



Article type : Article

Reference Intervals for Blood Analytes of Adult Aquarium-Housed Russian Sturgeon

(*Acipenser gueldenstaedtii*)

Stephen E. Cassle,^{1*} Roy P.E. Yanong,² Deborah B. Pouder,² Carlos Rodriguez,³ Natalie Mylniczenko,³ Patrick M. Thompson,⁴ Natalie K. Stilwell,⁵ Kathy J. Heym,⁶ Todd Harmon,³ and Nicole I. Stacy⁷

¹ Aquatic Animal Health Program, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, Florida 32610, USA

² Tropical Aquaculture Laboratory, Institute of Food and Agricultural Sciences, School of Forest Resources and Conservation, University of Florida, 1408 24th Street SE, Ruskin, Florida 33570, USA

³ Disney's Animals, Science and Environment, 2901 Osceola Parkway, Bay Lake, FL 32830, USA

⁴ Whitney Laboratory for Marine Bioscience, University of Florida, 9505 North Ocean Shore Boulevard, St. Augustine, Florida 32080, USA

⁵ Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, 501 DW Brooks Drive, Athens, Georgia 30602, USA

⁶ Center for Veterinary Medicine, United States Food and Drug Administration, 7500 Standish Place, Rockville, Maryland 20855, USA

⁷ Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, Florida 32610, USA

*Corresponding author. Email: scassle@icloud.com

DISCLAIMER: This article reflects the views of the author and should not be construed to represent the FDA's views or policies.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/AAH.10116](https://doi.org/10.1002/AAH.10116)

This article is protected by copyright. All rights reserved

ABSTRACT: Russian or Danube sturgeon (*Acipenser gueldenstaedtii*) are an important, critically endangered roe-producing species. Despite a wealth of knowledge pertaining to other members of *Acipenseridae* there is very limited published information regarding baseline blood analytes in Russian sturgeon. The objectives of this study were 1) to establish reference intervals for a suite of hematological and biochemical data and 2) to compare plasma chemistry data to two point-of-care (POC) cartridges, tested on the VetScan iSTAT® 1 analyzer, that use heparinized whole blood for the assessment of clinically normal, aquacultured adult Russian sturgeon sedated with eugenol (Aqui-S® 20E) at a single institution. Reference intervals are reported. The calculated hematocrit measured by the POC analyzer tended 4-5% lower than the spun packed cell volume (PCV), confirming the importance of spun PCV as a reliable measurement of red blood cell mass. Various analytes, notably whole blood urea nitrogen, glucose, sodium, total carbon dioxide, chloride, ionized calcium, and anion gap, were significantly different by both POC cartridges. This study successfully produced reference intervals for blood analytes in adult Russian sturgeon under managed care and creates a foundation for future studies into the effects of extrinsic and intrinsic factors, and into variations of analytical methodologies on blood analytes in this species.

[A] Introduction

Russian or Danube sturgeon (*Acipenser gueldenstaedtii*) are long-lived benthic cartilagenous fish species from the family *Acipenseridae* with a natural distribution in the Black Sea basin. According to the International Union for Conservation of Nature (IUCN), this species is critically endangered and owes the decimation of populations by 70% in the past few decades to habitat destruction (e.g. river dams), over-fishing, illegal fishing and poaching across the natural distribution, and water pollution from oil and industrial wastes. Recovery in wild populations from these stressors is complicated by long generational gaps due to later maturation of both males (8-13 years) and females (10-16 years) (IUCN, 2010).

Despite the economic importance of this roe-producing species and the dire state of populations in the wild, there is a paucity of information or studies looking at baseline health

variables in this species. Reference intervals of standard hematological and biochemical analytes are crucial for health status determination and animal monitoring in all species including fish (DiVincenti et al., 2013a), and are useful for assessing individuals, a group of individuals, or representatives of a given population. Reference intervals have been established for several other species of sturgeon, including lake sturgeon (*Acipenser fulvescens*), shovelnose sturgeon (*Scaphirhynchus platorynchus*), shortnose sturgeon (*Acipenser brevirostrum*), Pallid (*Scaphirhynchus albus*), Amur (*Acipenser schrenckii*), Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), and Chinese sturgeon (*Acipenser sinensis*) (DiVincenti et al., 2013a; Sepulveda et al., 2012; Knowles et al., 2006; Matsche and Gibbons, 2012; Shahsavani et al., 2010; Matsche, 2014; Shi et al., 2006). In some cases, when both wild-caught and species under managed care (e.g. aquarium-housed or tank-housed) were available, a comparison could be made between the groups or species (DiVincenti et al., 2013b; Sepulveda et al., 2012; Shahsavani et al., 2010; Shi et al., 2006). To the authors' knowledge, no reference intervals have been established for aquacultured adult Russian sturgeon to date. The objectives of this study were to establish reference intervals for a suite of hematological and biochemical data and to compare two cartridges using point-of-care (POC) whole blood testing to each other and each to plasma chemistry data for the assessment of clinically normal, aquacultured Russian sturgeon sedated with eugenol (Aqui-S® 20E, AquaTactics Fish Health, Kirkland, WA) at a single institution.

[B] Methods

All sample collection protocols were approved by the Institute of Food and Agricultural Sciences (IFAS) protocol # 001-11FAS, by the University of Florida's Institutional Animal Care and Use Committee (IACUC# 201706823), and by Disney's Animal Care and Welfare Committee. Fish were maintained in a well-established, indoor recirculating freshwater exhibit tank system at the WDW Walt Disney Resort, Epcot Land Pavilion, where they had been held as a group without other fish species for three and one-half years. The water quality parameters (dissolved oxygen 7.89-8.66 mg/L, pH 7.45-7.83, and total ammonia nitrogen <0.22 mg/L) were consistent in the temperature controlled (24.3-25.1 °C) system with a photoperiod of 14 hours light, 10 hours dark. Each fish was visually evaluated by veterinary clinicians and animal husbandry staff for overt abnormalities at time of sampling, including external lesions (e.g., wounds) and abnormal behavior (e.g., erratic swimming patterns, seclusion). The animals were fasted for 12 hours prior to sample collection.

Twenty three adult mixed sex fish, averaging approximately 22 kilograms in weight and 140 centimeters in length, were gathered from their culture tank by dip net and transferred to one of two circular 200 gallon anesthetic tanks (Tank A and Tank B) containing 757 liters of water from the original system and Aqui-S® 20E (50 mg/L active ingredient/eugenol; FDA-CVM Investigational New Animal Drug (INAD) #11-741) for sedation. Aqui-S® 20E was used through an INAD exemption during the current study as it is the only legally available sedative for food fish in the family Acipenseridae in the United States; it has an investigational drug withdrawal period of zero hours and 72 hours for wild and aquacultured sturgeon, respectively (USFWS, 2020). The following time points were recorded for each fish: time into anesthetic bath; time to handling as determined by the inability of the individual fish to maintain normal orientation; time to reach light anesthetic plane (Stage 4 of 6; Sneddon, 2012); blood draw start and stop time; time placed into sedative free water; and time to recovery, as determined by the ability to return to normal orientation. Each fish was identified with a previously implanted transponder in the dorsal musculature. All fish were handled during a single anesthetic event on the same day. Water quality parameters [temperature (°C), dissolved oxygen (mg/L), carbon dioxide (mg/L), pH, total ammonia nitrogen (mg/L), nitrite (mg/L), chloride (mg/L), total alkalinity (mg/L), and total hardness (mg/L)] were monitored approximately every 90 minutes throughout the four hour procedure. Water quality samples were taken prior to addition of Aqui-S® 20E and subsequently after three to five fish had been anesthetized in each tank. Tank water was not changed during the four hour procedure as the water quality parameters did not change and the time to anesthesia for each fish did not increase.

Once the fish could be handled, it was turned to dorsal recumbency in the anesthetic tank. Blood was drawn immediately posterior to the anal fin from the ventral hemal arch using a 20 ml syringe attached to an extension set. The 18 gauge 1.5 inch needle, extension set, and syringe were flushed with 10 ml of 1000 Unit/ml sodium heparin that was thoroughly expelled at least 10 times before blood sampling. Approximately 12 ml of blood was drawn from each fish (less than 1% of total body weight). After venipuncture was complete, the fish was weighed (kilograms) and the total length (centimeters) measured. The animal was then returned to the original culture tank where it was guided through the water column by a recovery team member until the fish was able to right itself

and swim on its own. The time required to handle the fish, time to anesthesia, total time in sedation, return time to the culture tank, and time to recovery were recorded (h:mm:ss).

Heparinized, whole blood samples from the syringe were used to complete immediate point-of-care testing using a portable point-of-care analyzer Vetscan iSTAT®1 (Abaxis Inc., Union City, California, <https://www.abaxis.com/i-stat-1-handheld-analyzer>) (iSTAT POC). The iSTAT POC is the most commonly utilized POC system in fish species and was therefore selected for this study (Stoot et al., 2014). Cartridge one (Cartridge 1) for the iSTAT POC used the CG8+ cartridge [Hematocrit (Hct), Hemoglobin (Hgb), Ionized Calcium (iCa), Glucose (Glu), Sodium (Na), Potassium (K), Potential for Hydrogen (pH), Partial Pressure of Carbon Dioxide (PCO₂), Bicarbonate (HCO₃), Total Carbon Dioxide (TCO₂), Base Excess, Partial Pressure of Oxygen (PO₂), Saturated Oxygen (sO₂)] and cartridge two (Cartridge 2) used the Chem8+ cartridges [(Hct, Hgb, Blood Urea Nitrogen (BUN), Creatinine (Crea), iCa, Glu, Chloride (Cl), Na, K, TCO₂, Anion Gap (AG)] (Abbott Point of Care, Inc., Princeton, NJ). Temperature correction of pH, PCO₂, and PO₂ was performed on each sample by manually entering the most recently recorded water temperature of the anesthetic tank into the iSTAT POC analyzer per manufacturer's instructions. Due to the potential effects of transfer of samples, sample temperature fluctuations, and extended time to processing, validation of blood gas data was not completed.

Whole blood samples were then aliquoted into four ml lithium heparin tubes (BD Vacutainer™, BD, Oakville, ON, Canada), cryotubes (Thermo Scientific™ Nalgene™ General Long-Term Storage Tubes, Thermo Fisher Scientific, Carlsbad, CA), and capillary tubes (Fisher Scientific Microhematocrit Capillary Tubes, Non-Heparinized, Thermo Fisher Scientific, Carlsbad, CA). Two blood films were immediately prepared from each fish. Capillary tubes were spun (Combo V24 Centrifuge, LW Scientific, Lawrenceville, GA) at 12,000g for five minutes and the spun packed cell volume (PCV), plasma color, and total solids determined by refractometer (LW Scientific Specific Gravity Refractometer, LW Scientific, Lawrenceville, GA) using standard methods (Campbell, 2012).

Immediately after all other samples were harvested, whole blood heparinized samples were spun at 14,000g for five minutes (Knowles et al., 2006). Plasma was divided into 0.5 ml aliquots, sent overnight on cold packs, and submitted for further analyses that were performed within 24 hours.

Plasma was analyzed on the Siemens Dimension Xpand Plus Integrated Chemistry System (Siemens Medical Solutions USA, Malvern, PA) at the University of Florida College of Veterinary Medicine Veterinary Diagnostic Laboratories for the following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), calcium (Ca), phosphorus (Ph), BUN, Glu, cholesterol (Chol), triglycerides (Trig), magnesium (Mg), gamma-glutamyltransferase (GGT), creatine kinase (CK), Na, K, Cl, TCO₂, AG, and uric acid. Blood films were stained with Wright-Giemsa stain (Harleco®, EMD Millipore, Billerica, MA) and reviewed for quantitative and morphological assessment, including the following: WBC estimate using the formula by Weiss (1984), 200-WBC differential, and morphological evaluation of WBC, RBC, and thrombocytes. Plasma samples were submitted to the Michigan State University, Veterinary Diagnostic Laboratory (Lansing, MI) for quantification of ionized calcium on an ion-selective analyzer, the NOVA 8 (Nova Biomedical, Waltham, MA).

Reference intervals were established using the guidelines and reported in standard international units from the Clinical Laboratory Standards Institute and the American Society for Veterinary Clinical Pathology (Friedrichs et al., 2012). The Reference Value Advisor was used to evaluate each analyte (Geffre et al., 2011). Data were first visually inspected for normality (normal distribution) by use of a histogram and box plot as well as procedurally by the Tukey method and outliers were removed. Outliers were detected for ALT (n=1), AST (n=2), Glu (n=1), Trig (n=2), GGT (n=2), CK (n=2), AG (n=1), and uric acid (n=4) and removed for further analysis (Friedrichs et al., 2012). Due to the sample size, the data were Box-Cox transformed and reference limits were reported with a robust method calculating the 90% confidence intervals on the lower and upper limits using the bootstrap method. Whenever an analyte was tested by more than one technique, those analytes were compared for statistically significant differences using the paired t-test method given that the number of samples was insufficient for method comparisons. The results of both cartridges were compared to each other and to plasma chemistry data. The compared analytes included: PCV and Hct, Hgb, BUN, Glu, Na, K, Cl, TCO₂, iCa, and AG. A p-value of 0.05 was set for statistical significance and calculations were performed using SAS 9.3 (SAS, Inc, Cary, North Carolina).

[C] Results

Twenty-three animals were sampled during this study. All fish were judged to be clinically normal by physical appearance and body condition, but the sex of each was not determined. The mean body weight and total length (+ standard deviation) were 22.25 (+7.64) kilograms and 140.14 (+14.62) centimeters, respectively. The anesthetic times for each fish were recorded. The mean time (+ standard deviation, range) were as follows: time to handling 0:01:37 (0:00:27; 0:00:42 to 0:02:37), time to reach sedation 0:02:27 (0:00:59; 0:01:37 to 0:05:56), total time in sedation 0:05:54 (0:02:00; 0:03:23 to 0:11:27), and the time to recovery 0:09:03 (0:04:32; 0:01:45 to 0:20:42).

The housing tank water parameters for the prior two months to sampling were as follows: dissolved oxygen 7.89-8.66 mg/L, pH 7.45-7.83, temperature 24.3-25.1 °C, and total ammonia nitrogen <0.22 mg/L. The housing area had a photoperiod of 14 hours light and 10 hours dark. During the sample collection, the mean temperature was 24.9 °C (Tank A) and 24.7 °C (Tank B) for the two anesthetic tanks and ranged from 24.2 °C to 25.5 °C for both anesthetic tanks. All water quality parameters in both anesthesia tanks remained within acceptable limits during the procedures (i.e. within the variance of previously established tank parameters). The mean values for water quality (Tank A and Tank B, respectively) were as follows: dissolved oxygen 8.38 and 8.31 mg/L, carbon dioxide 8.8 and 8.8 mg/L, pH 8.0 and 8.0, total ammonia nitrogen 0.0 and 0.0 mg/L, nitrite 0.0 and 0.0 mg/L, chloride 50.0 and 50.0 mg/L, total alkalinity 114 and 102.6 mg/L, and total hardness 128.3 and 119.7 mg/L.

Hematology and iSTAT POC data are presented in Table 1 by method of analysis, number of samples, mean, median, standard deviation, lower and upper limits of the reference interval and 90% confidence interval (CI) around the lower and upper reference intervals are presented. Both spun PCV, hematocrit (Hct), and hemoglobin (Hgb) obtained from the iSTAT POC system are reported.

Microscopic evaluation of red blood cells (RBC) showed very low numbers of cells without a nucleus (i.e., extruded nucleus), erythroplastids, and mild anisocytosis and rare polychromasia. Absolute numbers of various types of white blood cells (WBC) are reported. Lymphocytes were the predominant cell type followed by neutrophils. Basophils were absent in any of the samples. Of note, the neutrophils in all the fish appeared segmented, and a few had Döhle bodies and/or minimal cytoplasmic basophilia. Immature neutrophil stages were not observed in any of the samples. Thrombocyte numbers and morphology appeared adequate, although some clumping, likely as result

from heparin, was noted. The plasma color showed various shades of yellow and hemolysis was absent in all samples.

Blood chemistry analyte reference intervals are also presented in Table 1. The mean, median, standard deviation, lower and upper limits of the reference interval and 90% CI around the lower and upper reference intervals are included, except for creatinine and uric acid. Creatinine data obtained on the iSTAT POC analyzer were below the detection limit of the analyzer and, subsequently, were all reported as <0.2 (i.e., the detectable range for this machine). The uric acid sample size was too small (i.e., n <20) to establish the 90% CI around the upper and lower reference intervals; therefore, the provided descriptive values should be interpreted with caution. For analytes that were water temperature corrected, pH and PO₂ were visually lower and PCO₂ was higher for uncorrected data.

For comparison to other sturgeon species, Table 2 presents published reference ranges for four groups of sturgeon. Both hematological and biochemical analytes are shown for adult cultured Russian sturgeon of this study, juvenile cultured shortnose sturgeon, and aquarium-housed and wild-caught adult lake sturgeon (Knowles et al., 2006; DiVincenti et al., 2013a; DiVincenti et al., 2013b). When possible, a simple comparison, but no statistical comparison, between the data was made in the discussion section. The following analytes had no comparison values in any of the studies for other species: ALT, BUN, TCO₂, and AG.

Finally, the results of paired analysis are presented in Table 3 as comparison data for analytes that were measured on more than one system or multiple testing modalities within the same system (e.g., two different cartridges for the iSTAT POC). Significant differences ($p < 0.05$) are annotated in each column. Most importantly, spun PCV was significantly higher (on average 4-5% higher) than corresponding Hct values reported on the iSTAT POC system ($p < 0.0001$ for both Cartridge 1 and Cartridge 2). Plasma chemistry BUN ($p < 0.0001$, Cartridge 2 only), AG ($p < 0.0001$, Cartridge 2 only), and Na ($p < 0.0006$ for Cartridge 1 and $p < 0.006$ for Cartridge 2) were statistically significantly higher than those data from the iSTAT POC system. Plasma glucose was higher than whole blood glucose using the iSTAT POC ($p < 0.0001$ for both Cartridge 1 and Cartridge 2). Whole blood Cl ($p < 0.0002$, Cartridge 2 only) and iCa were statistically significantly higher ($p < 0.0001$) for both Cartridge 1 and Cartridge 2 than in plasma.

[D] Discussion

This is the first report of hematological, plasma biochemical, and whole blood iSTAT POC data for adult Russian sturgeon under managed care. Hematological and biochemical data are clinically important in the health assessment of various fish species in due consideration of the potential effects of various extrinsic and intrinsic factors on blood analytes in a species of interest. The establishment of reference intervals of blood analyte data from a group under defined environmental conditions or from representatives of a population allows for comparison of data when similar analytical methodologies are used, and offers an important tool for the interpretation of blood data from individuals or a group of animals in health and disease.

Hematology data of adult Russian sturgeon were comparable with data from other species but showed presumptive analytical methodology-related differences. The PCV for many fish species ranges from 20 to 45% and is an important end point used to determine health status (Hrubec, 2000; Campbell, 2012). Based on this study, the spun PCV for Russian sturgeon fell within the established range of other fishes and is comparable to PCV ranges previously reported in other sturgeon species. The difference in spun PCV and Hct from either POC cartridge (CG8+ and Chem8+) was likely associated with methodological differences (e.g., presence of nucleated red blood cells, nRBC, in fish). Other factors including a lower TP and electrolyte differences, as the iSTAT POC uses electric conductivity (i.e., different between nRBC and non-nRBC in mammals), electrolytes, and body/sample temperature differences could lead to a lower reported Hct. According to the manufacturer of the iSTAT POC, the reported Hct will decrease by approximately 1% for each decrease of 1 g/dL of total protein (TP) (Abbott, 2011). Based on this assumption, the Hct reading for fish could read 1-3% lower based on TP determined by refractometer in this study. In the case of the spun PCV to Hct comparison, there is strong evidence that Hct by iSTAT POC would result in an inaccurate assessment of RBC mass and thus result in misinterpretation that could affect clinical decision making. Therefore, a spun PCV should always be performed as reliable part of the hematological assessment of fish, and Hct reference intervals should be interpreted with caution. The observed low numbers of erythroplastids were presumably consistent with aged RBC. Mild anisocytosis and rare polychromasia were considered normal due to continuous RBC maturation in peripheral blood of fish as documented in other teleost species (Campbell, 2012).

Leukogram data can significantly enhance health assessment information in fish. Because fish have nucleated RBCs that cannot be differentiated from WBC by hematology analyzers, a method of automated WBC quantification has not yet been developed (Knowles et al., 2006). Manual methods are necessary to determine WBC counts, differential, and morphology. Lymphocytes reportedly are the most prevalent WBC followed by the neutrophil in many teleost fish species (Hrubec, 2000). This has also been noted in wild lake sturgeon (DiVincenti et al., 2013a). In the current study, the total number of WBCs and absolute numbers of each type of WBC was comparable to the other species reported except with juvenile shortnose sturgeon (Knowles et al., 2006). A strict interpretation of this difference is difficult but could be related to age/life stage differences in the species (i.e., juvenile versus adults), methods for WBC enumeration, and the identification of cell types because lymphocytes were reported as both large and small varieties in the shortnose sturgeon study (Knowles et al., 2006). The observation of few Döhle bodies and/or minimal cytoplasmic basophilia in Russian sturgeon neutrophils were considered normal for the species, given that all fish were considered clinically normal based on physical examination. Leukogram trends in an individual patient are an essential part of routine examinations and for monitoring during disease and treatment.

While hematological analytes can be influenced by both intrinsic and extrinsic factors, biochemical analytes in fish species are more susceptible to these effects (DiVincenti et al., 2013a). For example, CK and Glu can rapidly change due to stress, handling techniques, and blood processing techniques (e.g., extended or delayed centrifugation of plasma samples). When compared to wild-caught lake sturgeon, tank-housed fish had lower plasma and whole blood glucose concentrations, suggestive of less stressful handling techniques (i.e., quick capture, fish being accustomed to staff performing daily tank cleaning and/or effects from sedation), effects from various sedatives, or from fasting (Feng et al., 2011; DiVincenti et al., 2013). Finally, electrolytes have a wide range of results, especially when considering marine versus freshwater species, as fish use these elements to maintain osmoregulation (Greenwall et al., 2003). These endpoints can also be affected by age, sex, reproductive status, permeability of electrolytes across gill membranes, presence of a spiral valve and changes to the alimentary tract, added environmental stressors, as well as feed composition (i.e., presence of plant proteins) (Knowles et al., 2006; He et al., 2009; Matsche and Gibbons, 2012; Wei et al., 2019). For example, juvenile teleost fish that are actively growing will utilize more calcium and

phosphorus to develop bone, leaving less available in circulation (He et al., 2009; Asadi et al., 2010; Genz et al., 2013; Norris et al., 2013).

With these many variables in mind, this study provided important information about reference intervals for various biochemical analytes of adult Russian sturgeon. As mentioned above, many of the analytes showed good concordance with data from previously published studies of other species. The analytes below were comparatively different between Russian sturgeon and the other species (Knowles et al., 2006; DiVincenti et al., 2013a; DiVincenti et al., 2013b). Plasma calcium was comparatively higher in fish examined in the current study compared to other sturgeon species, which may be associated with interspecies variation, nutritional state, dietary differences, sex, external stressors, or life cycle stage (Matsche and Gibbons, 2012; DiVincenti et al., 2013). Other studies have shown that temperature and environmental factors can have an effect on this tightly controlled analyte in other sturgeon species (Knowles et al., 2006; Sadati et al., 2011).

Free-ranging fish in open waters are possibly affected by various stressors, e.g., environmental contaminants, and may exhibit higher plasma enzyme activities due to effects on tissues (DiVincenti et al., 2013). However, as noted by Anderson et al. (2011), enzymes customarily associated with the mammalian liver are not tissue specific in fish. Detectable amounts of ALT were found in all tissues of red lion fish (*Pterois volitans*), with the highest in liver and heart (Anderson et al., 2011). Therefore, tissue enzyme activities across studies are likely affected by many variables, including habitat, diet, handling, and anesthetic effects. In this study, the concurrent high activities of CK and AST were likely associated with muscle injury during handling and/or sampling. Plasma uric acid of Russian sturgeons appeared lower than compared to lake sturgeon, but accurate reference intervals were not possible due to the small sample size. The effects of Aqui-S® 20E on blood analytes in this study could not be determined. Carbon dioxide, a drug determined to be of low regulatory priority by the FDA for use in aquaculture, could not be used in this study due to the potential for interference with the plasma chemistry analytes being evaluated (Oberg et al., 2015; USFWS, 2020). All animals used in this study received the same dose and no control (i.e. carbon dioxide treatment) animals were used to assess a possible difference due to the potential for negative metabolic effects of carbon dioxide. To that end, other anesthetic agents reportedly affect certain biochemical analytes and metabolism of anesthetic agents varies by organ affected. Plasma glucose, ALT, and AST were higher

in Siberian sturgeon sedated with MS-222 than control animals without sedation (Feng et al. 2011). However, in that same study, clove oil (with the active ingredient eugenol) did not have significant effects on blood analytes when compared to control animals. Matsche (2014) showed that MS-222 as well as the temperature of the water system has varying effects on erythrocytes, total protein, and monovalent electrolytes in Atlantic sturgeon. It is presumed that the anesthetic reagent used in this study, eugenol (Aqui-S® 20E), likely caused differences in biochemical analytes. Further study is necessary to determine the effects of various anesthetic agents and length of anesthesia on normal blood reference intervals, but this study provides a starting baseline.

The current study also sought to compare whole blood POC analysis with plasma chemistry analysis to determine the utility of this diagnostic tool in health assessments of Russian sturgeon. When determining whether or not the test can be utilized, several factors must be considered. Many of the analytes that showed a statistically significant difference in data may not necessarily be associated with clinical significance. For example, Glu, Na, Cl, K, and iCa showed statistically significant differences, but when individual results from iSTAT POC tests were compared to plasma chemistry reference intervals, none fell outside this range. This is of particular importance for whole blood glucose by iSTAT POC analyzer as it is continuously consumed by RBC (i.e., decrease by 7% in 24 hours; Eisenhawer et al., 2008) and thus could produce a lower average in both cartridges compared to plasma samples. When considering the difference, although not clinically relevant since the heparin was likely present only in trace amounts, the ionized calcium may have been affected by the presence of heparin used in the collection method (Shin et al., 2006). When collecting samples for either calcium or magnesium, care should be taken to ensure excess heparin is removed and unable to bind these analytes. In contrast, plasma BUN and AG were statistically significant between the test methods but when comparing individual values to the reference interval, 52% and 13% of individuals fell outside the range, respectively. This variation could be clinically relevant and results should be interpreted with caution. With respect to the temperature correction of pH, PCO₂ and PO₂, this step is considered standard practice and crucial to avoid misinterpretation of blood gas results in poikilotherm species. Given the lower surrounding water and therefore body temperature of the Russian sturgeon in this study as compared to the expected temperature for mammalian species at which the iSTAT POC analyzer operates (i.e., approximately 25°C compared to 37°C), the reported

uncorrected values would be lower for pH and PO₂ and higher for PCO₂. These visual differences in analytes illustrate how the solubility of blood gases (i.e., leftward shift in hemoglobin dissociation curve for lower temperatures) is affected by water/body temperature in poikilotherms. This important factor should be considered when using POC systems with ectothermic teleost fish since uncorrected data may alter interpretations with regard to clinical significance and treatment in individual patients. While POC systems, including the iSTAT analyzer, have been validated for a variety of fish, individual species and analyte validation is recommended given the original intended animal use (i.e., mammals) (Stoot et al., 2014)

This study provides reference intervals for blood analytes of the Russian sturgeon, *Acipenser gueldenstaedtii*. While some analytes showed obvious differences for each species, the majority of these data reported herein were similar when compared to other sturgeon species. This study illustrates the importance of spun PCV versus calculated Hct and leukogram evaluation. The biochemical and methodological differences observed in this study warrant further study, including how anesthetic protocols and other pre-analytical and analytical factors may affect these analytes.

[E] Acknowledgements: The authors thank the Veterinary Diagnostic Laboratories at the University of Florida for sample analysis; Jane Davis (formerly of WDW) and staff for assistance with fish husbandry; Hugh Mitchell of AquaTactics for donation of Aqui-S® 20E, and Jim Bowker and Molly Bowman who provided assistance through the USFWS Aquatic Animal Drug Approval Partnership for INAD #11-471.

[F] Literature cited

- Asadi F., A. Hallajian, A. Shahriari, P. Asadian, and M. Pourkabir. 2010. Serum electrolyte and nonelectrolyte status in freshwater juvenile Persian sturgeon *Acipenser persicus*. Journal of Aquatic Animal Health 22(3):167-73.
- Campbell, T. 2012. Hematology of fish. Pages 298-312 in Veterinary Hematology and Clinical Chemistry, 2nd ed., Thrall, M.A., G. Weiser, R. Allison, and T. Campbell, Eds., John Wiley & Sons Inc., Ames, Iowa.
- DiVincenti, L., J. Wyatt, H. Priest, D. Dittman, R. Klindt, D. Gordon, A. Preston, T. Smith, and C. Bowman. 2013. Select hematologic and plasma chemistry values of wild lake sturgeon

(*Acipenser fulvescens*) in the St. Lawrence River, New York. Veterinary Clinical Pathology 42:19–26.

- DiVincenti, L. H. Priest, K. Walker, J. Wyatt, D. Dittman. 2013. Comparison of select hematology and serum chemistry analytes between wild-caught and aquarium-housed lake sturgeon (*Acipenser fulvescens*). Journal Zoo and Wildlife Medicine 44(4): 957-964.
- Eisenhower E., C. Courtney, R. Raskin, and E. Jacobson. 2008. Relationship between separation time of plasma from heparinized whole blood on plasma biochemical analytes of loggerhead sea turtles (*Caretta caretta*). Journal of Zoo Wildlife Medicine 39:208–215.
- Feng, G., P. Zhuang, L., Zhang, B. Kynard, X. Shi, M. Duan, J. Lui, X. Huang. 2011. Effects of anesthetic MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon (*Acipenser baerii*). Journal of Applied Ichthyology 595-599.
- Friedrichs, K., K. Harr, K. Freeman, B. Szladovits, R. Walton, K. Barnhart, and J. Blanco-Chavez. 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Veterinary Clinical Pathology 41:441–453
- Geffre, A., D. Concordet, J. Braun, and C. Trumel. 2011. Reference value advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Veterinary Clinical Pathology 40:107–112.
- Genz J, B. Carriere, and W. Anderson. 2013. Mechanisms of calcium absorption by anterior and posterior segments of the intestinal tract of juvenile lake sturgeon. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 166(2):293-301.
- Greenwall, M., J. Sherrill, and L. Clayton. 2003. Osmoregulation in fish. Mechanisms and clinical implications. Veterinary Clinics of North America Exotic Animal Practice 6(1):169-189.
- He X., P. Zhuang, L. Zhang L, and C. Xie. 2009. Osmoregulation in juvenile Chinese sturgeon (*Acipenser sinensis* Gray) during brackish water adaptation. Fish Physiology and Biochemistry 35(2):223-230.

Hrubec T. and S. Smith. 2000. Hematology of fish. Pages 1120-1125 in B. Feldman, J. Zinkl, N. Jain, editors. Schalm's Veterinary Hematology, 5th edition. Lippincott Williams & Wilkins, Philadelphia.

International Union for Conservation of Nature. 2010. Sturgeon more critically endangered than any other group of species. Available: <https://www.iucn.org/content/sturgeon-more-critically-endangered-any-other-group-species>

Knowles, S., T. Hrubec, S. Smith, and R. Badal. 2006. Hematological and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). Veterinary Clinical Pathology 35:434–440.

Matsche M. and J. Gibbons. 2012. Annual variation of hematology and plasma chemistry in shortnose sturgeon, *Acipenser brevirostrum*, during a dam-impeded spawning run. Fish Physiology and Biochemistry 38(6):1679-1696.

Matsche M. 2014. Evaluation of tricaine methanesulfonate (MS-222) as a surgical anesthetic for Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus*. Journal of Applied Ichthyology 27(2): 600-610.

Norris, D., and J. Carr. 2013. Regulation of Calcium and Phosphate Homeostasis in Vertebrates. Pages 501-527 in Vertebrate Endocrinology, 5th Edition. Elsevier, Amsterdam.

Oberg, E., K. Perez, L Fuiman. 2015. Carbon dioxide is an effective anesthetic for multiple marine fish species. Fisheries Research 165:22-27.

Sadati, M., M. Pourkazemi, M. Shakurian, M. H. Hasani, H. Pourali, M. Pourasaadi, and A. Yousefi. 2011. Effect of daily temperature fluctuations on growth and hematologic parameters of juvenile *Acipenser baerii*. Journal of Applied Ichthyology 27:591– 594.

Sepulveda, M., T. Sutton, H. Patrick, and J. Amberg. 2012. Blood chemistry values for shovelnose and lake sturgeon. Journal of Aquatic Animal Health 24:135–140.

Shahsavani, D., H. Kazerani, S. Kaveh, and H. Gholipou-Kanani. 2010. Determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus* Pallas, 1771) during spring season. Comparative Clinical Pathology 19:57–61.

Shi, X., D. Li, P. Zhuang, F. Nie, and L. Long. 2006. Comparative blood biochemistry of Amur sturgeon, *Acipenser schrenckii*, and Chinese sturgeon, *Acipenser sinensis*. Fish Physiology and Biochemistry 32:63–66.

Shin, C., C. Chang, and J. Kim. 2006. Liquid heparin anticoagulant produces more negative bias in the determination of ionized magnesium than ionized calcium. Yonsei Medical Journal 47:191-195.

Sheddon L. 2012. Clinical anesthesia and analgesia in fish. Journal of Exotic Pet Medicine 21(1): 32-43.

Stoot, L., N. Cairns, F. Cull, J. Taylor, J. Jeffrey, F. Morin, J. Mandelman, T. Clark, and S. Cooke. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. Conservation Physiology, 2(1):1-21.

United States Fish and Wildlife Service. 2020. Aquatic Animal Drug Approval Partnership, Investigational New Animal Drug, Aqui-S® 20E INAD #11-741. Available:
<https://www.fws.gov/fisheries/aadap/inads/AQUI-S20E-INAD-11-741.html>

Wei H. P. Chen, X. Liang, H. Yu, X. Wu, J. Han, L. Luo, X. Gu, and M. Xue. 2019. Plant protein diet suppressed immune function by inhibiting spiral valve intestinal mucosal barrier integrity, anti-oxidation, apoptosis, autophagy and proliferation responses in amur sturgeon (*Acipenser schrenckii*). Fish and Shellfish Immunology 94:711-722.

Weiss, D. 1984. Uniform evaluation and semiquantitative reporting of hematologic data in veterinary laboratories. Veterinary Clinical Pathology 8(2):27-31.

Accepted Article

Table 1.—Hematological, whole blood iSTAT point-of-care (POC), and plasma biochemical reference intervals (RI) in Standard International Units of adult aquarium-housed Russian sturgeon (*Acipenser gueldenstaedtii*). Routine manual hematology (RMH) using whole blood, iSTAT POC (POC) using whole blood, and plasma chemistry (PC). Data distribution was parametric for all analytes.

Analyte	Unit	Analysis Method	Sample Number	Mean	Median	Standard Deviation	Minimum	Maximum	Lower limit		Upper limit		90% CI for lower limit		90% CI for upper limit	
									Reference Interval	Reference Interval	Reference Interval	Reference Interval	90% CI for lower limit	90% CI for upper limit	90% CI for lower limit	90% CI for upper limit
Packed Cell Volume	%	RMH	23	25	25	4	17	33	16	34	14	19	32	37		
Hematocrit (iSTAT CG8+)	%	POC	23	20	20	3	13	26	11	26	7	14	24	27		
Hematocrit (iSTAT Chem8+)	%	POC	23	21	21	4	14	28	13	28	11	15	26	30		
Hemoglobin (iSTAT CG8+)	g/L	POC	23	68	68	11	44	88	39	89	27	50	83	93		
Hemoglobin (iSTAT Chem8+)	g/L	POC	23	71	71	12	48	95	46	97	40	53	89	104		
Total estimate WBC ^a	10 ⁹ /L	RMH	23	8.05	7.3	1.71	6	11.5	5.44	13.77	5.27	5.92	10.99	19.26		
Segmented neutrophils	10 ⁹ /L	RMH	23	2.75	2.73	0.84	1.09	4.14	0.85	4.47	0.48	1.52	3.98	4.87		
Lymphocytes	10 ⁹ /L	RMH	23	4.35	4.13	1.38	1.63	7.64	1.65	7.50	1.00	2.38	6.33	8.56		
Monocytes	10 ⁹ /L	RMH	23	0.27	0.26	0.12	0.07	0.56	0.063	0.56	0.00	0.11	0.47	0.66		
Eosinophils	10 ⁹ /L	RMH	21	0.53	0.56	0.36	0.07	1.44	0.02	1.64	0.00	0.12	1.24	2.02		
Alanine aminotransferase	Units/L	PC	22	33	25	19.5	14	76	12	137	10	14	65	316		
Aspartate aminotransferase	Units/L															
pyridoxal 5-phosphate																
Total Protein	g/L	PC	23	58.50	58.00	10.00	39.00	77.00	37.50	80.00	32.20	43.90	74.10	85.30		
Calcium	mmol/L	PC	23	2.89	2.38	1.03	1.95	5.40	1.72	4.81	1.63	1.86	4.22	5.71		
Phosphorus	mmol/L	PC	23	2.90	2.91	0.47	2.16	4.07	2.01	4.02	1.80	2.24	3.65	4.44		
Blood Urea Nitrogen	mmol/L	PC	23	2.68	2.86	0.64	1.43	3.93	1.46	4.11	1.25	1.82	3.61	4.46		
Glucose	mmol/L	PC	22	3.56	3.47	0.64	2.33	4.83	2.34	5.09	2.11	2.65	4.51	5.62		
Cholesterol	mmol/L	PC	23	1.96	2.02	1.07	0.08	4.27	0.07	4.64	0.00	0.52	3.83	5.43		
Triglycerides	mmol/L	PC	21	6.58	6.61	1.65	4.27	10.43	3.79	10.77	3.39	4.47	9.39	12.23		
Magnesium	mmol/L	PC	23	0.87	0.86	0.10	0.74	1.15	0.73	1.15	0.71	0.76	1.03	1.35		
Gamma-	Units/L	PC	21	7	5	3.2	5	14	1	14	1	2	10	17		

glutamyltransferase

Creatine Kinase	Units/L	PC	21	6,004	5,128	4,060	1,229	18,725	1,061	18,035	777	1,775	12,688	24,410
Sodium	mmol/L	PC	23	141	139	6	131	152	129	155	126	132	150	161
Potassium	mmol/L	PC	23	3.3	3.4	0.3	2.8	3.8	2.7	3.9	2.6	2.9	3.7	4.0
Chloride	mmol/L	PC	23	115	115	5	103	126	104	126	100	107	122	129
Total CO ₂	mmol/L	PC	23	6.50	7.00	2.10	2.00	10.00	2.10	10.90	0.90	3.30	9.60	12.20
Anion Gap	mmol/L	PC	22	23	23	3	16	28	16	30	14	18	28	32
Uric Acid	μmol/L	PC	19	15.46	11.90	7.73	5.95	35.69	3.57	38.66	-- ^b	-- ^b	-- ^b	-- ^b

^a Weiss D. 1984. Uniform evaluation and semiquantitative reporting of hematologic data in veterinary laboratories. Veterinary Clinical Pathology 8(2):27-31.

^b n < 20 too small to establish CI using bootstrap method.

Table 2.—Comparison of hematology and plasma chemistry data from various sturgeon species in the literature in Standard International Units.

Analyte	Units	<i>Species</i>	<i>A. gueldenstaedtii</i>	<i>A. brevirostrum</i>	<i>A. fulvescens</i>	<i>A. fulvescens</i>
		Common name	Russian sturgeon	Shortnose sturgeon	Lake sturgeon	Lake sturgeon
		Life Stage	Adult	Juvenile	Adult	Adult
Hematologic Reference Intervals		Sample Number	(n=23)^a	(n=44)^b	(n=30)^c	(n=52)^d
Packed Cell Volume	%		16-34	24-46	16-42	17-38
Hemoglobin	g/L		39-89	57-87	--	--
Total estimate WBC	10 ⁹ /L		5.44-13.77	28.38-90.79	3.84-26.7	2.7-23.2
Segmented neutrophils	10 ⁹ /L		0.85-4.45	3.76-33.6	0.55-7.91	0.19-6.1
Lymphocytes	10 ⁹ /L		1.65-7.5	11.19-67.00	1.53-15.43	1.4-14.00
Monocytes	10 ⁹ /L		0.063-0.56	0-7.14	0-0.54	0.055-1.7
Eosinophils	10 ⁹ /L		0.019-1.64	0-1.54	0-1.82	0-0.56
Plasma Biochemical Reference Intervals		Sample Number	(n=23)	(n=77)	(n=30)	(n=52)
Alanine aminotransferase pyridoxal 5-phosphate	Units/L		12-137	--	--	--
Aspartate aminotransferase pyridoxal 5-phosphate	Units/L		165-661	62-209	113-547	223-1178
Total Protein	g/L		37.5-80	28-60	18-53	20-44
Calcium	mmol/L		0.43-1.20	0.41-0.76	0.41-0.53	0.48-0.7
Phosphorus	mmol/L		0.65-1.3	0.52-0.85	0.48-1.226	0.72-1.88
BUN	mmol/L		1.46-4.11	--	--	--
Glucose	mmol/L		2.33-5.11	2.05-4.11	0.89-3.05	2.89-14.76
Cholesterol	mmol/L		0.07-4.64	1.09-3.44	--	--
Triglycerides	mmol/L		3.79-10.77	--	0-532	1.07-5.12
Magnesium	mmol/L		0.73-1.88	0.66-0.95	--	--
Gamma-glutamyltransferase	Units/L		1-14	--	1-51	6-30

Creatine Kinase	Units/L	1,061-18,035	--	0-5,720	35,540-775,995
Sodium	mmol/L	129-155	124-141	120-141	123-151
Potassium	mmol/L	2.7-3.9	2.9-3.7	2.3-3.6	2.3-4.2
Chloride	mmol/L	104-126	106-121	106-124	95-123
TCO ₂	mmol/L	2.1-10.9	--	--	--
Anion Gap	mmol/L	16-30	--	--	--
Uric Acid	μmol/L	0-38.66*		0-5.95	1.19-249.82

References

^a Current Study

^b Knowles et al., 2006

^c DiVincenzi et al., 2013b

^d DiVincenzi et al., 2013a

* Limited number of samples (n<20)

-- No Information Presented

Table 3.—Comparison of blood analytes by plasma chemistry and iSTAT POC (whole blood) in Russian sturgeon (*Acipenser gueldenstaedtii*)

Analyte	Units	Whole Blood	Plasma Chemistry	Cartridge 1 (CG8+)	Cartridge 2 (Chem 8+)
Packed Cell Volume	%	25 + 4.3	--	--	--
Hematocrit	%			20 + 3 ^a	21 + 4 ^b
Hemoglobin	g/L	--	--	68 + 11	71 + 12
BUN	mmol/L	--	2.68 + 0.62	--	1.46 + 0.5 ^b
Glucose	mmol/L	--	3.56 + 0.64	2.78 + 0.42 ^{a,c}	2.89 + 0.42 ^{b,c}

Sodium	mmol/L	--	141 + 6	135 + 2 ^{a,c}	137 + 2 ^{b,c}
Potassium	mmol/L	--	3.3 + 0.27	3.3 + 0.22 ^c	3.24 + 0.21 ^c
Chloride	mmol/L	--	115 + 5	--	120 + 3 ^b
TCO ₂	mmol/L	--	6.5 + 2.1	7.4 + 0.7 ^c	6.2 + 0.7 ^c
Ionized Calcium	mmol/L	--	1.102 + 0.094	1.276 + 0.091 ^a	1.268 + 0.093 ^b
Anion Gap	mmol/L	--	23 + 3	--	15 + 2 ^b

^a Statistically Significant Difference
between plasma chemistry and CG8+

^b Statistically Significant Difference
between plasma chemistry and
Chem8+

^c Statistically Significant Difference
between CG8+ and Chem8+