Title: Blood analyte changes of wild caught adult Almaco Jack (*Seriola rivoliana*) in response to acclimation to recirculating aquaculture systems and hyposalinity treatment.

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INTRODUCTION:

Blood sampling is a non-invasive technique that provides a sample matrix for various analyses, including hematology and plasma chemistry, that are commonly used for diagnosis and monitoring of individuals or groups of animals in health and disease (Filho et al. 1992; Campbell 2012). Although baseline and reference data of blood analytes have been established for numerous species in veterinary medicine, they still need to be defined for a variety of commercially important cultured fish species (Berillis 2017). As described by Fazio (2019), fish blood analysis has become an important diagnostic tool in aquaculture for assessing the health status of fish in response to changes related to water quality, nutrition, and disease. The use and validation of standardized, non-lethal, and inexpensive methodologies for monitoring fish health are necessary for optimizing husbandry protocols for intensive aquatic animal production.

Variations in blood can be caused by intrinsic (e.g., age/life stage) and extrinsic (e.g., water quality, seasonality, and handling stress) factors (Clauss et al. 2008). In addition to providing baseline health data of an individual animal or a group of animals, hematology and plasma chemistry end points can be used to identify the effects of environmental stressors (Burgos-Aceves et al. 2019), metabolic changes (Eddy and Handy 2012), and underlying or early onset of disease (Grant 2015; Fazio 2019). Establishing baseline blood analyte data can be important in evaluating the health of individual stocks as shown in studies with commercially farmed fishes. Such examples include dietary inclusion trials with juvenile yellowtail Seriola quinqueradiata (Ren et al. 2008), comparison of blood chemistry data to elucidate the cause of death in bluefin tuna

Thunnus orientalis including assessments of fish exhibiting normal and abnormal swimming behavior (Honryo et al. 2019), and documenting stress responses and impact of transport on juvenile yellowtail kingfish Seriola lalandi (Moran et al. 2008). Despite the potential of blood analyte evaluation as a tool to assist in monitoring the health of marine fishes, baseline data is lacking for many commercially important marine finfish species.

Similar to other commercially farmed Seriola species, Almaco Jack have a fast growth rate, high market value, and are increasingly well-regarded among chefs for their versatility in both cooked and raw preparations (Roo et al. 2014; Fernández-Palacios et al. 2015; Sicuro and Luzzana 2016). Growth in the aquaculture sector has led to product diversification and thus, intensive culture of new high-value finfish like Almaco Jack. As such, domesticated broodstock need to be established along with health management protocols that ensure reliable production of high-quality gametes and juveniles. The objective of this study was to compare hematology and plasma chemistry data for adult wild-caught Almaco Jack (Seriola rivoliana) at time of capture and again by 16 weeks following a period of acclimation to a recirculating aquaculture system and hyposalinity treatment.

MATERIALS AND METHODS:

Broodstock Collection and Maintenance

A total of 30 adult fish were caught via hook and line in the eastern Gulf of Mexico, approximately 120 miles offshore (salinity, 35 ppt) from Madeira Beach, Florida. Blood was collected from a random subset of these wild adult Almaco Jack (n=13) immediately after capture. A second, but different subset of these fish (n=12) were then sampled at 16 weeks post capture (following acclimation to the new captive environment). The authors chose to only sample a subset of fish for this trial due to concerns regarding the impact of handling stress and blood sampling on spawn capability. All fish from which samples were collected were visually examined and found to be apparently healthy without any external injuries or other overt abnormalities. Blood (3 ml) was taken from the caudal vein using a 23-gauge needle attached to a heparinized syringe. No sedation was used prior to blood collection. Heparin was thoroughly expelled from the syringe before use and transferred into a vacutainer tube coated with lithium heparin (Sigma-Aldrich, St. Louis, MO). The time elapsed between hooking the fish and blood collection ranged from 22-28 minutes for wild caught specimens. Fish were transported from Madeira Beach to Mote

Aquaculture Research Park (MAP) in Sarasota, Florida, using a specialized live-hauler designed with four individual compartments (capacity, $1m^3$ each, filled with natural seawater at 35 ppt), oxygenation, and separate controls for each. The total transport time from the point of capture to stocking at MAP was approximately 15 hours. Upon arrival to MAP, all fish were immersed in a freshwater bath for 10 minutes to remove external parasites. Fish were placed in an indoor, photoperiod (12H light) and temperature (26°C) controlled recirculating tank system. The system consisted of a green, fiberglass tank ($28m^3$) equipped with solids filter, bio-filter, a protein skimmer, and UV sterilization. Salinity was maintained at 35 ppt for the first two weeks of acclimation. This was followed by a 45-day hyposalinity (15 ppt) exposure, a procedure used to control monogenean parasites found during initial health examinations (Rigos et al. 2001). In addition to monogeneans, digenean parasites were also observed; both groups are commonly identified in Seriola sp. (Ogawa 2015; Hirazawa et al. 2016, 2017; Valles-Vega et al. 2019). The same sampling methods for collection of blood were used with both groups of fish. A total of 16 weeks elapsed between the time the fish were initially brought to MAP and the blood samples were taken from a second subset of fish (now newly acclimated broodstock). Fish were presumed to have successfully adjusted to their captive environment as evidenced by their continued growth throughout the acclimation period and observed volitional spawning documented two weeks following the completion of the hyposalinty treatment (Patrick et al. 2019). Throughout the entire time from capture to sampling at 16 weeks, fish were fed a daily diet of squid (50%) and threadfin herring Opisthonema oglinum (50%) at 3% of the total tank biomass.

Hematology and Blood Chemistry

All collected blood samples were kept cold from the time of collection and processed within a maximum of 24 h post sampling. Packed cell volume (PCV), total solids (TS), and visual plasma color were assessed after centrifugation (Combo V24T Centrifuge, LW Scientific Inc., Lawrenceville, Georgia, USA) of a capillary tube for fish at time of capture. Total solids were determined by clinical refractometer (Master-SUR/Nα Clinical Automatic, Atago USA, Inc., Bellevue, Washington, USA). The following procedures were performed for both subsets of fish. Two blood films were prepared from each sample, air-dried, and stained with Wright-Giemsa (Harleco®, EMD Millipore, Billerica, Massachusetts, USA). Blood film evaluation included a white blood cell (WBC) estimate (Weiss 1984), a 200-WBC differential, and blood cell

morphological evaluation. Whole blood was then centrifuged and plasma harvested and immediately frozen at ultra-cold freezer temperature (-80ºC). Within one month after sampling, frozen plasma samples were shipped to the University of Miami Avian & Wildlife Laboratory (Miami, Florida USA). Plasma was analyzed using a dry slide chemistry analyzer Ortho 250XR (Ortho Clinical Diagnostics, Rochester, New York USA). The following were assessed: hemolysis index, lipemia, anion gap, aspartate aminotransferase (AST), blood urea nitrogen (BUN), bicarbonate (CO2), calcium, chloride, cholesterol, creatine kinase (CK), gamma-glutamyl transferase (GGT), glucose, potassium, magnesium, phosphorus, sodium, total protein, triglycerides, uric acid, and calculated osmolality. Plasma protein electrophoresis was performed using SPIFE 3000 system (Helena Laboratories, Beaumont, Texas USA) to measure the concentrations of 6 fractions. The gels were run according to manufacturer's instructions and protein fractions were quantified using gel electrophoresis and laser densitometry as described previously (Christiansen et al. 2015). Each protein fraction (protein fractions 1-6) was calculated using the percentage of the fraction multiplied by the total protein concentration.

Statistical Analysis

Data were visually examined for potential outliers and no values had to be excluded from the dataset. Blood analyte data are reported as mean, median (minimum, maximum). Distributions of blood analytes were compared between blood obtained at time of capture and again at 16 weeks post capture by using the non-parametric Wilcoxon Rank Sum test. The proportions of Almaco Jack with a hemolysis index = 1 were compared between groups by using a chi-square test. Values of $p < 0.05$ were considered significant. All analyses were conducted using Statistix 10 for Windows (Analytical Software, Tallahassee, Florida).

RESULTS

Among fish sampled, an increase in growth (mean body weight, g) was observed (19%) between the initial (May) and final (September) health evaluations of fish from each time point. At the time of capture, Almaco Jack (n=13) weighed an average of 3,276 ± 504 g (58.85 ± 3.4 cm fork length; 63.2 ± 3.4 cm total length). Captive held fish (n=12) weighed an average of 4,007.2 ± 370 g (62.8 ± 2.7 cm fork length; 62.8 ± 4.1 cm total length).

Blood data is presented in Table 1. Plasma color was clear for most samples. More wild Almaco Jack (9/13; 69%) had a hemolysis index = 1 than acclimated (5/11; 45%); but this difference was not significant (P = 0.23). All wild (13/13) and acclimated (12/12) Almaco Jack had lipemia index = 0. Representative images of blood cell morphology are shown in Figure 1. Blood film evaluation revealed minimal thrombocyte clumping and WBC clumping was absent. Lymphocytes were the predominant WBC type. Heterophils had a variable number of rod-shaped, orange granules and pale blue cytoplasm, and there was mild left-shifting. Red blood cells were consistently mature (i.e., absence of polychromasia). Thrombocytes appeared adequate in number with low numbers of small thrombocyte aggregates present in all fish. Compared to acclimated fish, wild-caught fish had significantly higher ($P < 0.05$) absolute white blood cell (WBC) counts, while acclimated fish had lower sodium, chloride, and calculated osmolality, blood urea nitrogen, and higher calcium, calcium to phosphorus ratio, cholesterol, glucose, total protein, plasma protein fractions (except for fraction 1), potassium, and triglycerides. Figure 2 shows two representative electrophoretograms of wild-caught and acclimated Almaco Jack without hemolysis.

DISCUSSION:

The information presented herein documents baseline blood data for wild-caught Almaco Jack from the Gulf of Mexico and describes their physiological responses 16 weeks after capture and 6 weeks post hyposalinity treatment. Prophylactic techniques, such as incremental salinity changes, are sometimes administered in aquaculture to limit the introduction of pathogens to the captive environment when new fish are introduced to an established system (Segawa et al. 2000; Brazenor and Hutson 2015). The observation of only subtle hematological changes was somewhat unexpected, given that stress of captivity could have shown some effects. Although the observed significant difference in absolute WBC may not be clinically relevant and within expected variation of analytical precision, especially given the lack of differences in specific WBC types, this finding may be explainable by biological variation and/or non-specific antigenic stimulation in wild fish. This may have resulted from parasitic accumulation in wild fish, as monogeneans and digeneans are common in wild populations of Seriola sp. (Sharp et al. 2004; Hirazawa et al. 2010; Tamaru et al. 2016). An additional consideration for lower WBC concentrations in acclimated fish includes possible immunosuppression associated with stress in captivity. Although stress was not manifested in the leukogram (i.e., lack of heterophilia and/or lymphopenia), higher glucose in

acclimated fish could suggest a stress response or effects from dietary differences. However, artifactual alterations in plasma glucose need to be considered due to processing within 24 hours and possible consumption by red blood cells, although a recent study of another non-mammalian vertebrate species with nucleated red blood cells showed that plasma glucose was consistent in blood samples that were refrigerated up to 48 hours (Kunze et al. 2020).

The second subset of fish were sampled after a completed prophylactic treatment. Changes in sodium, chloride, and calculated osmolality suggest osmoregulatory adjustments occurred following this extended treatment. Since blood was sampled 6 weeks after hyposalinity treatment, data presented herein likely reflect an adjustment from 35 to 15 ppt and back again to 35 ppt. Electrolytes were still not adjusted 6 weeks after completion of the hyposalinity treatment, indicating that adjustment of electrolyte hemostasis takes at minimum 6 weeks.

The identified plasma biochemical differences between wild-caught and acclimated fish indicate responses to dietary, physiological variation, and/or environmental changes. Of notable interest were prominent changes in plasma lipids and triglycerides after acclimation. These findings presumptively resulted from differences in diet (i.e., dietary composition and/or quantity) as fish were transitioned from their natural diet (Manooch and Haimovici 1983; Barreiros et al. 2003) to a fresh frozen diet consisting of Atlantic thread herring (Opisthonema oglinum) and squid. Based on the gut content analysis of this species in the wild, Almaco Jack can be considered almost exclusively piscivorous, though they are opportunistic feeders. Lipid and fatty acid composition of diet reportedly are well known factors affecting fish reproductive success and survival of offspring (Izquierdo et al. 2001, 2015). Results from this study highlight the need for further research on optimizing egg and larval quality through management of nutrition for Almaco Jack. In addition to dietary considerations, higher plasma calcium and calcium to phosphorus ratio in acclimated fish may reflect differences in environmental calcium compared to their natural habitat or physiological variation of protein-bound calcium in actively reproductive females as supported by higher total protein (Campbell 2012). Protein fractions have previously been reported in other fish species, including 6 and 5 fractions in Rainbow trout Oncorhynchus mykiss (Manera and Britti 2008) and Koi Cyprinus carpio (Christiansen et al. 2015), respectively. Considerations for potassium variations in this study suggest dietary differences, environmental changes (e.g., cutaneous loss), or effects from hemolysis although minimally expected by the utilized chemistry analyzer in this study.

The observed plasma biochemical data suggest that Almaco Jack can adjust quickly to environmental and dietary manipulation as evidenced by the observation of natural spawning among broodstock within five months of collection (Patrick et al. 2019). This study is the first to report baseline hematology and plasma biochemistry data for Almaco Jack and describes consequent changes following prophylactic hyposalinity treatment and a period of acclimation. The data herein document novel and relevant health information regarding physiological responses of Almaco Jack to their new environment as captive broodstock. These findings serve as a baseline for development of broodstock health management protocols needed for commercial aquaculture.

CONFLICT OF INTEREST STATEMENT: The authors of this manuscript have no financial or personal relationships with other people or organizations that could influence or bias the content of the paper.

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Table 1. Comparison of blood analytes between wild Almaco Jack (Seriola rivoliana) at time of capture (May) and 16 weeks post capture (September) following a period of acclimation to a

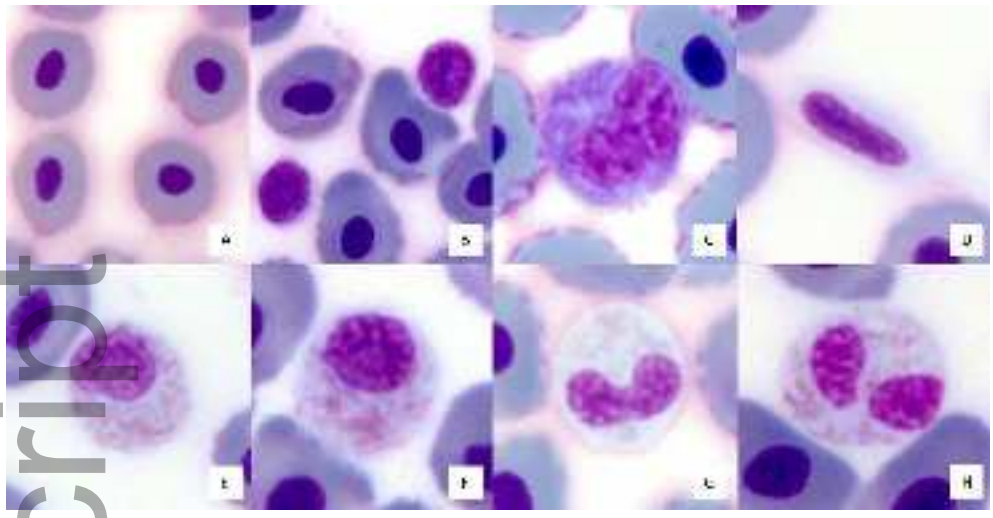
Variable	Unit	Wild (At Transfer) N = 13	Captive (16 weeks post capture) N = 12	p
Packed cell volume	%	44, 44 (30, 56)	NP	ND
Total solids	g/dL	5.5 (5.0, 6.4)	NP	ND
White blood cell estimate[1]	$x10^3/\mu l$	25.9, 25.9 (19.2, 32.5)	21.6, 22.0 (15.5, 27.1)	0.04
Mature heterophils[1]	$x10^3/\mu l$	3.1, 3.1 (1.6, 5.9)	2.6, 2.4 (1.5, 4.6)	0.28
Immature heterophils[1]	$x10^3/\mu l$	0.3, 0.3 (0.0, 1.2)	0.2, 0.1 (0.0, 0.5)	0.38
Lymphocytes[1]	$x10^3/\mu l$	17.0, 18.0 (13.0, 22.0)	14.7, 14.5 (11.0, 19.0)	0.06
Monocytes[1]	$x10^3/\mu l$	5.5, 4.8 (3.7, 9.6)	4.2, 4.3 (2.5, 6.4)	0.09
Eosinophils[1]	$x10^3/\mu l$	0.1, 0.0 (0.0, 0.4)	0.0, 0.0 (0.0, 0.0)	0.99
Basophils[1]	$x10^3/\mu$	0.0, 0.0 (0.0, 0.0)	0.0, 0.0 (0.0, 0.0)	0.99
Anion gap	mmol/L	33.6, 14.0 (10.0, 214.0)	26.3, 27.0 (15.0, 36.0)	0.07
Aspartate aminotransferance[2]	U/L	45, 34 (15, 96)	58, 52 (10, 145)	0.59
Blood urea nitrogen	mg/dL	10.4, 11.0 (5.0, 15.0)	4.0, 4.0 (2.0, 7.0)	< 0.01
CO_2	mmol/L	9.1, 9.0 (5.0, 13.0)	11.0, 11.0 (9.0, 13.0)	0.07
Calcium	mg/dL	15.4, 14.8 (12.2, 20.5)	20.9, 19.6 (18.9, 24.7)	< 0.01
Calcium to phosphorus ratio	-	1.4, 1.4 (1.1, 1.9)	1.9, 1.9 (1.5, 2.4)	<0.01
Chloride	mmol/L	172, 172 (164, 178)	154 (151, 155)	< 0.01
Cholesterol	mg/dL	235, 236 (152, 327)	357, 364 (235, 409)	< 0.01
Creatine kinase	U/L	1720, 1300 (242, 3981)	1689, 1650 (161, 3263)	0.93
Gamma-glutamyl transferase	U/L	5, 5 (5, 7)	5, 5 (5, 5)	0.99
Glucose	mg/dL	77, 77 (51, 96)	110, 106 (77, 164)	< 0.01
Potassium	mmol/L	1.7, 1.4 (1.0, 3.1)	2.9, 2.6 (1.7, 5.1)	< 0.01
Magnesium	mg/dL	4.4, 4.4 (3.0, 5.3)	4.5, 4.5 (3.8, 5.5)	0.65
Phosphorus	mg/dL	11.0, 10.2 (6.8, 16.8)	11.1, 11.3 (8.3, 13.2)	0.32
Sodium	mmol/L	201, 199 (188, 221)	191, 190 (182, 199)	< 0.01
Total protein	g/dL	4.7, 4.6 (3.3, 6.1)	7.0, 6.8 (5.9, 8.4)	< 0.01
Triglycerides	mg/dL	48, 36 (17, 129)	275, 248 (136, 435)	< 0.01

Uric acid	mg/dL	0.3, 0.4 (0.3, 0.4)	0.4, 0.4 (0.4, 0.4)	0.99
Osmolality, calculated		382, 377 (357, 420)	362, 361 (346, 376)	< 0.01
Total Protein	g/dL	4.3, 4.4 (2.6, 5.7)	7.0, 6.8 (5.9, 8.4)	< 0.01
Fraction 1	g/dL	0.0 (0.0, 0.0)	0.0 (0.00, 0.01)	0.18
Fraction 2	g/dL	1.4 (1.1, 1.6)	2.11 (1.9, 2.2)	< 0.01
Fraction 3	g/dL	0.7 (0.6, 0.8)	1.21 (1.1, 1.3)	< 0.01
Fraction 4	g/dL	0.9 (0.8, 1.0)	1.44 (1.2, 1.7)	< 0.01
Fraction 5	g/dL	0.8 (0.7, 1.0)	1.44 (1.2, 1.7)	< 0.01
Fraction 6	g/dL	0.3 (0.3, 0.4)	0.68 (0.5, 0.7)	< 0.01

recirculating aquaculture system. All blood samples were obtained 6 weeks following an anti-parasitic hyposalinity treatment. Data are reported as mean, median (minimum, maximum). NP = not performed.

[1]n = 13 wild Almaco Jacks and n = 10 captive Almaco Jacks

[2]n = 13 wild Almaco Jacks and n = 11 captive Almaco Jacks



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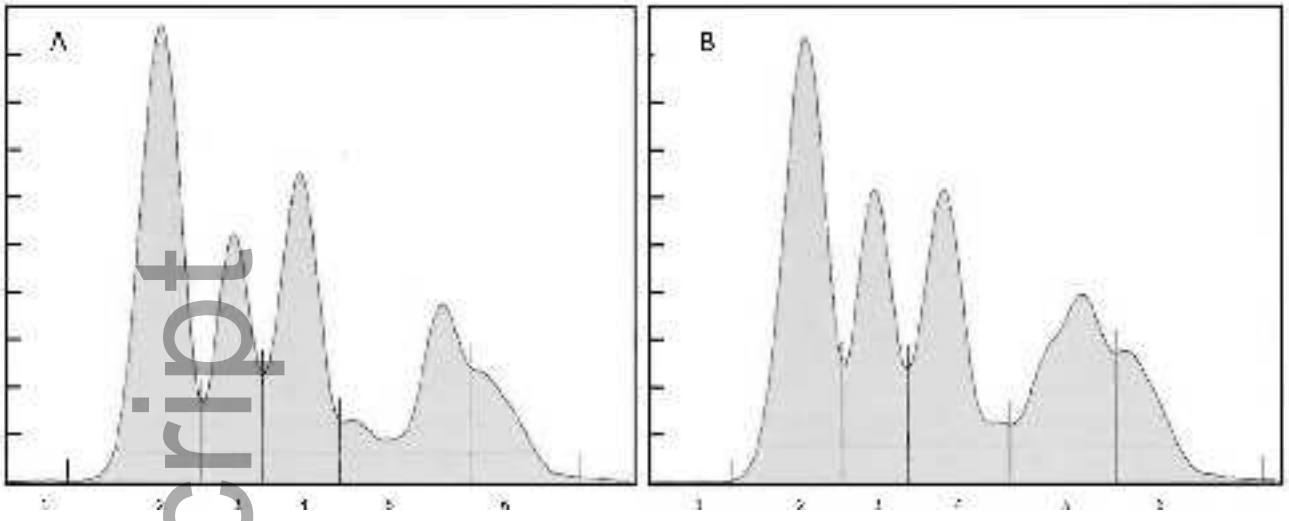

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