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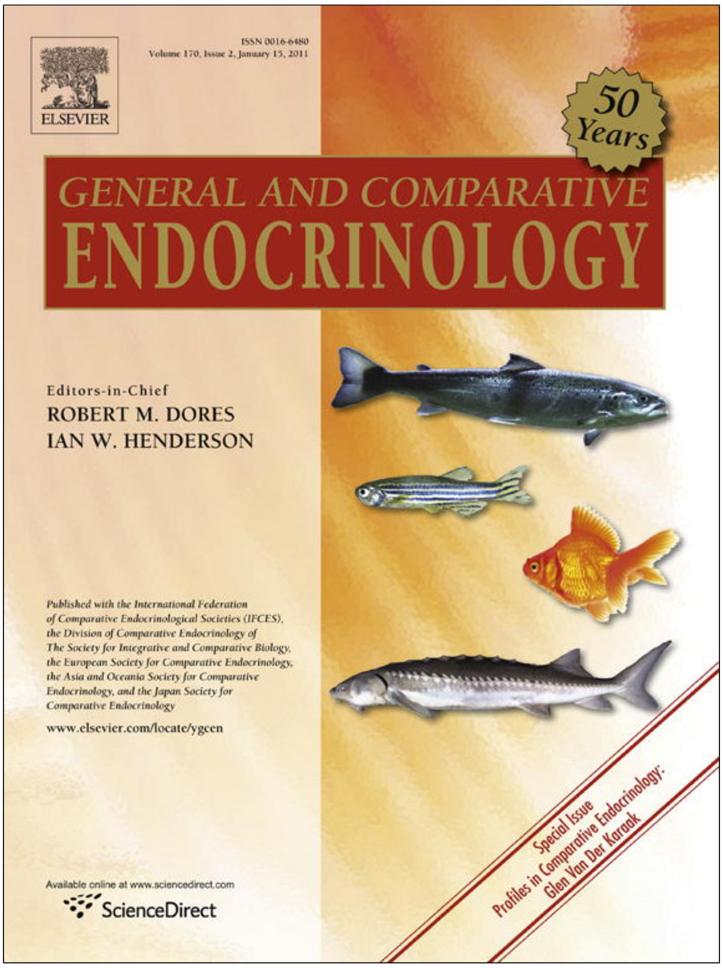

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Importance of environmental endocrinology in fisheries management and aquaculture of sturgeons

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ABSTRACT

Less is known about the reproductive endocrinology of sturgeons compared to modern teleosts. However, tools to assess the reproductive endocrinology and effects of environmental factors on reproduction do exist. This review utilizes case studies to describe the parameters involved in environmental endocrinology and the management and recovery efforts for the phylogenetically ancient sturgeon and paddlefish (Clade Chondrostei). Specifically, we discuss the use of environmental endocrinology to determine sex and stage of maturity and identify oviposition on spawning grounds, the importance of understanding endocrine disruption pathways, the challenges and benefits of assessing stress in wild populations of sturgeon, and three major physiological events in the reproductive development of farmed sturgeon understanding of which appears to be crucial for improving sturgeon aquaculture.

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1. Introduction

The field of fish reproductive endocrinology has made significant progress over the last several decades [68,141]. Through a better understanding of the reproductive neurobiology and endocrinology of fishes, many of the parameters used to determine how the endocrine system controls reproduction and how environmental factors control gametogenesis, spawning, and early development of fishes have been identified. Even though less is known about the reproductive endocrinology of sturgeons compared to modern teleosts, tools to assess the reproductive endocrinology and effects of environmental factors on reproduction do exist.

The majority of chondrostean species world-wide are threatened or endangered [13]. Sturgeons and paddlefishes are phylogenetically ancient ray-finned fish that are important for both science and education, aquaculture and fisheries, and they are good indicator species for ecosystem function because of their life history characteristics (i.e., long-lived and late-maturing).

Like teleosts, sturgeons have a biological clock controlled by endogenous and environmental factors (Fig. 1) [e.g., 31,36,113,132]. The clock may begin with sex differentiation, but knowledge of this event in sturgeon is largely limited to morphological studies [38,50,91]. Puberty is a hallmark in the onset of a reproductive cycle and the timing of puberty depends, as in teleosts [111], on age and body size, accumulated energy (e.g., lipids), and environmental cues. The first reproductive cycle is activated by neuroendocrine signals, with endogenous factors acting as the gates to first maturation [36]. Gametogenesis and spawning are controlled by energy balance, environmental factors, and the neuroendocrine reproductive axis [14,31,36,37,79,88]. The relative importance of the environmental factors controlling reproduction and the magnitude of change of these factors required to initiate key gametogenic stages and a spawning event have not yet been well defined for many chondrostean species. It will be important to understand the specific role of environmental factors in driving gametogenesis and spawning to address the mismatch that may occur with global climate change.

1.1. Neuroendocrine system controlling reproduction

Though less is known about neuroendocrine regulation of reproduction in chondrosteans then in teleosts, there is a general similarity in regulatory mechanisms between the two clades of ray-finned fish (Fig. 2) [36,79]. Little is known about the neurotransmitters and neurohormones participating in signal transduction in sturgeon, although there are several immunochemical studies on localization of neuropeptides and neurotransmitters in the sturgeon brain [23,66,93]. Two forms of gonadotropin-releasing hormone, a mammalian GnRH1 (mGnRH) and a chicken GnRH2 have been identified in sturgeons [66,67,108]. Synthetic mGnRHa is effectively used to induce ovulation and spermiation in captive breeding of sturgeon and paddlefish. The GnRH and neurotransmitter dopamine interact on

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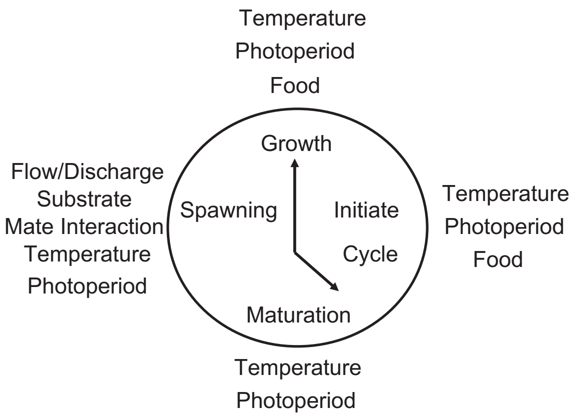

Fig. 1. Endogenous and environmental factors controlling the biological clock in sturgeons. The effect of photoperiod on growth has not been studied, but the observations on cultured sturgeon suggest a strong effect.

the pituitary gonadotropes to regulate synthesis and release of gonadotropins, GTHs [89]. Two pituitary gonadotropins, stGTH I and stGTH II were first described in farmed white sturgeon (*Acipenser transmontanus*) [79] and later characterized as molecular and functional homologues of mammalian FSH and LH in Siberian (*Acipenser baeri*), Russian (*Acipenser gueldenstaedti*), and Chinese (*Acipenser sinensis*) sturgeons [17,57,95]. The FSH controls the initiation of a gametogenic cycle and vitellogenesis in females [e.g., 77–79], while LH stimulates events resulting in gamete maturation and ovulation and spermiation [79,80]. Except for a single study indicating the inhibitory role of dopamine [89], little is known about regulation of gonadotropin release in sturgeon compared to teleosts [see 141]. Kisspeptins, gamma aminobutyric acid (GABA),

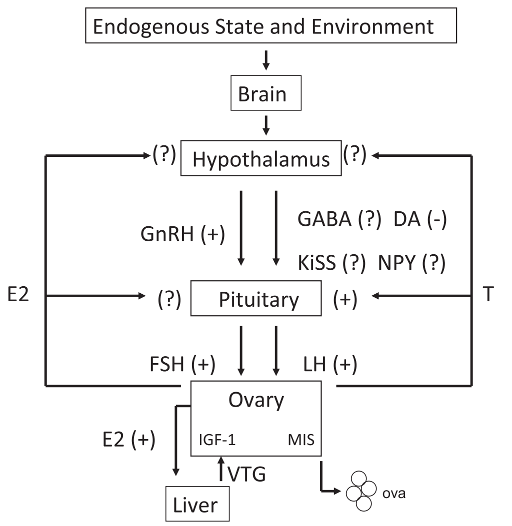

Fig. 2. Hypothalamo-pituitary–gonadal axis in sturgeon females. Little is known about the neurotransmitter and neurohormone modulation (gamma aminobutyric acid, GABA, kisspeptins, KiSS, neuropeptide Y, NPY) of gonadotropin-releasing hormone (GnRH) or pituitary gonadotropins (follicle stimulating hormone, FSH, and leutinizing hormone, LH). However, dopamine (DA) has been found to inhibit gonadotropin release. GnRH (mammalian GnRH1) stimulates the synthesis and release of FSH or LH and is used for induction of ovulation and spermiation in captive breeding of sturgeon. The FSH controls development and growth of ovarian follicle and vitellogenesis (vitellogenin, Vtg) by stimulating secretion of estradiol-17β, E2. LH stimulates oocyte maturation and ovulation by stimulating synthesis of the maturation inducing steroid, MIS. The positive and negative feedbacks of sex steroids (testosterone, T, and E2) on the hypothalamus and pituitary are not well known, except of the positive effect of the exogenous T on accumulation of FSH and LH in the pituitary. The insulin-like growth factor, IGF-1, and its receptor were characterized in sturgeon and are believed to play a major role in regulation of vitellogenesis.

neuropeptide Y (NPY), and gonadal growth factors play important roles in regulation of pituitary gonadotropins in teleosts [68], but they have not been adequately investigated in chondrosteans.

The sex steroids testosterone (T), 11-ketotestosterone (11-KT), estradiol-17β (E2), progesterone (P4), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) have been measured in sturgeon plasma during gametogenesis [e.g., 2,6,27,29,31,39,105,120,124,126,127,131,132,134]. The role of these steroids in reproduction of chondrostean fish appears to be very similar to that in teleosts; however, the hormonal "cross talk" known for teleosts [74] is not well understood in chondrosteans. In sturgeons, plasma sex steroid concentrations are undetectable or low until gonadal differentiation [39]. Concentrations of plasma T and 11-KT increase in males at proliferation of spermatogonia and in females before the onset of vitellogenesis [e.g., 29,39,80]. Concentrations of plasma androgens remain elevated throughout spermatogenesis [e.g., 2,29,120,124,126] and oogenesis [e.g., 3,29,120,124,126,131] and decrease following ovulation or spermiation [e.g., 124,131,132,134]. Plasma E2 concentrations are elevated during vitellogenic growth of the oocytes and gradually decrease prior to ovulation as a switch in production from C18 and C19 to C21 steroids occurs [e.g., 3,102,132,134]. However, concentrations of both E2 and T in the female's circulation have to remain above a basal level for gamete maturation and spawning to occur [110,132]. The identity of the putative maturation inducing steroid (MIS) has not yet been determined in sturgeons; however, several C21 steroids may act as an MIS, including P4, 17,20β-P, 20β-S, and 11-deoxycortisol [102,104,105,127,130]. Though the concentrations of circulating steroids may differ among sturgeon species and at various locations, the pattern of steroid profiles throughout the reproductive cycle is similar in different species [see 124]. In summary, our knowledge of sex steroids in chondrostean fish is limited to their profiles during the reproductive cycle, but little is known about their function in regulation of gonadotropins to elicit spawning behavior. One study revealed positive feedback of T on accumulation of FSH and LH in the sturgeon pituitary [90], but there have been no similar studies with E2 and growth factors. Recent molecular characterizations of the StAR protein in white sturgeon [62] and insulin-like growth factor (IGF-1) in sterlet (*Acipenser ruthenus*) [138,139] added important new tools for future research aimed to understand "cross-talk" between the gonads, pituitary and brain in sturgeons.

Vitellogenin (Vtg), the egg yolk precursor protein, has been investigated in sturgeons [e.g., 11,12,47,56,69]. The Vtg is synthesized in the liver in response to circulating E2 and other estrogenic chemicals, released into the bloodstream, sequestered within developing oocytes via receptor-mediated endocytosis, and enzymatically converted into egg yolk proteins stored in the egg cytoplasm as crystalline yolk platelets. In contrast to teleosts, embryonic egg yolk in sturgeon is intracellular and retains crystalline structure throughout early development [15,32]. Two components of female-specific serum proteins (FSSP) have been identified in the hybrid sturgeon bester (*Huso huso* × *Acipenser ruthenus*); the major FSSP was identified as Vtg, and the identity of the minor FSSP has not yet been verified [56]. In bester, three yolk proteins were identified: yolk protein 1 shared properties of lipovitellin identified in teleosts, yolk protein 2 was identified as β′-component identified in teleosts, and yolk protein 3 exhibited common characteristics of phosvitin identified in teleosts [56,73]. Partial molecular characterization of white sturgeon Vtg revealed lipovitelline and phosvitin moieities [11]. The changes in plasma concentrations of Vtg in a normal ovarian cycle have been well studied and correlated with progression of ovarian development in several species of sturgeon [e.g., 3,27,69,134]. As in teleosts, hepatic synthesis of Vtg can be induced in both sexes of sturgeon and at different stages of ontogeny by exogenous estrogens and xenoestrogens.

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Interestingly, the treatment of white sturgeon females approaching puberty (weight 8–14 kg) with slow-release E2 implants, alone or combined with GnRHa treatment, induced Vtg secretion but did not stimulate ovarian follicle development and Vtg uptake by the oocyte [78].

1.2. Neuroendocrine system controlling the stress response

Less is known about the neuroendocrine control of the stress response and roles of allostasis and hormesis in chondrosteans compared to teleosts [see 100]. However, the cortisol response has been described in several sturgeon species in response to a stressor [e.g., 7,8,10,64], and cortisol was identified as the primary glucocorticoid in pallid sturgeon (*Scaphirhynchus albus*) [122]. It is assumed that stressors induce an immediate release of catecholamines followed by stimulation of the hypothalamo-pituitary–interrenal axis (HPI) in sturgeons. Activation of the HPI axis lags behind the catecholamine response due to the time needed for neural stimulation of corticotropin releasing factor from the hypothalamus that induces synthesis and secretion of corticotropic hormone from the pituitary. The pituitary, in turn, stimulates synthesis and secretion of glucocorticoid hormones from the interrenal tissue. Elevated plasma catecholamines and glucocorticoids constitute the primary stress response and may be followed by secondary and tertiary stress responses changing metabolism and affecting physiological functions and performance of the organism. The stress response in green sturgeon (*Acipenser medirostris*) was found to be modified by time of day and temperature; the magnitude of cortisol and lactate were significantly higher when stress was imposed at night time, and the lactate response was significantly extended at 11 °C compared to 19 °C [64]. The magnitude of cortisol in stressed sturgeons does differ according to the stressor and by species [see 122]. This does not, however, infer that lower cortisol concentrations in some sturgeon species are insufficient to elicit a stress response as changes in plasma glucose and lactate were seen in these studies.

2. Role of environmental endocrinology in fisheries management

2.1. Sex and stage of maturity

Successful management of sturgeon populations requires knowledge of the stock composition with regard to sex and maturational stage. Sturgeon females and males are distinctly different in their life history patterns within a population and among species [see 13]. Because sturgeon do not express external sex dimorphism, it is essential to be able to determine sex and stage of maturity for management of sturgeon populations.

The pattern of steroid production in sturgeons allows for discrimination of sex and stage of maturity less-invasively using blood plasma rather than a gonadal biopsy. This is beneficial when working with threatened or endangered species. In subadult and adult white sturgeon of the Columbia River, plasma T and E2 were found to be the best predictors of sex and stage of maturity with 88% of the non-reproductive females, 72% of the non-reproductive males, 98% of the vitellogenic and ripe females, and 96% of the maturing and ripe males correctly identified [126]. In the adult population of white sturgeon below Bonneville Dam in the Columbia River, 93% of the non-reproductive females, 100% of the non-reproductive males, 98% of the vitellogenic and ripe females, and 100% of the maturing and ripe males were correctly identified using plasma T and E2 [126]. In the classification of green sturgeon into six groups of sex and maturity, plasma T, 11-KT, and E2 plus fork length (FL) or total length (TL) were found to be the best predictors. The derived discriminant functions led to the correct classification of 100% of the non-reproductive females, 73% of the non-reproductive males, 100% of the vitellogenic females, 100% of the spermiating males, 77% of the post-ovulatory females, and 61% of the post-spermiated males (M. Webb and D. Erickson, unpublished data). In Persian sturgeon (*Acipenser persicus*), plasma T and E2, with either fish age, length, or weight led to the correct classification of 100% of the non-reproductive females, 100% of the mid-spermatogenic males, 100% of the post-vitellogenic (ripe) females, and 86% of the ripe males [74]. When only plasma T and E2 were used for predicting reproductive status in Persian sturgeon, 92% of the non-reproductive females, 88% of the mid-spermatogenic males, 100% of the post-vitellogenic (ripe) females, and 86% of the ripe males were correctly classified. These results indicate the usefulness of the endocrine profiles in sturgeons as a tool to assess sex and stage of maturity for fisheries management.

Under ideal circumstances, the misclassification rates of individuals among classes of maturity would be determined for each species. However, when classification functions derived for white sturgeon to predict sex and stage of maturity were applied to a small number of lake sturgeon (*Acipenser fulvescens*), comparable classification rates were found [27]. When the white sturgeon classifications were applied to predict sex in adult stellate sturgeon (*Acipenser stellatus*), males were overestimated due to the extremely high concentrations of plasma T when compared to other sturgeon species [18]. Hence, it is important to describe the sex steroid profiles in a population and estimate the error rate associated with the use of predicting sex and stage of maturity on at least a small subset of a population. Special consideration for the use of this tool should be made in populations that may be exposed to environmental contaminants (see Section 2.3).

Ultrasonography [21,26,81,114,133] and endoscopy [34,63,133] have also been applied to determine sex and stage of maturity in sturgeons. However, the correct classification of sex and stage of maturity was lower compared to the use of plasma sex steroids; and non-gravid females and stage of maturity in males were difficult to identify. The Fourier transform infrared spectroscopy of blood plasma coupled with multivariate analysis was used to differentiate pre-vitellogenic, vitellogenic, and late vitellogenic females and females undergoing follicular atresia as well as predict the oocyte polarization index (a morphologic metric of spawning readiness) [72].

2.2. Identification of oviposition in wild sturgeon populations

Many sturgeon species spawn in moderately turbid, fast flowing rivers, and their fertilized eggs are attached to the rocky substrate in the river bed. This environment makes collection of newly fertilized eggs difficult and prevents researchers from identifying spawning events and locations. A combination of endocrine parameters and changes in body weight effected by postspawning gonadal regression have recently been employed to identify successful oviposition of female pallid and shovelnose sturgeon (*Scaphirhynchus platorynchus*) in the Missouri River [48,97]. Changes in circulating sex steroid profiles from post-vitellogenesis to post-ovulation have been described in white sturgeon [131], pallid sturgeon [48], shovelnose sturgeon [31,97,134], green sturgeon [124], lake sturgeon [1,75], stellate sturgeon [9,102], and sterlet [105]. Live weight decreased after oviposition by 12–22% in pallid sturgeon [48], by 6–22% in shovelnose sturgeon [97], and by 20–30% in shortnose sturgeon *Acipenser brevirostrum* (B. Kynard, unpublished data). Identification of the stage of maturity of the female prior to spawning season and collection of blood plasma and data on body weight are the key components to the success of this analysis. If a significant decrease in sex steroids is seen without a concomitant change in body weight within approximately 2 weeks of the suspected spawning season, follicular

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atresia has ensued since the early stage of atresia does not significantly affect the inner compartment of the egg [70,131,132]. The benefit of using the significant decrease in sex steroid concentrations in combination with a decrease in body weight is that a repeated examination of gonadal tissue through invasive means is not necessary which is often valuable in studies with threatened or endangered species. If the fish cannot be recaptured within 2 weeks of the suspected spawning date, this method may not be applicable because a decrease in live weight may occur due to ovarian regression. Richards and Guy [97] found that one month after peak spawning season (water temperatures of approximately 21 °C) the loss of live weight in shovelnose sturgeon that failed to spawn and underwent follicular atresia ranged 5–18%. The use of endocrine parameters in this manner, while not able to demonstrate successful fertilization of eggs, has provided fishery managers with the evidence that a normal oviposition had taken place. Because sturgeon undergo follicular atresia under non-optimal environmental conditions [70,131,132], it has long been assumed that females will not lay the eggs in the absence of optimal environmental conditions and a suitable mate. Surely, the timing of the loss in body weight associated with follicular atresia at different temperatures should be determined for sturgeon species prior to application of this method. The use of ultrasonagraphy in this instance may prove very useful as the classification of ripe females is very high [21,26,81,114,133], though the classification rates of females undergoing follicular atresia compared to post-ovulatory females would need to be determined.

2.3. Assessing endocrine disruption

Intersexes have been identified in wild populations of sturgeon in the San Francisco Bay white sturgeon [20], Columbia River white sturgeon [40,128,129], Missouri River shovelnose sturgeon [30], Mississippi River shovelnose sturgeon [26,52], Hudson River Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon [4,119], and in captive populations of shortnose sturgeon [42,53], Russian sturgeon [59], and sterlet [135]. The proportions of intersexes in sampled populations ranged from less than 1% to 29% in shovelnose sturgeon of the middle reach of Missouri River [30]. The natural rate of intersexes in sturgeon populations is not well known, however the elevated rate of intersexes in the Missouri and Mississippi River shovelnose sturgeon (29%) appears to be driven by endocrine disrupting environmental contaminants [30,52].

Only a few studies have examined the endocrine profiles of intersex sturgeon. Jackson et al. [59] found that in intersex Russian sturgeon (pre-vitellogenic females with testicular germinal tissue in the ovary) plasma E2 concentrations were slightly higher compared to normal females; however, no statistical differences were seen. Plasma concentrations of 11-KT were significantly higher in fish with intersex gonads compared to normal females and were similar to normal males. The expression of FSH β-subunit revealed mRNA levels in intersex fish similar to those in normal males and significantly lower than in normal females, while LH β mRNA levels were higher in intersex fish and normal males compared to normal females. These results indicate that endocrine parameters in intersex Russian sturgeon were more similar to normal males but differed from those in normal females even though the predominant mass of gonads in the intersex fish was composed of ovarian tissue [59].

In the lower Columbia River white sturgeon, negative correlations between plasma T concentrations and total DDT, pesticides, and PCBs in gonads and livers of males [40] were found. As well, negative correlations between plasma T and 11-KT concentrations and muscle mercury content [125], and plasma E2 concentration and liver mercury content of females and males [125] were found. Feist et al. [40] found only 3 intersexes out of 158 fish in the dataset, while Webb et al. [125] found no intersexes present. The lower concentrations of plasma androgens detected in males with higher gonad and liver contaminant loads appeared to be due to the up-regulation of liver cytochrome P450 isozymes resulting in increased rates of steroid metabolism [40]. It is interesting that the misclassification rate of non-reproductive males as non-reproductive females in this population was high (22%) [126] suggesting that it is important to consider the potential influence of environmental contaminants when using steroid hormones to assess sturgeon population structure.

Elevated plasma Vtg, which is naturally synthesized in the liver in response to E2 secretion by the ovarian follicle in sexually maturing females, has been documented in wild male and wild immature female sturgeon [40]. Endocrine disrupting chemicals, such as 4-nonlyphenol [140], phytoestrogens [65], and ethynylestradiol [85] have been found to induce Vtg synthesis in male and immature female sturgeon in the laboratory. Circulating concentrations of Vtg is a useful tool to identify endocrine disruption in the field; however identification of the constituent that affects vitellogenin production may be difficult. For example, some contaminants present in environmental mixtures, such as PCBs, PAHs and dioxins, can suppress the action of environmental estrogens and inhibit vitellogenin synthesis in sturgeon [86].

2.4. Assessing stress

Assessing stress in a field study can be extremely challenging. It is known that stress can affect reproduction in various ways, and stressors experienced in one developmental stage can have an effect during latter developmental stages [see 100] potentially confounding our ability to determine if a single variable is responsible for early, delayed or failed reproduction or poor gamete quality. A case study that demonstrates these difficulties as well as the value in assessing stress load in the field involves the catch-and-release fishery for adult white sturgeon below Bonneville Dam in the Columbia River.

Catch-and-release fisheries for adult white sturgeon exist in the Columbia, Snake, Fraser, and the Sacramento Rivers. From 2000 to 2004, blood samples were collected from 305 adult white sturgeon handled during a study to determine the maturation cycle below Bonneville Dam [123,126]. Fish were captured with the aid of sport fishing guides by hook and line. As a result of the capture method, the opportunity arose to look at the relationship between plasma cortisol concentrations and play time (M. Webb and B. Cady, unpublished). Play time was determined for each fish caught by sport fishers and was defined as the time a fish was hooked to when the fish was brought on the boat for sampling. A blood sample was taken within 5 min of landing each fish. Hook marks and scars were counted in the buccal cavity of each fish, and each fish was examined for overall well-being. Plasma cortisol was measured by radioimmunoassay following the protocol of Foster and Dunn [46] and modified by Redding et al. [96]. Simple linear regression was used to determine if a relationship existed between plasma cortisol and play time. The analysis revealed a significant positive relationship between play time and plasma cortisol (Fig. 3), however, the R^2 value was low ($R^2 = 0.187$).

Plasma cortisol concentrations in unstressed wild white sturgeon have been found to be 13.4 ± 3.4 ng/ml (C. Kern, J. North, T. Rien, unpublished), while concentrations in unstressed captive white sturgeon were 8.6 ± 4.5 ng/ml [10]. The mean cortisol concentrations were significantly higher in the sport-caught white sturgeon below Bonneville Dam (39.58 ± 1.77 ng/ml, range 1.70–238.27 ng/ml; M. Webb and B. Cady, unpublished) compared to basal concentrations reported by Kern, North, and Rien (unpublished) and [10]. The low R^2 value appears to be driven by high cortisol concentrations in fish with short play times. This demonstrates

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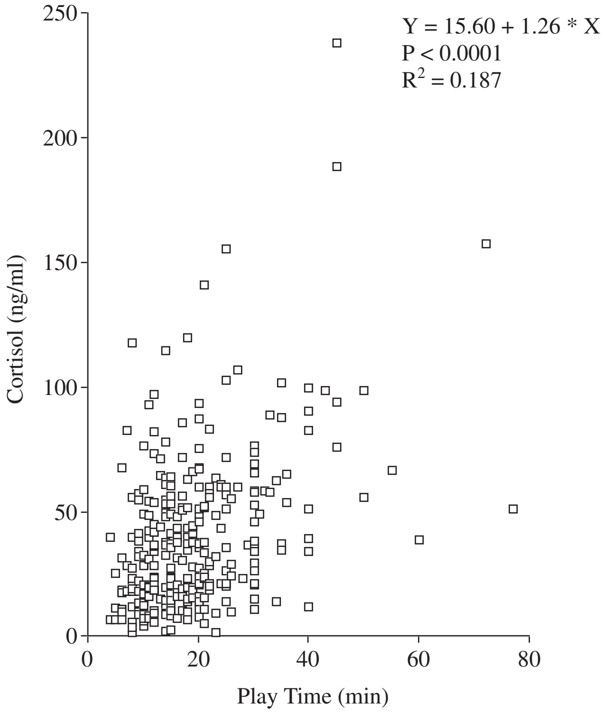

Fig. 3. Adult white sturgeon plasma cortisol concentrations versus play time (n = 305). Fish were caught in the catch-and-release sport fishery below Bonneville Dam in the Columbia River (2000–2004).

the challenge in interpreting this data. A significant number of the fish with high cortisol concentrations and short play times were found to have multiple hook scars in the buccal cavity due to being handled multiple times within the sport fishery season. A specific example of the difficulty in interpreting this data involves examination of two fish captured with the shortest play time. These fish were captured within 4 min of hooking by a sport fisher; one fish had a plasma cortisol concentration of 6.82 ng/ml, while the other fish had a plasma cortisol concentration of 40.20 ng/ml. The fish with the lower cortisol concentration did not have any other hook scars or marks and appeared to be in good condition. The fish with the higher cortisol concentration (40.20 ng/ml) had a large abdominal growth (tissue source/cause undetermined) and appeared in poor condition. The higher cortisol concentration in this fish compared to the fish also caught in 4 min indicates the importance of the physiological state of the animal prior to being captured by hook-and-line. Though in field settings it is difficult to control previous experiences, the value of assessing the plasma cortisol concentrations in these white sturgeon captured by hook-and-line is in bringing attention to the importance of fishing regulations that promote short play times and a realization that average cortisol concentrations of white sturgeon handled in the catch-and-release fishery are significantly higher than basal. It would be most interesting to determine the effects of this fishery on reproduction as in teleosts it has been shown that stress can impede reproduction by reducing gamete and progeny quality [e.g., 16,87,101,103], increasing the incidence of gonadal atresia [e.g., 24,25,87], and compromising immune function [e.g., 49]. Hypoxia and hypercapnia elicited catecholamines and cortisol responses and affected growth, food consumption, and cardiovascular function in white sturgeon [19,28]. Stress may also lead to direct mortality [99]. Because of the life history characteristics of sturgeon (longevity, late maturation, and long spawning intervals), the effects of the adult catch-and-release sport fishery on the white sturgeon population below Bonneville Dam may not become fully evident for 20 or more years as declining recruitment to broodstock reduces production [33].

3. Sturgeon aquaculture

First artificial reproduction of sturgeon (sterlet) was carried out in Russia in 1869 [84] and was followed by successful artificial breeding of other species in North America [98] and Western Europe [32]. The early era of fish endocrinology provided a critical tool for artificial propagation of chondrosteans, hormonal induction of ovulation and spermiation in sturgeon [see 92]. This technique made it possible to establish hatchery stocking programs for the Caspian Sea sturgeon [32] and North American paddlefish [94] whose natural reproduction was greatly reduced by dams.

As sturgeon stocks decline and the list of endangered species grows, a goal of sturgeon aquaculture is shifting from propagation and stocking to the commercial farming and conservation hatcheries. Sturgeon farming is rapidly evolving as a major supplier of caviar to the international market [137]. To our knowledge, at least ten countries of Europe, Asia, and North and South America are currently farming sturgeon for caviar. As in aquaculture of salmon and trout, the environmental endocrinology is a critical player in aquaculture of sturgeon. Endocrine studies illuminate regulatory mechanisms that are essential for sturgeon breeding and caviar production, such as sex differentiation, puberty, sexual maturation, and the relationships between the environment and reproductive performance. Farming sturgeon for caviar is based on a complex production system with continuous generation and maintenance of the large, multi-age female cohorts. The production system is expensive but profitable due to the high price and demand of caviar on the world markets [71,121].

Rearing and breeding in captivity has changed the pattern of growth and reproductive development of sturgeon, although most changes were probably induced by the culture environment. In the following paragraphs, we briefly discuss some key physiological events in the life history of farmed sturgeon understanding of which appears to be critical for further development of sturgeon aquaculture.

3.1. Sex determination and differentiation

Current annual production of 10–15 tons of caviar by the white sturgeon farms in California and Idaho requires 4000–5000 mature females available each year. Males mature at age 3–5 and most females mature at 7–9 years. Due to a late sex differentiation (18–22 months) and a lack of external sexual dimorphism in white sturgeon, farmers have to grow mixed-sex populations (1:1 sex ratio) until approximately 3 years of age, when they are able to determine sex in thousands of fish using surgical examination or ultrasound and cull off males for the much less profitable meat sale. Obviously, monosex (all-female) breeding could provide significant economic advantage for caviar farming. However, the sex determination system and sex differentiation physiology in sturgeons remain enigmatic [60].

Sex differentiation has been characterized morphologically in several species of sturgeon [see 50], but the information on the endocrine secretion in early life stages of sturgeon is very fragmentary and limited to a few studies [38,39,109]. A long-term oral treatment of juveniles with E2, starting from the age 3 months, is generally successful in inducing phenotypic sex reversal in sturgeons [41,83] but is unlikely to be applied in caviar production. Interestingly, Grandi et al. [51] reported feminization effect of short-term E2 immersion treatment before hatch in the Adriatic sturgeon (*Acipenser naccarii*), which may deserve further investigation.

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No morphologically or behaviorally (pairing in meiosis) distinct sex chromosomes were found in cytogenetic studies with sturgeons [44,115,116]. Several attempts to identify sex-specific molecular markers in different species were not successful [115 for white sturgeon; 54,55 for Atlantic sturgeon; 61 for beluga; 136 for four species of g. Acipenser].

The sex transmission experiments with gynogenetic and triploid offspring [117] were more revealing, suggesting a potential female heterogamety in white [118], shortnose [43] and Siberian [45] sturgeons, and in fertile hybrid bester [82]. In contrast to sturgeons, gynogenesis in paddlefish was initially reported to produce all-female offspring, with an anticipated straightforward approach to monosex breeding using sex-reversed gynogens [76,106]. However, sex transmission in the gynogenetic stock of paddlefish has recently been revisited by the authors, who reported approximately 19% of males in a larger population of sexually mature gynogens [107]. This is statistically similar to 18% males reported in gynogenetic offspring of white sturgeon and may indicate chromosomal ZZ/ZW sex determination system [118]. If such a system operates in chondrosteans, gynogenesis may potentially produce super-females (WW) which can be used for monosex breeding [118]. Unfortunately, there are no sex-specific markers to confirm this or similar [e.g., 5] hypotheses derived from the sex transmission experiments. It appears that the monosex breeding of sturgeons is unlikely to be established until molecular, cytogenetic and environmental aspects of sex differentiation are better understood in chondrosteans.

3.2. Endocrine and environmental regulation of puberty in cultured sturgeon

Sturgeons, particularly females, require a long time to reach first sexual maturity. The female white sturgeon in the Columbia River mature at 16–35 years of age and at a median age of 24 years [33]. In contrast, cultured white sturgeon mature at 6–14 years of age and at a median age of 8 years [36]. The fact that body weight at first maturation is similar (about 35 kg) in cultured and wild females indicates that the early puberty in cultured sturgeon is associated with growth and energy reserves, as in the other cultured fish [111,112]. One of the major problems in caviar production is highly asynchronous puberty and first maturation in females, extended over a period of several years in each year class. This necessitates repeated and labor-intensive sorting of mature and immature females using ovarian biopsy or endoscopy [58]. Synchronization of puberty in cultured sturgeon will be beneficial for sturgeon aquaculturists.

Puberty (defined as acquisition of the capacity to reproduce and functional competence of brain–pituitary–gonad axis) [111] is recognized in the sturgeon female by the increase in plasma concentration of sex steroids (T and E2), proliferation of granulosa cells, and the onset of chorion synthesis and vitellogenesis [35,36]. The brain peptides, neurotransmitters and ovarian growth factors, such as IGF-1, play important roles in these processes [68,111,139,141]. Body size and accumulated energy, including the lipid reserves of liver, muscle, and adipose tissue of gonads in sturgeon, may play important permissive roles in the onset of puberty, when the energy is diverted from somatic growth to reproduction. Obviously, metabolic and reproductive endocrine axes are interacting in regulation of puberty [111], but the knowledge of this interaction is practically absent in the sturgeon literature.

It is also known today that the onset of first reproductive cycle in fish is orchestrated by environmental signals, such as photoperiod [111], but the information about the role of environmental cues in activation of the first reproductive cycle is also absent in the sturgeon literature. As it was stated for monosex breeding, synchronization of puberty in cultured sturgeon will be a difficult task requiring fundamental studies of physiological, neuroendocrine and genetic mechanisms of puberty in sturgeon.

3.3. Role of environmental temperature in regulation of oogenesis

Seasonal environmental factors, photoperiod and temperature play important roles in gametogenesis and spawning of sturgeon [32], even though very little is known about their roles as environmental cues at the mechanistic level. One of the major hurdles in developing sturgeon aquaculture is high thermosensitivity of the sturgeon ovary during the late phase of oogenesis, particularly pronounced in the species or stocks starting their river spawning migration in the fall, such as the Russian sturgeon [32] or white sturgeon [131,132]. This high sensitivity to environmental temperature occurs during late vitellogenesis and germinal vesicle migration. For example, maturing white sturgeon females undergo ovarian follicular atresia if they are exposed to temperatures of 15 °C or above during the late fall and winter months before the spring spawning. Unlike follicular atresia during early vitellogenesis which is believed to maintain ovarian homeostasis in fish [73], temperature-induced follicular atresia in late vitellogenesis of sturgeon is manifested by the dramatic decrease in plasma sex steroids and rapid degeneration of the entire clutch of vitellogenic follicles [70,110]. Russian scientists have been familiar with this problem for a long time [32] and have developed a 'vernalization' technique (holding fish in chilled water) for the hatchery propagation of temperature-sensitive species of sturgeon [22,32]. While successful breeding of sturgeon is possible by maintaining low temperature (up to 4 °C) in a recirculation system with a few brood fish, holding thousands of large fishes for caviar production in such a system will be very costly. In this case, production of caviar may be affected in the regions where the cold water is not readily available during the spring and early summer.

The fact that slightly elevated temperature during the prespawning season results in decreases of plasma T and E2 followed by ovarian regression suggests that seasonal environmental temperature is a regulatory factor in the final stages of gametogenesis of sturgeon. It should be noted that massive ovarian regression and failure to spawn have been reported in wild sturgeon stocks affected by dam regulations and changes in the river thermograph [32]. Better understanding of the role of temperature as an environmental regulator of reproduction is important for both aquaculture and conservation of sturgeon.

4. Conclusions

Endocrine tools have been developed and can aid in the assessment of wild and cultured populations of sturgeon, many of which are threatened or endangered. The status of many sturgeon populations warrants further refinement and development of non-invasive or less-invasive tools to determine the physiological state of the animals. The current tools discussed within this paper utilize endocrine parameters to determine sex and stage of maturity, identify oviposition less-invasively, and assess stress load. With each less- or non-invasive technique, the researchers must clearly understand the influence of endocrine disruption as well as the challenges and assumptions of each tool. Through a better understanding of the reproductive neurobiology and endocrinology of sturgeons, the conservation endocrinology toolbox to aid fishery managers and culturists in population assessment can be developed and validated. Further progress in sturgeon neurobiology, endocrinology, and environmental physiology is needed to establish secure and successful aquaculture operations, with the objective to completely replace the sturgeon food products from fisheries with those produced by aquaculture.

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