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Potential Classification of Sex and Stage of Gonadal Maturity of Wild White Sturgeon Using Blood Plasma Indicators

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Abstract.—Because white sturgeon *Acipenser transmontanus* show no readily discernible external signs of gender, management agencies use surgical biopsies to determine the sex and stage of gonadal maturity of individuals. This procedure is highly invasive and can be difficult under field conditions. Therefore, gonadal tissue and blood were collected from white sturgeon captured in tribal and commercial fisheries (fishery fish) and by fish and wildlife agencies (oversize fish) in the Columbia River basin to develop a method of determining sex and stage of maturity using the blood plasma indicators testosterone (T), 11-ketotestosterone (KT), estradiol (E2), and calcium (Ca^{2+}). The sex and stage of maturity was determined by histology or by visual examination in maturing fish. Plasma sex steroid levels were measured by radioimmunoassay, and plasma Ca^{2+} was measured spectrophotometrically. White sturgeon showed sex- and maturity-specific levels of steroids and Ca^{2+}. Stepwise discriminant function analysis (DFA) was used to choose the best variable(s) for predicting sex and stage of maturity, and quadratic DFA was conducted to classify fish into two groups of sex or four groups of sex and stage of maturity. In the classification of the fishery plus oversize fish, plasma T and E2 were the best predictors of sex and stage of maturity. Of the 151 females and 106 males, 85% of the females and 79% of the males were correctly classified; 88, 72, 98, and 96% of the immature females, immature males, maturing females, and maturing males, respectively, were correctly classified. The greatest error of misclassification occurred with immature males classified as immature females. In the analysis of immature fishery fish only, plasma T and fork length led to the correct classification of 88% of the females and 86% of the males. In the analysis of oversize fish only, 100% of the females and 95% of the males were correctly classified using plasma T and E2; 93, 100, 98, and 100% of the immature females, immature males, maturing females, and maturing males, respectively, were correctly classified using these variables. The derived classification functions for the prediction of sex or sex and maturity may replace the need for surgical biopsies if the error associated with the misclassification of immature fish is acceptable.

The life history characteristics of long life and late maturity have sustained sturgeon, Acipenseriformes, populations since the end of the Jurassic (Grande and Bemis 1991); these same traits, however, are detrimental to populations faced with habitat alterations and overexploitation (Rieman and Beamesderfer 1990; Beamesderfer and Farr 1997). Most sturgeon populations worldwide are threatened or endangered, and efforts for protection and restoration are critical for their survival (Birstein 1993). Reproduction provides a key to the future success of sturgeon populations, and understanding reproduction becomes ever more crucial for successful management.

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Sturgeon are economically and culturally important in the Pacific Northwest and are harvested by recreational, tribal, and commercial fisheries throughout the Columbia River basin. Hydroelectric development of the Columbia River from 1938 to 1967 led to functionally discrete populations of white sturgeon *Acipenser transmontanus* (North et al. 1993) and reduced productivity in impounded sections of the river (Beamesderfer et al. 1995). The Lower Columbia River has four dams from the Snake River confluence to the mouth of the Columbia River. The Snake River has four dams from the Columbia River confluence to the Washington–Idaho border. Population characteristics of sturgeon between reservoirs and among reservoir and unimpounded populations (below Bonneville Dam extending to the ocean) differ significantly; therefore, the sturgeon fisheries are managed with stock-specific regulations (Beamesderfer et al. 1995; Beamesderfer and Farr 1997). The current sturgeon population in the unimpounded Columbia River is similar in size to that existing before overexploitation in the late 1880s (Beamesderfer and Farr 1997) and supports one of the most productive sturgeon fisheries in North America—and potentially, the world (DeVore et al. 1995). However, the continued productivity of sturgeon in both the unimpounded and impounded sections of the Columbia and Snake rivers is dependent on successful fisheries management.

Successful management of sturgeon populations requires knowledge of the stock composition with regard to sex and maturational status. Harvest rate guidelines are based on sustainable rates derived from sturgeon population models that are extremely sensitive to sex ratio, age at first maturity, and spawning frequency. Sturgeon are not obviously sexually dimorphic; therefore, sex and stage of maturity are currently determined through gonadal biopsy. This technique is invasive, and the collection of gonadal tissue from immature fish is often difficult, requiring histological confirmation. It would be beneficial for fisheries management to have a less invasive test for discriminating between the sexes and stages of maturity.

In sturgeon, plasma sex steroid concentrations are low or undetectable until gonadal differentiation. With the onset of meiosis, plasma concentrations of testosterone (T) and 11-ketotestosterone (KT) increase in both males and females (Cuisset et al. 1995; Moberg et al. 1995) and remain elevated throughout ovarian (Cuisset et al. 1995; Amiri et al. 1996b; Van Eenennaam et al. 1996; Webb et al. 1999; this study) and testicular (Cuisset et al. 1995; Amiri et al. 1996a; Van Eenennaam et al. 1996; this study) development. Plasma estradiol (E2) and calcium (Ca^{2+}) increase during the vitellogenic phase of ovarian development in sturgeon and decrease during the final stages of oocyte maturation (Linares-Casenave et al. 1994; Amiri et al. 1996b; Doroshov et al. 1997; Webb et al. 1999). Total plasma Ca^{2+} in cultured white sturgeon females has a significant linear relationship with plasma vitellogenin (R^2=0.96, N = 72) and was therefore used as an index of vitellogenin. Because plasma sex steroids and Ca^{2+} in sturgeon are sex-specific and maturity-specific, they may be used to differentiate males and females at various levels of development. Therefore, our objective was to develop a method to determine the sex and stage of maturity of wild white sturgeon using blood plasma indicators, sex steroids, and Ca^{2+}.

Methods

Fish sampling.—White sturgeon were sampled from the commercial, tribal, and recreational fisheries in the lower Columbia River (John Day, The Dalles and Bonneville reservoirs, and the unimpounded stretch below Bonneville Dam) in 1996 and 1997 (Table 1). These fish (N = 180) were within the legal size limit (96–152 cm fork length [FL]; mean [±SE] = 116 ± 1 cm). These fish are referred to herein as ''fishery fish.'' Paired gonad and blood samples were also collected from sturgeon outside of the legal size limit in McNary Reservoir on the Columbia River; above Ice Harbor Dam, Lower Monumental Dam, and Little Goose Dam on the Snake River; and in the Kootenai River between river kilometers (rkm) 205 and 215 (Table 1). The mean FL of these fish was 178 ± 3 cm. These fish are herein referred to as ''oversize fish.''

Tissue and blood collection and processing.—Gonad tissue was collected following the protocol of Conte et al. (1988) and stored in phosphate-buffered formalin. Gonad tissue collected from all fishery fish and 34% of the oversize fish was embedded in paraffin, sectioned at 7 μm, and stained by hematoxylin and eosin (Luna 1968). Slides were examined under a compound scope (10–100× power), and the germ cells were scored for stage of development according to the protocol of Van Eenennaam and Doroshov (1998). Stage-1 (differentiation of testis and ovary) and stage-2 (proliferation of spermatogonia and endogenous growth of the oocyte) fish were considered immature, whereas stage-3–6 males (onset of meiosis through spermiation) and stage-3–7 females (early

TABLE 1.—The number of white sturgeon, by stage of gonadal maturation and year, collected from the Columbia River estuary, various reservoirs in the Columbia and Snake rivers, and the Kootenai River between river kilometers 205 and 215.

Location	Year	Immature females	Immature males	Maturing females	Maturing males
Columbia River					
Estuary	1996	33	40	0	1
	1997	2	6	0	0
Bonneville	1996	3	0	0	0
	1997	13	8	1	2
The Dalles	1996	21	9	0	0
	1997	12	11	0	1
John Day	1996	5	1	5	0
	1997	11	5	0	1
McNary	1995	6	0	3	6
Snake River					
Ice Harbor	1996	0	0	2	0
Little Monumental	1997	0	0	2	0
Little Goose	1997	1	0	15	0
Kootenai River	1998	3	2	4	0
	1999	0	0	9	13
Total		110	82	41	24

vitellogenesis through ovulation) were considered maturing. Gonad tissue from the remaining 66% of the oversize fish were visually examined for sex and stage of maturity and scored according to Van Eenennaam and Doroshov (1998). Ovarian follicle diameter, measured using an ocular scale in a compound microscope, and degree of pigmentation of the oocyte were used to assess the developmental stage of females; coloration and presence or absence of lobed testes was used to assess the developmental stage of males (Table 2). Though coloration and the presence or absence of lobed testis was not an ideal method to distinguish immature from maturing males, the 15 males staged for development based on these criteria were correctly classified as immature or maturing males.

Blood was collected from the caudal veins with a heparinized Vacutainer. The plasma was separated by centrifugation and stored at −80°C until steroids were extracted and analyzed by radioimmunoassay (RIA) and Ca^{2+} was analyzed spectrophotometrically. Fork length (in some cases total length converted to FL using the formula described in DeVore et al. 1995) of each fish was measured (±0.5 cm).

Radioimmunoassays and plasma calcium measurements.—The steroids T, KT, and E2 were extracted from plasma following the method of Fitzpatrick et al. (1987) for RIA analysis. Briefly, 100 μL of plasma was extracted twice with 2 mL of diethyl ether. Tubes were vortexed vigorously with ether, and the aqueous phase was removed by snap-

TABLE 2.—Stages of gonadal development identified from biopsies of the oversize white sturgeon. Females were visually identified on the basis of ovarian follicle diameter and degree of pigmentation, males on the basis of the presence or absence of lobed testes.

	Developmental stage	Description
Females		
Previtellogenic	2	Small, translucent ovarian follicles < 0.6 mm
Early vitellogenic	3	Cream to grey colored ovarian follicles 0.6–2.1 mm
Mid-vitellogenic	4	Pigmented ovarian follicles 2.2–2.9 mm
Migratory nucleus	5	Fully pigmented ovarian follicles > 3.0 mm
Oocyte maturation	6	Ovulated ovary with few residual fully pigmented follicles
Postovulatory	7	Ovaries contain numerous empty postovulatory follicles and the next generation of oocytes
Males		
Immature	1–2	Testes with translucent smokey pigmentation, no folds or lobes
Maturing	4–5	Testes with folds and lobes

freezing in liquid nitrogen. Combined extracts were dried in a Speed Vac centrifuge, resuspended in 1 mL of phosphate-buffered saline with gelatin (PBSG), and 10 or 50 μL were assayed for each steroid. Recovery efficiencies for all steroids were determined by adding tritiated steroids to tubes containing plasma ($N = 4$), which were extracted as described above. The average recovery efficiencies were 86% for T, 84% for KT, and 76% for E2. All steroid assay results were corrected for recovery.

Plasma concentrations of T, KT, and E2 were measured by RIA as described in Fitzpatrick et al. (1986) and modified by Feist et al. (1990). All samples were analyzed in duplicate. A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L PBSG) was used for all assays to reduce nonspecific binding. The lower limit of detection was 1.25 pg/tube for all assays, except KT (3.12 pg/tube). The intra and interassay coefficients of variation for all assays were less than 5% and 10%, respectively. Steroid levels (determined by RIA) were validated by verifying that serial dilutions were parallel to standard curves and by analyzing selected samples by high performance liquid chromatography to show that steroids in plasma eluted at the same time as the standards and that concentrations (as reflected by peak height) were consistent with values derived by RIA.

Total plasma Ca^{2+} was analyzed using the Sigma Diagnostics kit (587; Sigma Co.). The Ca^{2+} was quantified using a Beckman DU-64 spectrophotometer.

Statistical analyses.—Plasma sex steroid and Ca^{2+} concentrations and fork lengths were compared between the four classes of sex and stage of maturity (immature females, immature males, maturing females, and maturing males) using one-way analysis of variance (ANOVA). Mean comparisons were conducted using the Bonferroni procedure.

Discriminant function analysis (DFA) was used to develop a set of discriminating functions to predict sex or sex and maturity. To attain multivariate normality, the logarithms ($\log_{10}$) of the variables T, KT, E2, and Ca^{2+} were considered for analysis. Fork length was not used in the analysis of fishery and oversize fish combined because the fish were not randomly collected from the river with regard to size (i.e., fishery fish were within a legal size limit, and the majority of these fish were immature; the fish collected outside of the fishery were significantly larger than the legal size limit, and the majority of these fish were maturing). Stepwise DFA was conducted using $\log_{10}$-transformed T, KT, E2, and Ca^{2+} concentrations to chose the best predictor(s) of sex or sex and stage of maturity. The significance level to enter and remain in the model was $\alpha = 0.05$. Quadratic DFA was then conducted with the variables chosen in the stepwise procedure to determine the number of observations and percent classified into the two groups of sex or four groups of sex and stage of maturity. The error rate associated with predicting sex or sex and maturity using the chosen discriminant functions was accomplished through cross-validation (see Khattree and Naik 2000). Briefly, one observation is eliminated from the data set and a discriminant rule is constructed from the rest of the data. The rule is used to classify the observation that was left out. This procedure is repeated for every observation, the numbers of misclassified observations for each group are counted, and the individual and overall error rates are computed. The DFA was also conducted on the immature fishery fish alone, the oversize fish alone, and females alone. Fork length was included in the analysis of the immature fishery fish because these fish were randomly selected within the slot size limit. All analyses were conducted using Statistical Analysis Systems for Windows, release 6.10 (SAS Institute, Cary, North Carolina). The classification function coefficients predicting the group (two groups of sex or four groups of sex and stage of maturity) to which new observations belong were generated using Statgraphics 3.0 (Statistics Graphics Corp.).

Results

Of the 257 fish examined in this study, 110 were immature females, 82 were immature males, 41 were maturing females, and 24 were maturing males. Of the 180 fish caught in the fisheries, only 5 males were maturing (stage 5) and 1 female was maturing (stage 4). The remaining fishery fish were immature (stages 1 and 2 for both females and males). Of the oversize fish, 40 of the 55 females and 19 of the 22 males were maturing. Histological examination and measurement of ovarian follicle diameter of oversize fish revealed 15 stage-2 females, 3 stage-3 females, 16 stage-4 females, and 21 stage-5 females. Six of the oversize maturing males were, histologically, stages 4–5. The remaining maturing males were distinguished by large lobed testes, several of which were spermiating.

Fork length differed significantly ($P < 0.0001$;

TABLE 3.—One-way analysis of variance conducted to determine whether there were differences between sex steroid (testosterone = T, 11-ketotestosterone = KT, estradiol-17β = E2) and calcium (Ca^{2+}) concentrations and fork length (FL) among the four groups of sex and stage of maturity.

Variable	df	MS	*F*	*P*
T	3	186,268.89	116.33	<0.0001
KT	3	103,392.33	165.11	<0.0001
E2	3	303.94	104.90	<0.0001
Ca^{2+}	3	750.82	32.57	<0.0001
FL	3	44,826.40	75.85	<0.0001

Table 3) among the four groups of sex and stage of maturity. Lengths of immature females (126 ± 3 cm) were significantly greater than those of immature males (117 ± 2 cm). Lengths of maturing fish were significantly greater than those of immature fish, and maturing females (179 ± 5 cm) were significantly longer than maturing males (161 ± 6 cm). Lengths of immature females (117 ± 1 cm) and males (114 ± 1 cm) caught in the fishery did not differ significantly.

Concentrations of plasma sex steroids and Ca^{2+} differed significantly ($P < 0.0001$; Table 3) among the four groups of sex and stage of maturity. The Bonferroni mean comparison tests revealed that

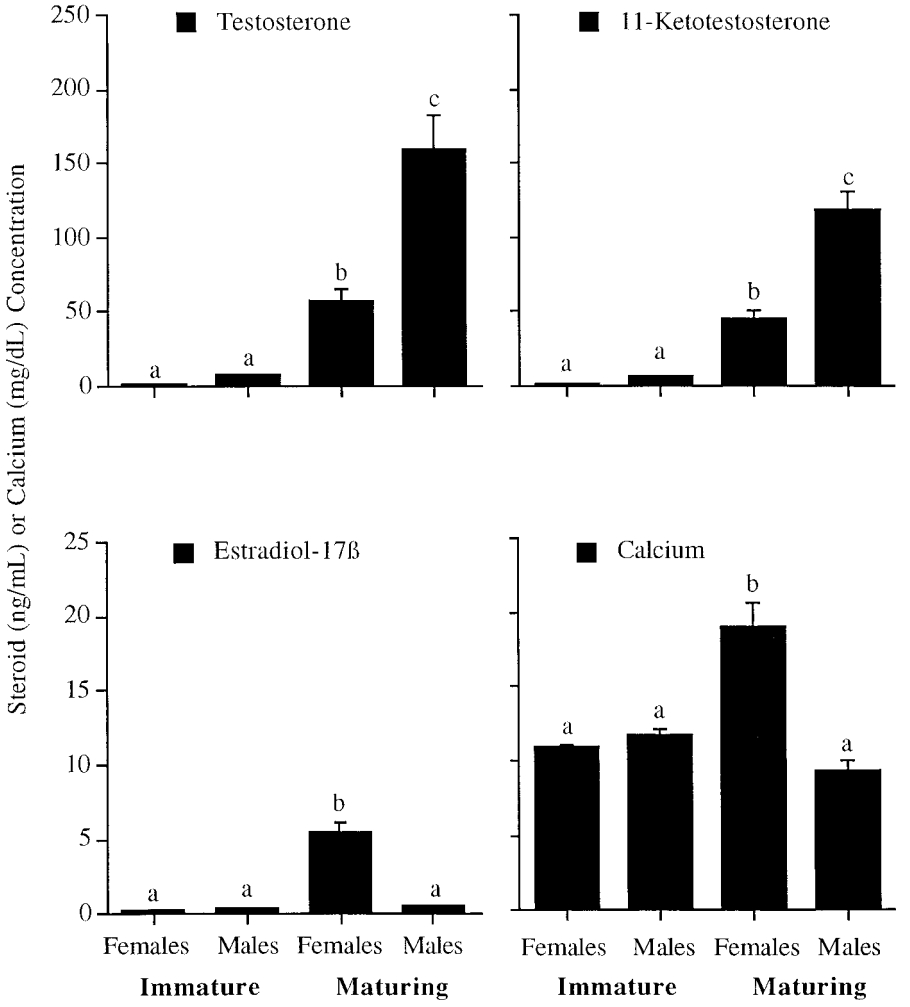

FIGURE 1.—Mean (+ SE) plasma sex steroid (testosterone, 11-ketotestosterone, and estradiol-17β) and calcium concentrations in immature and maturing white sturgeon males and females captured in the Columbia River basin. Means within panels having different letters were significantly different ($N = 110$ for immature females; $N = 82$ for immature males; $N = 41$ for maturing females; $N = 24$ for maturing males).

TABLE 4.—Results from stepwise discriminant function analysis predicting sex or sex and stage of maturity of white sturgeon captured in the Columbia River basin, which was conducted using fishery and oversize fish combined, immature fishery fish only, oversize fish only, and female fish only. The blood plasma indicators used in the analysis were testosterone (T), 11-ketotestosterone (KT), estradiol-17β (E2), and calcium (Ca^{2+}). In the analysis of fishery fish only, fork length (FL) was included as a variable; $P = 0.0040$ *, $P = 0.0001$ **.

Variable	Partial R^2	F-statistic	Wilks' lambda	Average squared canonical correlation
Sex; fishery and oversize				
T	0.13	38.44**	0.87**	0.13**
E2	0.31	111.62**	0.60**	0.40**
Sex and maturity; fishery and oversize				
T	0.79	317.62**	0.21**	0.26**
E2	0.63	144.83**	0.08**	0.46**
Sex; immature fishery				
T	0.52	187.41**	0.48**	0.52**
FL	0.05	8.50*	0.46**	0.54**
Sex; oversize				
T	0.56	95.01**	0.34**	0.66**
E2	0.23	22.19**	0.77**	0.23**
Sex and maturity; oversize				
T	0.78	87.59**	0.22**	0.26**
E2	0.74	67.72**	0.06**	0.50**
Maturity; females				
T	0.84	267.57**	0.15**	0.28**
E2	0.39	31.11**	0.09**	0.31**
KT	0.17	9.88**	0.78**	0.33**

TABLE 5.—Classification summary for determination of sex from the quadratic discriminant function analysis for white sturgeon in fishery and oversize fish combined (all fish), oversize fish only (using $\log_{10}$-transformed plasma testosterone and estradiol concentrations as predictors), and immature fishery fish (using $\log_{10}$-transformed plasma testosterone and fork length as predictors). Values in bold are the percentages of fish correctly classified, whereas values not in bold are the percentages of misclassified fish; sample sizes (N) are in parentheses.

Actual	Classified: Female	Classified: Male	Total
All fish			
Female	**85** (129)	15 (22)	100 (151)
Male	21 (22)	**79** (84)	100 (106)
Immature fishery			
Female	**88** (83)	12 (11)	100 (94)
Male	14 (12)	**86** (72)	100 (84)
Oversize			
Female	**100** (55)	0 (0)	100 (55)
Male	5 (1)	**95** (21)	100 (22)

plasma T and KT were not significantly different between immature fish but were significantly higher in maturing fish (Figure 1). Plasma concentrations of E2 and Ca^{2+} were significantly higher in maturing females compared with immature fish and maturing males (Figure 1).

The stepwise DFA of the fishery and oversize fish combined revealed that plasma T and E2 were the best predictors to distinguish female from male sturgeon (Table 4). The use of these two derived discriminant functions led to the correct classification of 85% of the females and 79% of the males, both exceeding the 50% probability of correctly classifying females and males by chance alone (Table 5). Overall, 82% of the fish were correctly classified by sex. The cross-validation of the model revealed an error rate of 15% (females) and 21% (males) using T and E2.

To distinguish fish by sex and maturational stage, plasma T and E2 were found to be the best predictors in the analysis of the fishery and oversize fish combined (Table 4). These derived discriminant functions led to the correct classification of 88% of the immature females, 72% of the immature males, 98% of the maturing females, and 96% of the maturing males (Table 6). By comparison, 25% of the total number of fish would be correctly classified into their respective groups by chance alone. Overall, 88% of the fish were correctly classified by sex and stage of maturity. The cross-validation of the model for sex and stage of maturity using the predictors T and E2 revealed error rates of 13% for immature females, 30% for immature males, 5% for maturing females, and 4% for maturing males.

In the DFA of the immature fish captured in the fisheries, plasma T and fork length were the best predictors of sex (Table 4). These variables led to the correct classification of 88% of the females and 86% of the males (87% overall correct classification). In the cross-validation of plasma T and fork length as predictors of sex in immature white sturgeon, classification errors were 12% for females and 15% for males.

Plasma T and E2 were chosen in the stepwise DFA of the oversize fish alone as the best predictors of both sex and sex and stage of maturity (Table 4). These variables led to the correct classification of 100% of the females and 95% of the

TABLE 6.—Classification summary for determination of sex and stage of maturity in fishery and oversize fish combined (all fish) and oversize fish only, as derived from the quadratic discriminant function analysis for white sturgeon using $\log_{10}$-transformed plasma testosterone and estradiol concentrations as predictors. Values in bold are the percentages of fish correctly classified, whereas values not in bold are the percentages of misclassified fish; sample sizes (N) are in parentheses.

	Classified				
Actual	Immature female	Immature male	Maturing female	Maturing male	Total
All fish					
Immature female	**88**	12	0	0	100
	(97)	(13)	(0)	(0)	(110)
Immature male	22	**72**	4	2	100
	(18)	(59)	(3)	(3)	(82)
Maturing female	0	0	**98**	2	100
	(0)	(0)	(40)	(1)	(41)
Maturing male	0	4	0	**96**	100
	(0)	(1)	(0)	(23)	(24)
Oversize fish					
Immature female	**93**	7	0	0	100
	(14)	(1)	(0)	(0)	(15)
Immature male	0	**100**	0	0	100
	(0)	(3)	(0)	(0)	(3)
Maturing female	0	0	**98**	2	100
	(0)	(0)	(39)	(1)	(40)
Maturing male	0	0	0	**100**	100
	(0)	(0)	(0)	(19)	(19)

TABLE 7.—Classification summary for determination of stage of maturity in all females from the quadratic discriminant function analysis for white sturgeon using log-transformed plasma testosterone, estradiol, and 11-ketotestosterone concentrations as predictors. Values in bold are the percentages of fish correctly classified, whereas values not in bold are the percentages of misclassified fish; sample sizes (N) are in parentheses. See Table 2 for the description of developmental stages.

	Classified				
Actual	Stage 2	Stage 3	Stage 4	Stage 5	Total
Stage 2	**100**	0	0	0	100
	(110)	(13)	(0)	(0)	(110)
Stage 3	0	**100**	0	0	100
	(0)	(3)	(0)	(0)	(3)
Stage 4	0	0	**88**	12	100
	(0)	(0)	(15)	(2)	(17)
Stage 5	0	0	24	**76**	100
	(0)	(0)	(5)	(16)	(21)

males (Table 5) as well as 93% of immature females, 100% of immature males, 98% of maturing females, and 100% of maturing males (Table 6). Overall, 95% of the fish were correctly classified by sex, whereas 98% were correctly classified by sex and stage of maturity. In the cross-validation of the model predicting sex in oversize fish, classification error rates were 5% for females and 18% for males. In the cross-validation of the model predicting sex and stage of maturity in these fish, error rates were 7% for immature females, 100% for immature males ($N = 3$), 2% for maturing females, and 0% for maturing males.

When only females were used in the DFA, plasma T, E2, and KT were chosen as the best predictors of stage of maturity (Table 4). These variables produced correct classifications of 100% for stages 2 and 3, 88% for stage 4, and 76% for stage 5 females (Table 7). The cross-validation of this model revealed 0% (stage 2), 100% (stage 3), 24% (stage 4), and 24% (stage 5) error rates. All 3 (100%) of the Stage 3 females were misclassified as Stage 4 females in the cross-validation.

The classification functions derived using fishery and oversize fish combined to predict the sex of an unknown individual were

$$-1.6727 + 2.3678(\log_{10}\mathrm{T}) - -3.5783(\log_{10}\mathrm{E2})$$

for females and

$$-5.2972 + 5.2524(\log_{10}\mathrm{T}) - 7.5539(\log_{10}\mathrm{E2})$$

for males.

These functions may be used to predict whether new observations are female or male. For example, a sturgeon with plasma concentrations of 0.9 ng/mL T and 0.5 ng/mL E2 has a value of -0.7039 using the classification function equation for females and -3.2636 using the classification function equation for males. The highest value of the two equations predicts the sex of the individual based on the discriminant analysis model (Tabachnick and Fidell 1996). Therefore, this individual would be classified as a female.

The classification functions for the four levels of sex and stage of maturity using fishery and oversize fish combined were

$$-4.1156 + 0.7728(\log_{10}\mathrm{T}) - 8.8389(\log_{10}\mathrm{E2})$$

and

$$-6.0205 + 5.8878(\log_{10}\mathrm{T}) - 9.1700(\log_{10}\mathrm{E2});$$

immature females and males, respectively, and

$$-11.7485 + 10.2755(\log_{10}\mathrm{T}) + 6.660(\log_{10}\mathrm{E2})$$

and

$$-18.9086 + 15.3687(\log_{10}\mathrm{T}) - 8.4362(\log_{10}\mathrm{E2})$$

for maturing females and males.

Again, it is the highest value from the four equations that determines the group of sex and stage of maturity to which an individual would be classified.

Discussion

In classifying white sturgeon by sex or sex and stage of maturity, plasma T and E2 were relatively reliable predictors. Using these variables, correct classifications were greatest for females (fishery plus oversize fish = 85% females, 88% immature females, and 98% maturing females; oversize fish = 100% females, or 93% immature females and 98% maturing females correctly classified). Correct classifications for males in the fishery plus oversize combined (79% for all males, 72% for immature males, and 96% for maturing males) was less accurate than for males in the oversize population alone (95% for all males, 100% for immature males, and 100% for maturing males). Similar characteristics have been used to distinguish between sexes and maturational stages for other fish species (e.g., Fitzpatrick 1985). In addition, Heppell and Sullivan (2000) used muscle concentrations of vitellogenin and KT to differentiate between males, adult females, and immature gag *Mycteroperca microlepis*. Five stages of ovarian development in female English sole *Pleuronectes vetulus* could be distinguished with approximately 70% accuracy using plasma concentrations of E2, vitellogenin, total protein, and triglycerides (Johnson and Casillas 1991).

Plasma concentrations of KT have previously been used to sex immature Siberian sturgeon *A. baeri* (Cuisset et al. 1991). We have found that plasma T or KT may be used to distinguish between male and female immature white sturgeon. Although the Bonferroni mean comparison tests showed no significant differences between plasma T or KT in immature white sturgeon, one-way ANOVA revealed significantly higher T ($P < 0.0001$) and KT ($P < 0.0001$) concentrations in immature males than immature females. In examining individual steroid concentrations, the critical threshold value of plasma T or KT to distinguish the sex of immature white sturgeon is 4 ng/mL; correctly classified immature females in the DFA had concentrations below this value, whereas correctly classified immature males had concentrations above this value. The misclassification of immature males as immature females resulted from a large portion of these males (34% for T, 44% for KT) having plasma concentrations of T or KT below 4 ng/mL. The level of KT that was used by Cuisset et al. (1991) to discriminate between immature Siberian sturgeon was 5 ng/mL. A fish with 5 ng/mL or more of KT was classified as a male; a fish with less than 5 ng/mL was classified as a female. If this discriminating level (5 ng/mL) was applied to the Columbia River data set, 40% (for T) or 54% (for KT) of the immature males would be misclassified as immature females. If the threshold value was lowered to 3 ng/mL, the misclassification of immature females increases from 5% (4 ng/mL) to 9% (3 ng/mL) for T and from 3% (4 ng/mL) to 7% (3 ng/mL) for KT. It therefore seems that a concentration of 4 ng/mL of plasma T or KT may be used to distinguish immature females and males, considering the error involved in misclassifying immature males as females.

To distinguish between immature and mature sturgeon, plasma concentrations of T or KT can be compared. Androgen concentrations were significantly higher in maturing fish than immature fish (Figure 1). Maturing females can be separated from maturing males using plasma E2 or Ca^{2+} because concentrations were significantly higher in maturing females than in all other groups (Figure 1). In maturing females, 93% had plasma E2 concentrations greater than 2 ng/mL, whereas all immature fish and maturing males had levels less than 2 ng/mL. Calcium concentrations were more variable. In maturing females, 62% had Ca^{2+} concentrations greater than 15 mg/dL, while only 5% of the immature fish and maturing males had concentrations greater than 15 mg/dL. The basal level of plasma Ca^{2+} in cultured white sturgeon females with previtellogenic ovaries was 9.89 mg/dL (Linares-Casenave et al. 1994), similar to levels detected in immature sturgeon (both sexes) and maturing males in the Columbia River basin. Use of the Sigma Diagnostics kit not only allows for quick determination of plasma Ca^{2+} but may be used in the field without a spectrophotometer. The kit is a colorimetric test, so maturing females may be detected by visually comparing the sample to the different dilutions of the provided standards. Because Ca^{2+} concentrations in females with ovarian follicles undergoing oocyte maturation will be close to basal level, this technique will most accurately identify vitellogenic females.

Sustainable harvest levels of sturgeon in the Columbia River are based on population models and fecundity estimates (DeVore et al. 1995). A key element of these models is spawning frequency. The DFA of female white sturgeon suggests that females can be distinguished by stage of maturity (previtellogenic, early vitellogenic, midvitellogen-

ic, and migratory nucleus) using plasma T, E2, and KT (Table 7). However, the duration of vitellogenesis and the spawning interval of white sturgeon in the Columbia River basin are currently unknown. If this information could be determined, the use of plasma sex steroids may provide a less invasive method for calibrating the current population models. We are currently working to collect a larger sample size of females within each stage of development to test and further improve upon this potential technique.

The DFA models were least effective in distinguishing white sturgeon males (sex only) or immature males (sex and maturity) in the fishery plus oversize data set. The large percentage of immature males (22%) misclassified as immature females in this data set was due to low circulating androgen concentrations. Only three of the oversize males were immature, hence the correct classification of immature and maturing males in the analysis of oversize fish alone (Table 6). When FL was included in the DFA of the immature fishery fish alone, the percentage of correctly classified males increased to 86% with 14% of the immature males misclassified as immature females. It is unclear at this time why wild white sturgeon males in stage 2 of gonadal development are not producing concentrations of T or KT of 4 ng/mL or higher. It appears that spermatogonia proliferation (Stage 2) in cultured white sturgeon is associated with increased circulating androgen concentrations, regardless of age or size (Fitzpatrick, unpublished data). In wild white sturgeon, this may not be the case and should be investigated.

Application of these discriminant analysis models to differentiate between sexes and maturational stages may be possible across species and should be tested because of the similar changes in steroid profiles among sturgeon. For example, the steroid concentrations detected in the Columbia River white sturgeon population were comparable to concentrations detected in Siberian sturgeon (Cuisset et al. 1995), the hybrid Bester, beluga *Huso huso* × sterlet *A. ruthenus* (Amiri et al. 1996a, 1996b), and Atlantic sturgeon *A. oxyrinchus* (Van Eenennaam et al. 1996). Barannikova et al. (1997) reported much lower serum concentrations of T in maturing, wild Russian sturgeon *A. gueldenstaedti*, while Bukovskaya et al. (1997) detected higher concentrations of T and KT in the serum of wild immature female Russian sturgeon compared with the levels detected in our study. Although differences in steroid concentrations exist between species, the plasma steroid profiles are similar throughout maturation in sturgeon and may be used in discriminating among the sexes and maturational stages of development.

Though error does exist in the classification of sex and stage of maturity of white sturgeon using blood plasma indicators, this technique currently has some advantages over the biopsy method. The biopsy method of identifying sex and maturity, although highly accurate under some circumstances (Doroshov et al. 1997), is subject to considerable error for those untrained or inexperienced with the technique, especially under field conditions. In 1995 and 1996, the sex of 62% and 74%, respectively, of the biopsy samples provided to us could not be identified because the samples contained only adipose tissue or were from other organs. This problem is not uncommon among sturgeon biologists and management agencies. Therefore, under certain circumstances, the error associated with misclassifying fish using plasma steroid levels is more accurate than collecting gonadal biopsies, which in fact may be adipose or other tissue.

The DFA revealed that plasma T and E2 can be used to distinguish between two groups of sex or four groups of sex and stage of maturity in white sturgeon; however, a large percentage of immature males were misclassified. The percent of misclassified immature males was reduced when FL was included in the DFA. Use of blood plasma indicators to distinguish sex in a maturing population of wild white sturgeon is reasonably accurate. It seems that the use of plasma sex steroids may also be useful in distinguishing various developmental stages of females. We are currently collecting a larger data set on which to test the model. Hence, use of these blood plasma indicators may replace the need for surgical biopsies in fisheries management if the error associated with the misclassification is acceptable. Further investigation needs to be conducted to determine if other plasma constituents may improve distinguishing the sex of immature fish.

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