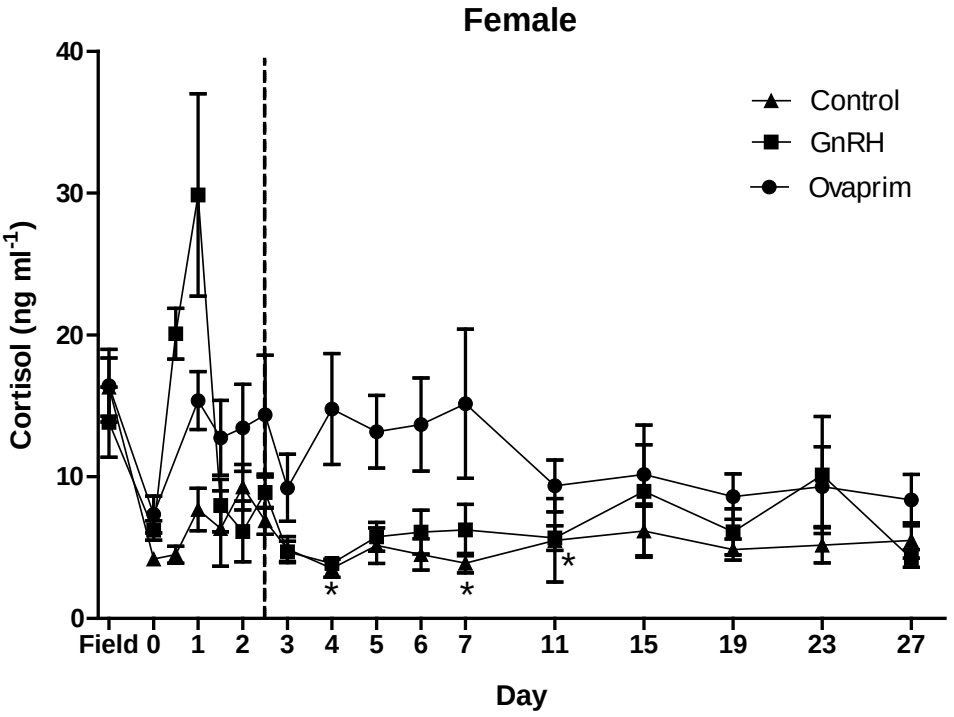
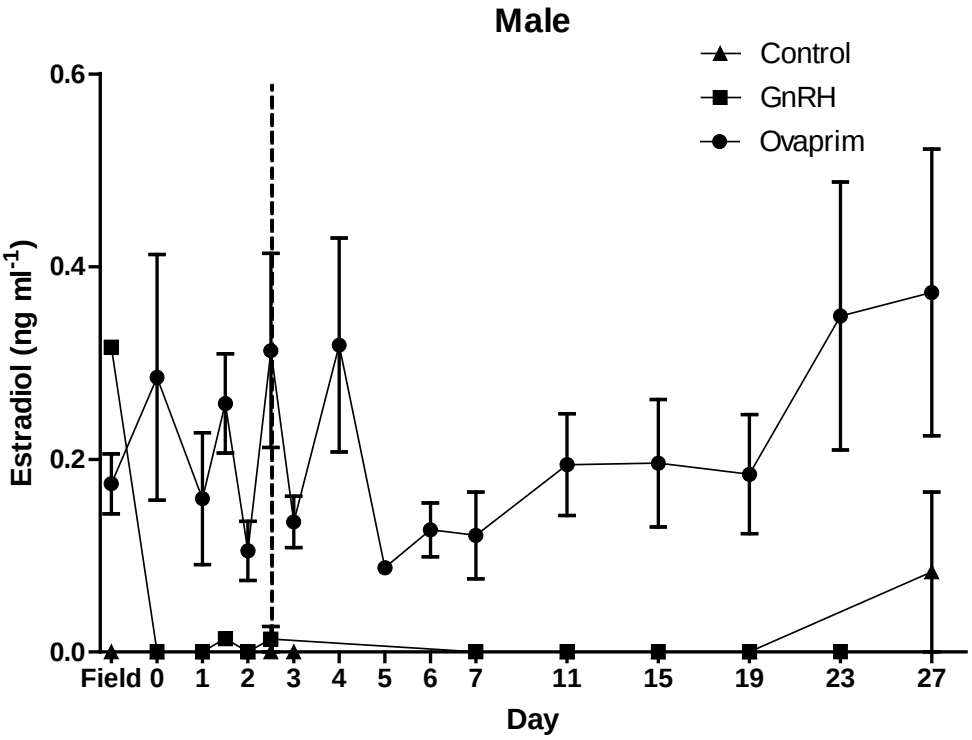

Female
Control
GnRH
Ovaprim
Cortisol (ng ml-1)
40
30
20
10
0
*
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day

Figure 6


Male
Control
GnRH
Ovaprim
Estradiol (ng ml-1)
0.6
0.4
0.2
0.0
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day

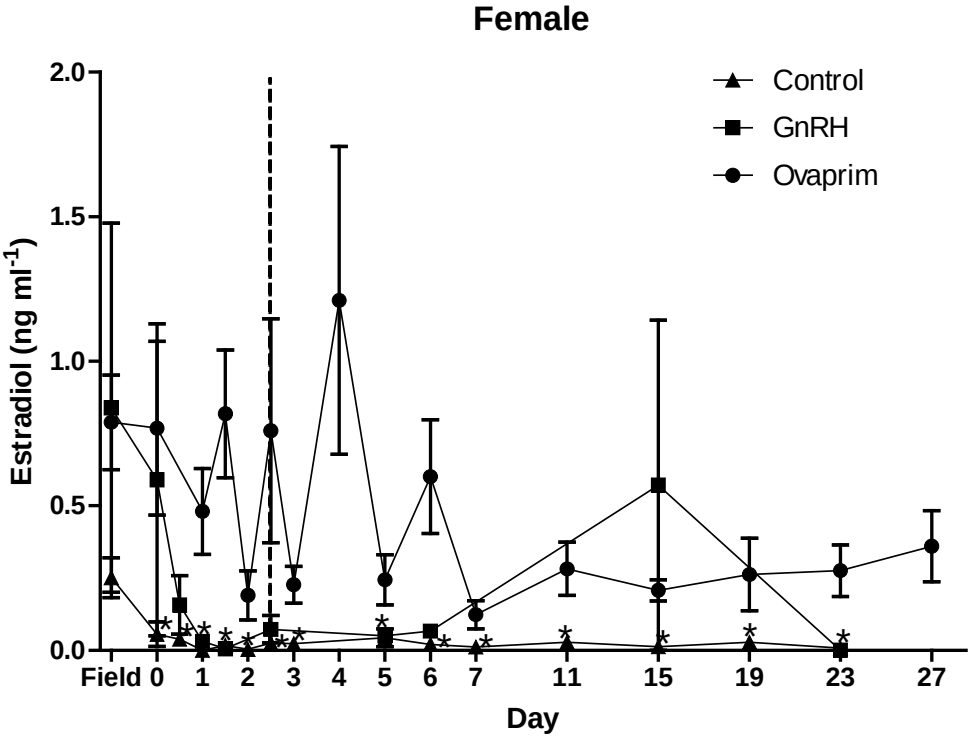

Female
Control
GnRH
Ovaprim
Estradiol (ng ml-1)
2.0
1.5
1.0
0.5
0.0
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day

Figure 7

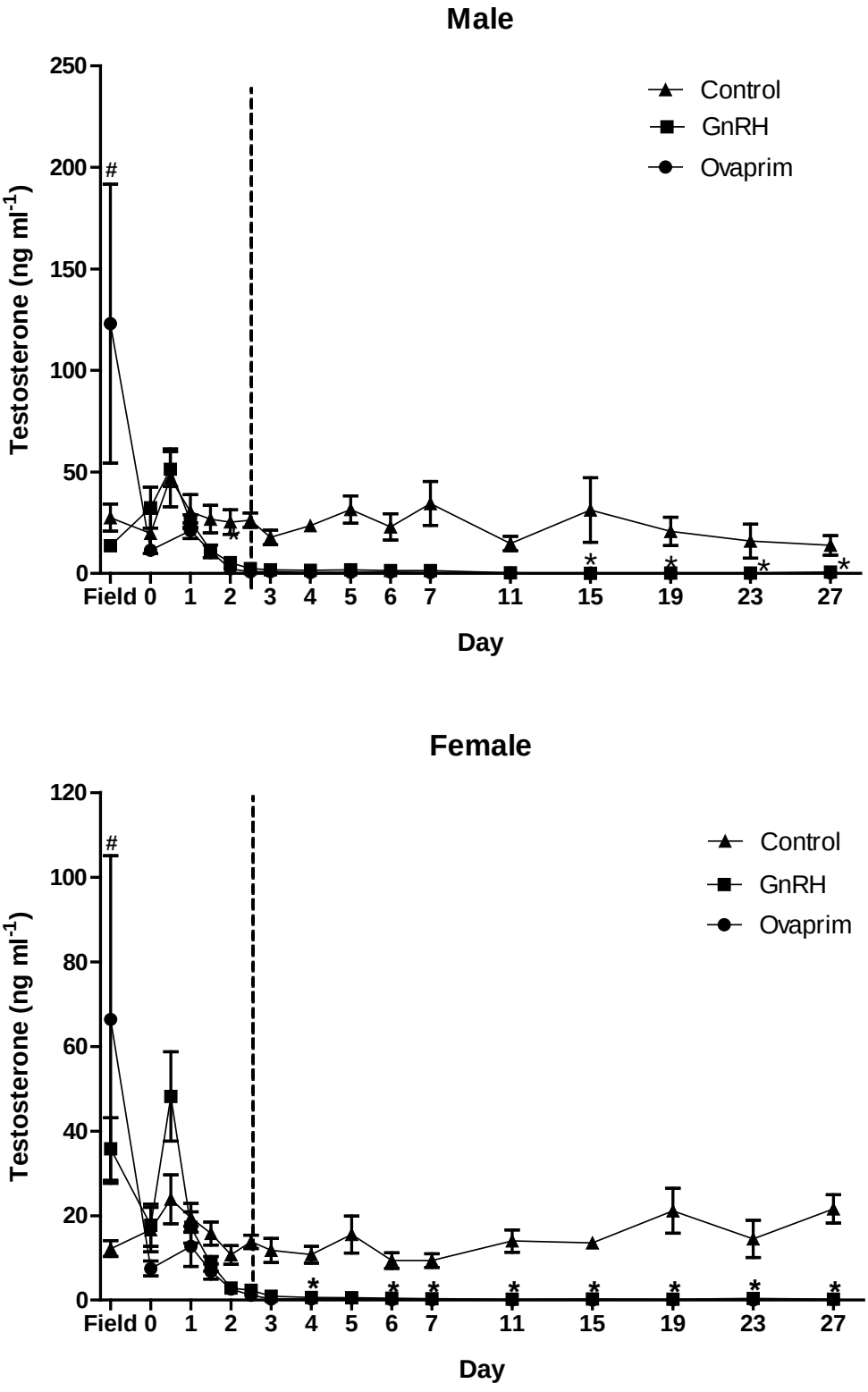

Male
Testosterone (ng ml-1)
250
200
150
100
50
0
Control
GnRH
Ovaprim
#
*
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day
Female
Testosterone (ng ml-1)
120
100
80
60
40
20
0
Control
GnRH
Ovaprim
#
*
Field 0 1 2 3 4 5 6 7 11 15 19 23 27
Day

Figure 8

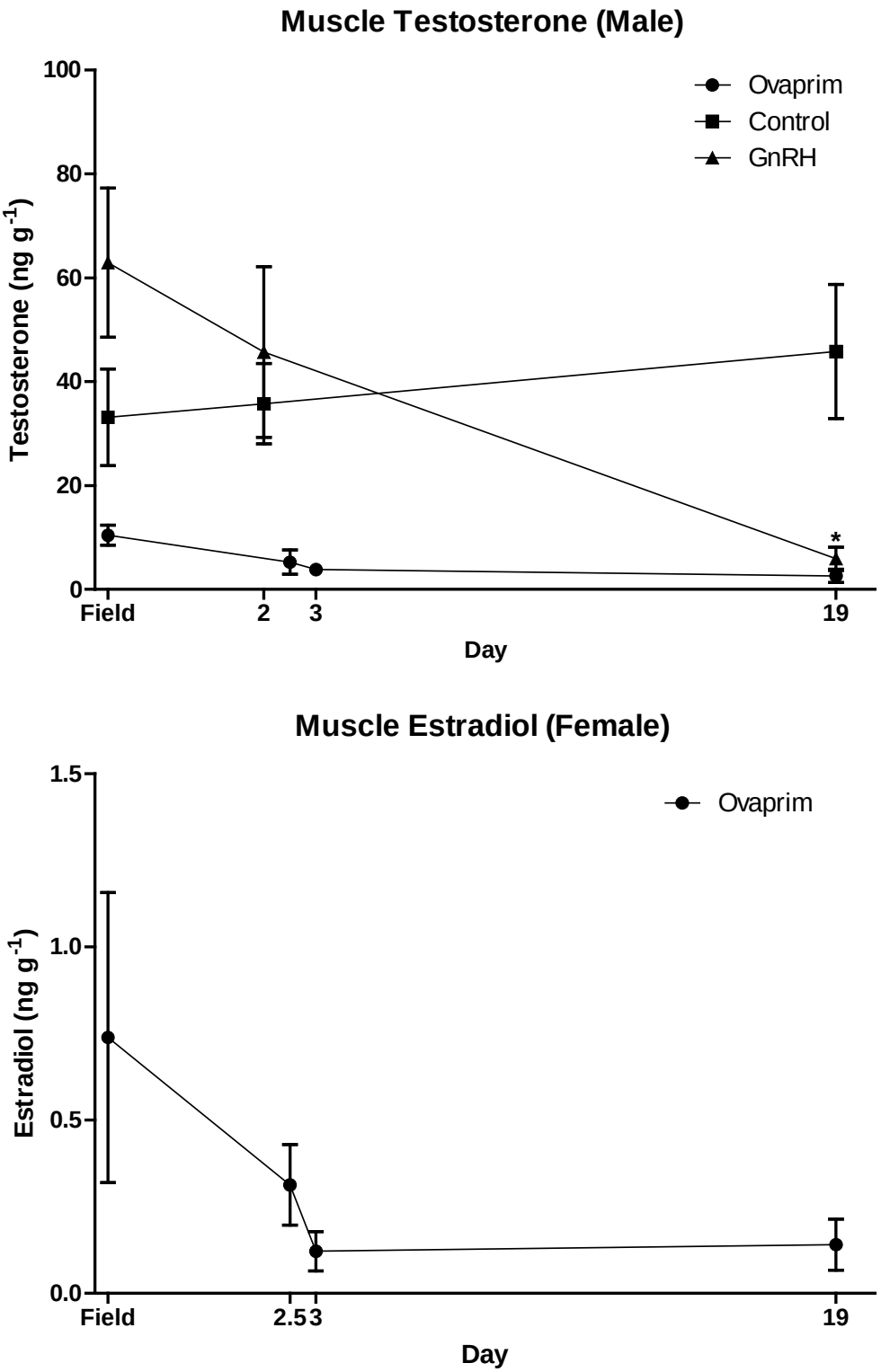

Muscle Testosterone (Male)
Ovaprim
Control
GnRH
Testosterone (ng g-1)
100
80
60
40
20
0
*
Field
2
3
19
Day
Muscle Estradiol (Female)
Ovaprim
Estradiol (ng g-1)
1.5
1.0
0.5
0.0
Field
2.5
3
19
Day

Figure 9

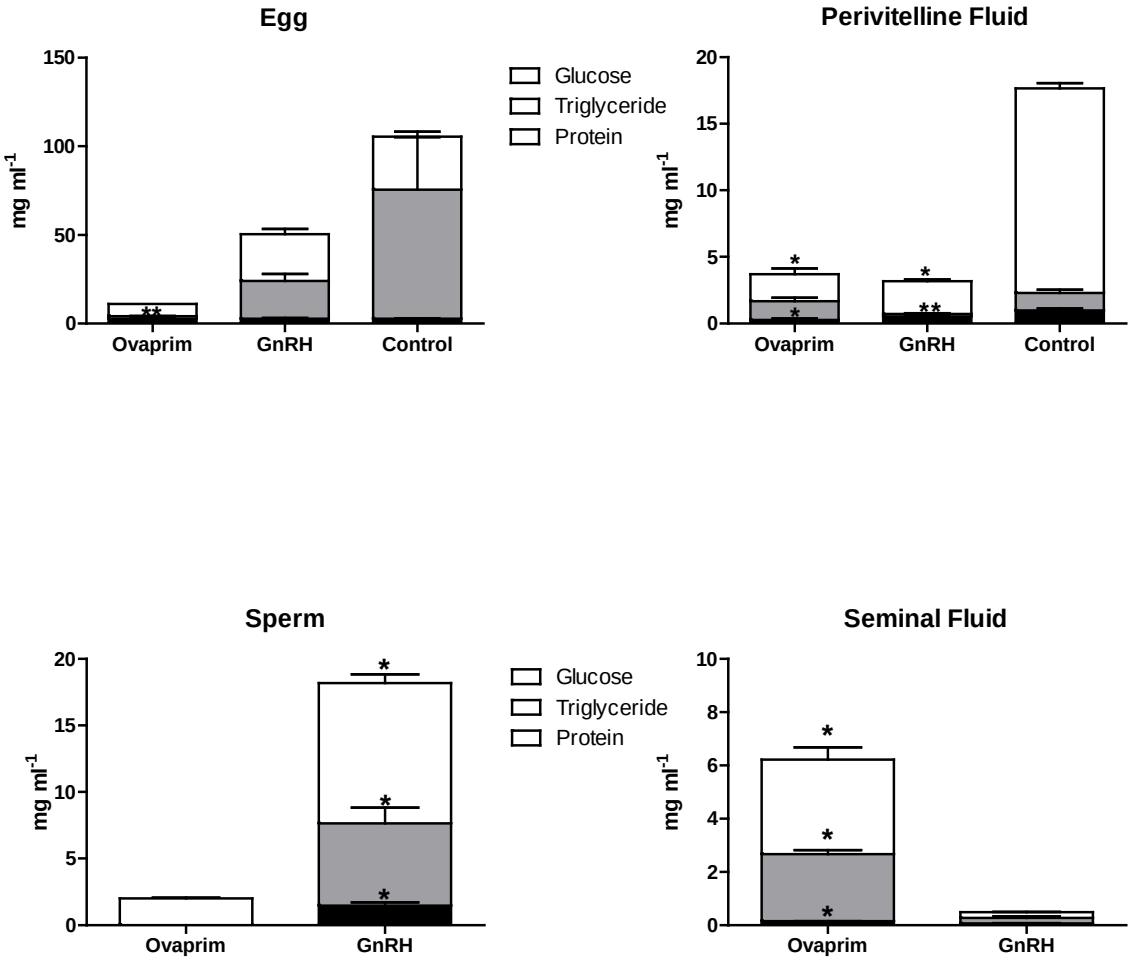

Egg
Perivitelline Fluid
Sperm
Seminal Fluid
Glucose
Triglyceride
Protein
mg ml-1
Ovaprim
GnRH
Control

Figure 10

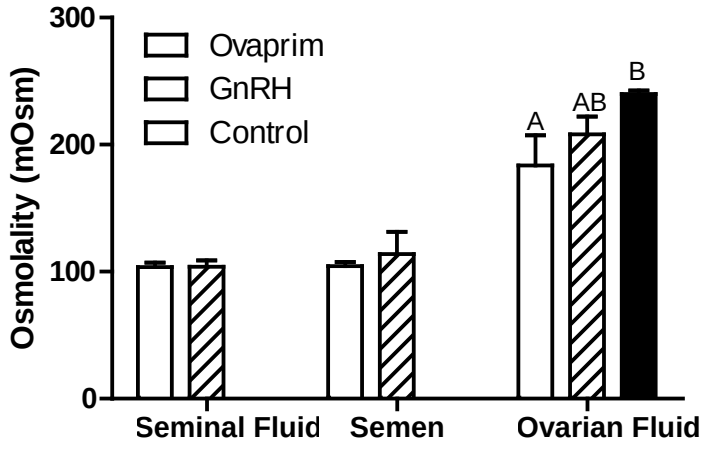

300
200
100
0
Osmolality (mOsm)
Ovaprim
GnRH
Control
A
AB
B
Seminal Fluid
Semen
Ovarian Fluid

Figure 11

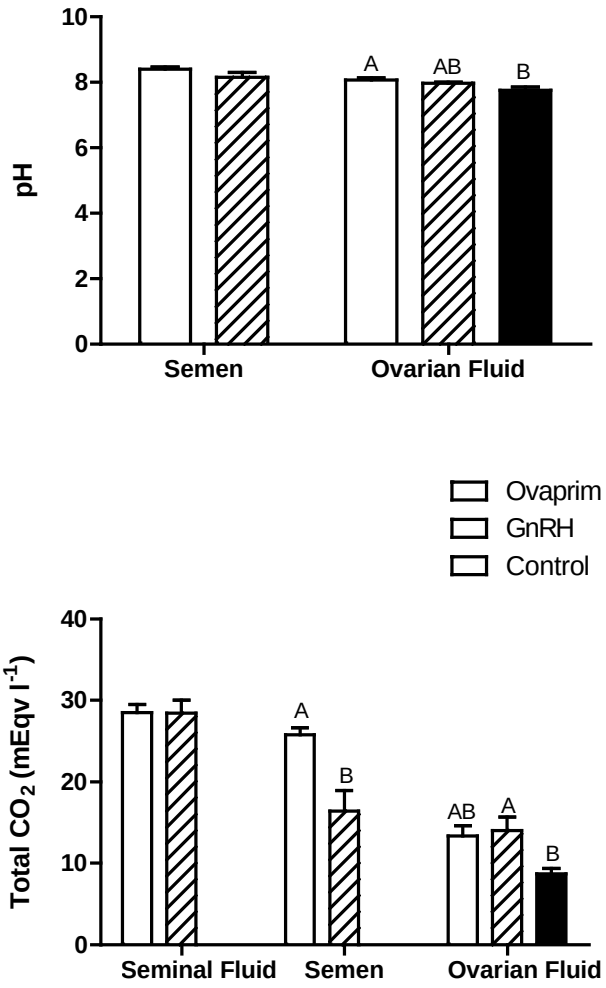

10
8
6
4
2
0
pH
A
AB
B
Semen
Ovarian Fluid
Ovaprim
GnRH
Control
40
30
20
10
0
Total CO_2 (mEqv l^{-1})
A
B
AB
A
B
Seminal Fluid
Semen
Ovarian Fluid

Figure 12

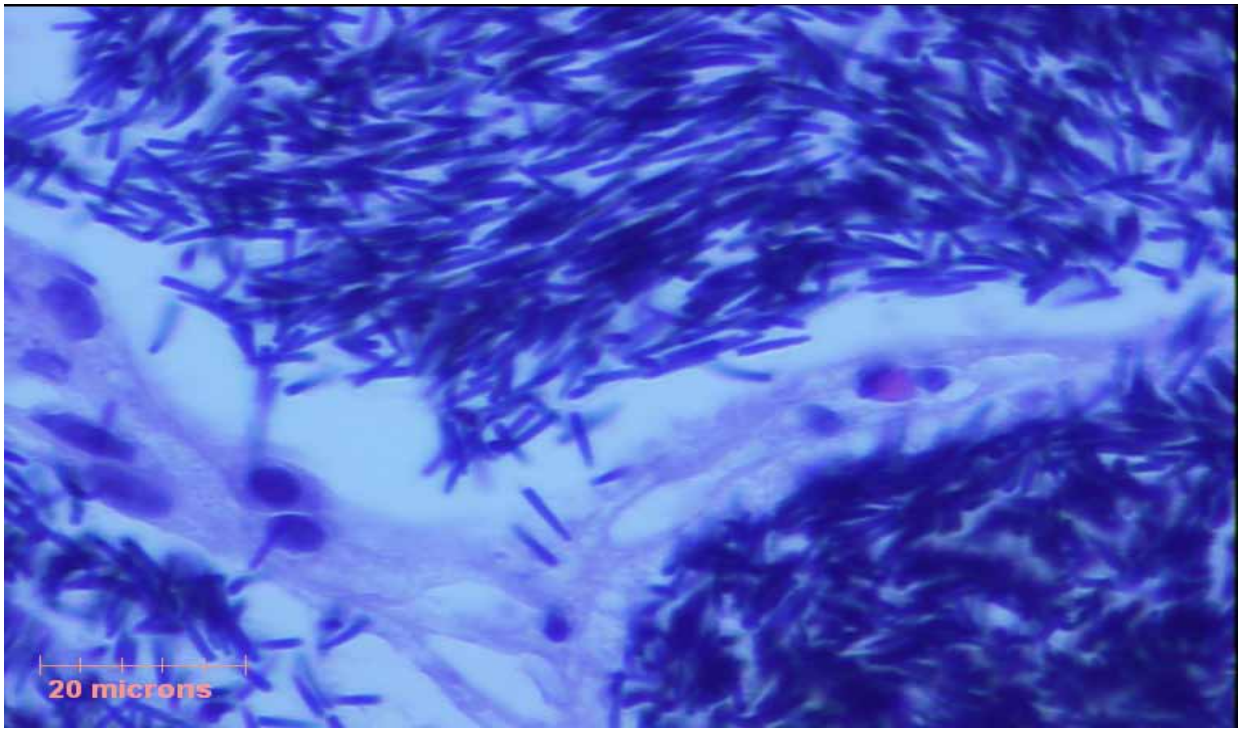


Figure 13

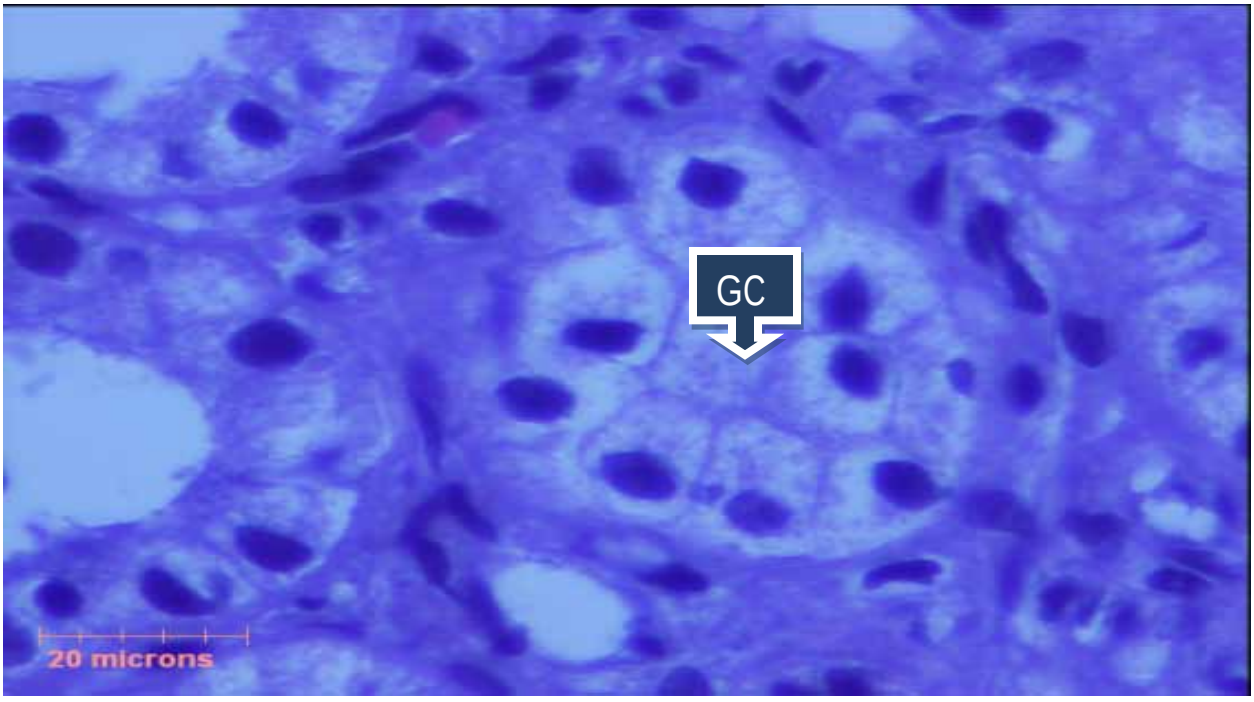


Figure 14

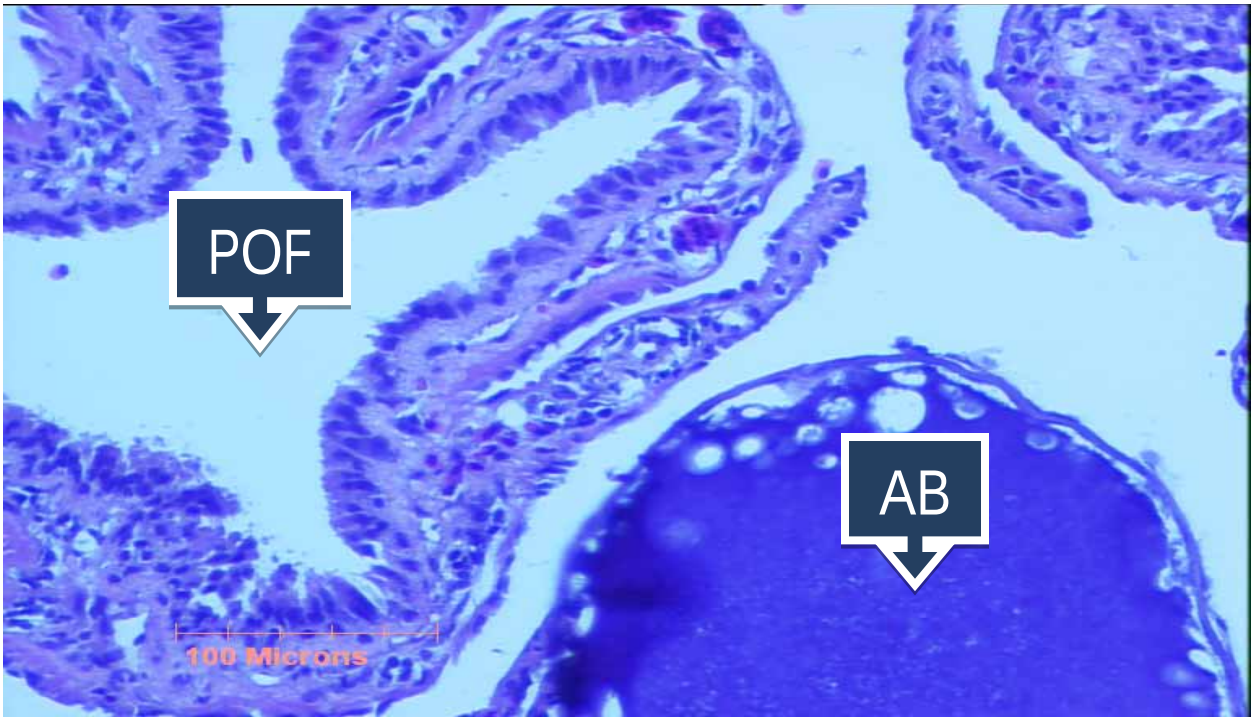
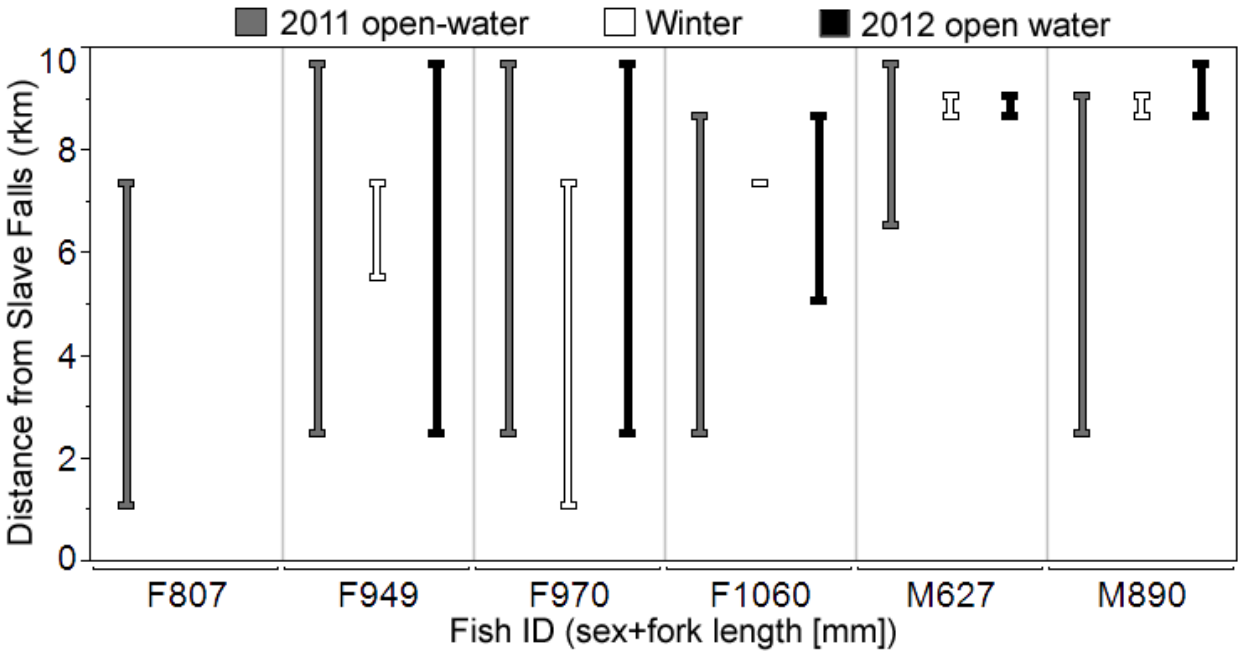


Figure 15


2011 open-water
Winter
2012 open water
Distance from Slave Falls (rkm)
0
2
4
6
8
10
F807
F949
F970
F1060
M627
M890
Fish ID (sex+fork length [mm])

References

Allen, P. J., Barth, C. C., Peake, S. J., Abrahams, M. V. and Anderson, W. G. (2009a). Cohesive behaviour shortens the stress response: The effects of conspecifics on the stress response in lake sturgeon, *Acipenser fulvescens*. *J. Fish Biol.* **74**, 90-104.

Allen, P. J., Webb, M. A. H., Cureton, E., Bruch, R. M., Barth, C. C., Peake, S. J. and Anderson, W. G. (2009b). Calcium regulation in wild populations of a freshwater cartilaginous fish, the lake sturgeon *Acipenser fulvescens*. *Comp. Biochem. Physiol.* **154A**, 437-450.

Baker, D. W., Peake, S. P. and Kieffer, J. D. (2008). The effect of capture, tagging and handling on haematological variables in wild lake sturgeon. *N. Am. J. Fish. Manag.* **28**, 296-300.

Barton, B. A., Morgan, J. D. and Vijayan, M. M. (2002). Physiological and condition-related indicators of environmental stress in fish, (ed. S. M. Adams), pp. 111-148. Bethesda, MD: American Fisheries Society.

Bayunova, L., Canario, A. V. M., Semenkova, T., Dyubin, V., Sverdlova, O., Trenkler, I. and Barannikova, I. (2006). Sex steroids and cortisol levels in the blood of stellate sturgeon (*Acipenser stellatus* Pallas) during final maturation induced by LH-RH-analogue. *J. Appl. Icthyol.* **22**, 334-339.

Counis, R., Laverriere, J. N., Garrel, G., Bleux, C., Cohen-Tannoudji, J., Lerrant, Y., Kottler, M. L. and Magre, S. (2005). Gonadotropin-releasing hormone and the control of gonadotrope function. *Reprod. Nutr. Dev.* **45**, 243-254.

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

Hayden, C. (2008). GnRH analogues: applications in assisted reproductive techniques *Eu. J. Endocrinol*. **159** S17–S25

Kah, O., Lethimonier, C., Somoza, G., Guilgur, L. G., Vaillant, C. and Lareyre, J. J. (2007). GnRH and GnRH receptors in metazoa: A historical, comparative, and evolutionary perspective. *Gen. Comp. Endocrinol.* **153**, 346-364.

McDougall, C.A., Blanchfield, P.J., Peake, S.J. and Anderson, W.G. (2013). Movement patterns and size class influence entrainment susceptibility of lake sturgeon *Acipenser fulvescens* in a small hydroelectric reservoir. Trans. Am. Fish. (Submitted)

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

Pincock, D. G. 2012. False detections: what they are and how to remove them. Vemco document #: DOC-004691. Available: http://www.vemco.com/pdf/false_detections.pdf (April 2013).

Semenkova, T., Barannikova, I., Kime, D. E., McAllister, B. G., Bayunova, L., Dyubin, V. and Kolmakov, N. (2002). Sex steroid profiles in female and male stellate sturgeon (*Acipenser stellatus* Pallas) during final maturation induced by hormonal treatment. *J. Appl. Ichthyol.* **18**, 375-381.

Sherwood, N. M., Doroshov, S. I. and Lance, V. (1991). Gonadotropin-releasing hormone (GnRH) in bony fish that are phylogenetically ancient: Reedfish (*Calamoichthys calabaricus*) sturgeon (*Acipenser transmontanus*), and Alligator Gar (*Lepisosterus spatula*). *Gen. Comp. Endocrinol.* **84**, 44-57.

Sherwood, N. M., Lovejoy, D. A. and Coe, I. R. (1993). Origin of mammalian gonadotropin-releasing hormones. *Endocr. Rev.* **14**, 241-253.

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiol. Rev.* **77**, 1997.

Williot, P., Gulyas, T. and Ceapa, C. (2002). An analogue of GnRH is effective for induction os ovulation and spermiation in farmed Siberian sturgeon, *Acipenser baerii*, Brandt. *Aquac. Res.* **33**, 735-737.

Appendix

Table A. Results of statistical analyses of concentrations of estradiol, testosterone, and cortisol in plasma collected from adult lake sturgeon injected at 0 and 20 h with Ovaprim^{TM} (0.5 ml kg^{-1}, n=5 males, 7 females), mammalian LHRH dissolved in Ringer's solution (GnRH, 30 µg kg^{-1}, n=5 each male and female), or Ringer's solution (control, n=4 each male and female), sampled serially over a 27-day time course. Observed values are illustrated in Figures 5-7.

Hormone	Sex	Treatment	P value (RM 1-way ANOVA)	Points (day) significantly different from value at capture
Estradiol	Female	Control	<0.001	all
		Ovaprim	0.064	
		GnRH	0.903	
	Male	Control	--	
		Ovaprim	0.051	
		GnRH	--	
Testosterone	Female	Control	0.03	none
		Ovaprim	0.004	all
		GnRH	<0.001	4,6,7,11,15,19,23,27
	Male	Control	0.402	
		Ovaprim	0.002	all
		GnRH	<0.001	15,19,23,27
Cortisol	Female	Control	0.04	4,7,11
		Ovaprim	0.135	
		GnRH	0.006	none
	Male	Control	<0.001	all
		Ovaprim	<0.001	15,27
		GnRH	<0.001	5,6,11,27