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Immunophysiology of Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus* (Mitchill), and the relationship to parasitic copepod, *Dichelesthium oblongum* (Abilgaard) infection

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Abstract

The copepod parasite, *Dichelesthium oblongum*, is known to infect the Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, within the area near New York city, USA, known as the NY Bight. The gross pathology associated with the juvenile and adult copepod stages along with the parasite's link in causing changes in sturgeon osmoregulatory capabilities has led us to investigate the host immunophysiology in relation to this host–parasite system. All the host variables, which included gill Na^+-K^+-ATPase activity, serum alkaline phosphatase (AP) and white blood cell differential counts, were affected in a non-linear manner by the copepod parasite. The parasites increased the host gill Na^+-K^+-ATPase activity and serum AP along with the percentage granulocytes while decreasing the percentage lymphocytes. A new method, developed to sample and preserve white blood cells in the field for future flow cytometry analysis, proved adequate. The effects of fish size, location and time of sampling were accounted for by the use of generalized linear models, and their effects on the host variables are discussed.

Keywords: Atlantic sturgeon, copepod parasite, flow cytometry, gill Na^+-K^+-ATPase, leucocyte, serum alkaline phosphatase.

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Introduction

The Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus* (Mitchill), is a diadromous species with a long life span (> 50 years), of which the majority is spent in salt water. Juveniles of this species are known to leave the river system within the first few years of life, and genetics suggest they may return to their natal rivers (Waldman, Hart & Wirgin 1996; Grunwald *et al.* 2008), following maturation (12–14 years (Van Eenennaam & Doroshov 1998)), to spawn at intervals, which may be > 3 years (Boreman 1997). There is currently an information gap regarding the movements and habitat usage for juvenile Atlantic sturgeon upon leaving their natal river, but what is known suggests significant migrations across varying osmotic environments. Osmotic competence is therefore extremely important for adeptly negotiating nearshore environments during spring and autumn months and because the energetic demands required for undergoing long migrations are significant. Stressors these juveniles encounter within and around these habitats may have severe repercussions, as elevated levels of the stress hormone cortisol have been shown to disrupt the osmoregulatory capacity and reduce the immunocompetence of other fish species (Pickering & Duston 1983; Pickering & Pottinger 1989; Bonga 1997). For Atlantic sturgeon, there is little known about their immunophysiology within hypersaline environments.

Marine-phase Atlantic sturgeon migrations are thought to be limited to narrow corridors of waters

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< 20 m in depth along the eastern coast of North America, and aggregations of these fish have been observed in discrete areas at the mouths of large bays or estuaries (Dunton *et al.* 2010).

High densities of fish in relatively shallow waters, such as these, may enhance the transmission of pathogens between Atlantic sturgeon. In particular, ectoparasites, such as *Dichelesthium oblongum* (Abildgaard), which contribute to osmoregulatory stress, are known to have > 90% prevalence on hosts in aggregatory areas in the New York Bight (Fast *et al.* 2009). The effectiveness of compensatory mechanisms to offset ion loading and osmoregulatory stress as seen under these conditions, such as gill ion transport (Na^+-K^+-ATP pumps, etc.) are currently unknown (Fast *et al.* 2009). Furthermore, direct (immunomodulatory secretions) and/or indirect (chronic stress-induced) effects these infections may have on immunocompetence are also unknown.

Here, we describe non-lethal methods for analyzing both physiologically and immunologically important indicators in Atlantic sturgeon and how these change under infection-level variation, while accounting for differences in fish size and location and time of sampling. A fixation method for blood cells was developed for the determination of white blood cell differential counts from field samples through flow cytometry analysis and sorting. The interaction of biological and physical inputs on the immunophysiology of the host will be discussed.

Materials and methods

Fish sampling

Juvenile Atlantic sturgeon were captured by bottom trawling using an 80' (23.4 m) otter trawl aboard the RV 'Seawolf' within juvenile/immature ocean habitat off Sandy Hook, NJ, Jones Beach, NY, and Rockaway Beach, NY and previously described in Fast *et al.* (2009). Briefly, 10–20 min tows were conducted during four trips from 2007–2008, each one consisting of several days, beginning on the following dates: October 16, 2007; November 15, 2007; September 15, 2008; and November 21, 2008. All Atlantic sturgeon collected were immediately sampled or placed in live wells for 0–60 min prior to sampling. Blood samples were collected first to minimize alterations in blood chemistry, followed by measurement of weight and length, collection of ectoparasites (10% neutral-buffered formalin) and taking of gill biopsy samples. Following these procedures and tagging, fish were released (15–20 min procedure).

Blood and serum collection

To minimize any manipulation induced changes in serum chemistry, blood samples were collected shortly after fish were brought aboard the vessel, prior to all other examinations. Blood samples were taken from the caudal vein (ca. 1–5 mL) with a 5 cc syringe and 18 G-1 needles. Blood (ca. 1–3 mL) was aliquoted into one or two 1.5 mL microcentrifuge tubes and placed on ice to clot. The remainder of the blood (2 mL) was placed into a 5 mL vacutainer containing 10 000 units of sodium heparin and placed on ice for later blood cell analysis.

Serum analysis

For serum analysis, blood was allowed to clot, on ice, for up to 6 h and the transparent liquid fraction (i.e. the serum) collected after a 10–20 min centrifuge at 14 000 ***g***. Serum samples were immediately frozen at −80 °C until analysed. Serum samples (alkaline phosphatase, AP) were submitted to the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, and all other serum analyses results have been reported elsewhere (Fast *et al.* 2009).

Flow cytometry analysis

Blood samples for the flow cytometry analysis were collected in September 2008 off Jones Beach, NY (n = 11) and in November 2008 off Rockaway Beach, NY (n = 12) and Sandy Hook, NJ (n = 13). The samples were first processed to remove as many of the red blood cells as possible. Three hundred microlitres of the uncoagulated blood was added to 900 μL of cell lysis solution from Promega's Wizard®Genomic DNA Purification Kit. Following the kit protocol, the solution was inverted 5–6 times to mix and allowed to incubate at room temperature for 10 min inverting 2–3 times once during the incubation. After incubation, the mixture was centrifuged for 30 s at 14 000 ***g***. The supernatant was removed by pipette, and the pellet at the bottom, containing mainly the remaining white blood cells, was resuspended by adding 300 μL of 10% neutral-buffered formalin and

flicking the tube (i.e. rapidly and repeatedly tapping the bottom of the tube with a finger) until the pellet was resuspended. The samples were kept refrigerated at 2–4 °C until analysis. At the time of analysis, the formalin-fixed blood samples were resuspended and 100 μL of the blood cell-formalin mixture was added to 2.5 mL of phosphate buffered saline and gently mixed. Flow cytometric analyses were performed using a Becton Dickinson FACSCalibur flow cytometer and sorter equipped with a 488 nm laser. Filtered (0.22 μm) sea water was used as sheath fluid. Forward light scatter (FS) and log side scatter (SS) signals were collected for at least 10 000 particles from each individual. Differential blood cell counts, expressed as a percentage of the total, were assessed based on cell size (FS) and cell complexity (SS). Discernible subpopulations were gated electronically using bitmaps, and the ratio of the cells within each bitmap to the whole cell population was calculated (Fig. 1). The identity of each cell type was microscopically confirmed in representative samples following physical sorting.

Blood smear preparation and analysis

Blood smears were prepared by placing a drop of the un-coagulated blood on a microscope slide and spreading the drop of blood across the slide with another clean microscope slide. The smears were allowed to dry at room temperature for 1–2 h prior to fixation and staining using a Wright's Giemsa staining method. Leucocytes ($n \geq 100$) were counted in triplicate (i.e. three different areas of the same blood smear microscope slide) on a subsample of fish ($n = 18$) to compare the white blood cell (WBC) differential counts from the smears to the WBC differential counts from the flow cytometry analysis. The blood smears were viewed using a Nikon ECLIPSE E200 light microscope at 1000× magnification with oil immersion.

Photographs of the leucocytes and thrombocytes ($n = 100$ each) were taken using an Insight digital camera (Diagnostic Instruments Inc.) attached to the light microscope, processed by Spot Advanced software version 3.5 (Diagnostic Instruments Inc.), and measured for length, width and area using ImagePro Plus® software version 6.0 (Media Cybernetics Inc.).

Gill measurement of Na^+-K^+-ATPase

Gill samples were collected, homogenized and analysed using a modification of methods described by McCormick (1993). Owing to sampling restrictions, this assay was only carried out on fish sampled in 2007, off Rockaway Beach, NY ($n = 9$) and Jones Beach, NY ($n = 4$) in October 2007 and off Rockaway Beach ($n = 11$) in November. Briefly,

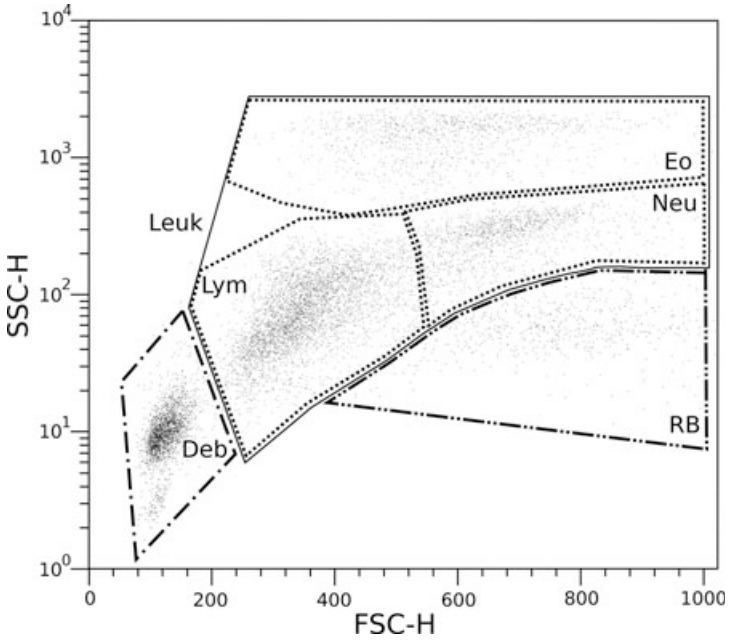

Figure 1 Example of a flow cytometry graph in which the x-axis (FSC-H) is the forward light scatter (i.e. cell size) and the y-axis (SSC-H) is the side scatter (i.e. cell complexity) on a log scale. Discernable subpopulations of leucocytes (- - - - -) were gated electronically using bitmaps within the leucocyte gate (Leuk,——) for eosinophilic granulocytes (Eo), lymphocytes (Lym) and neutrophilic granulocytes (Neu). The red blood cells (RB,—·—·—) and debris (Deb,— - —) gates are also included for completeness.

4–5 partial filaments were trimmed from the 1st gill arch of the left branchial chamber using clean, alcohol-dipped and flame-sterilized scissors and forceps for each fish and immediately frozen in the field on dry ice for later processing at the lab. An assay mixture of solution A (4 U lactate dehydrogenase, 5 U pyruvate kinase, 2.8 mM phosphoenolpyruvate, 0.7 mM ATP, 0.22 mM NADH and 50 mM imidazole, pH 7.5) and a mixture of solution B (sol A + 0.5 mM ouabain) were mixed separately with a salt solution (189 mM NaCl, 10.5 mM $MgCl_2$, 42 mM KCL and 50 mM imidazole, pH 7.5) in a 3:1 ratio (150:50 μL) and kept on ice. Just before use (2–3 min), the assay mixture was placed in a 25 °C water bath. Samples were then thawed on ice and immersed in 4 mL SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and 1 mL SEID buffer (0.5 g of sodium deoxycholate in 100 mL SEI) and were homogenized using a polytron homogenizer for 1–2 min. This mixture was then centrifuged at 5000 ***g*** for 30 s to remove insoluble material and 10 μL of homogenate added to each of four wells of a 96-well microplate on ice (remaining homogenate used for protein determination). Into duplicate wells for each sample, 200 μL of solution A + salt solution was added and 200 μL of solution B + salt solution added to the other two. The plate was placed in a temperature-controlled microplate, and the linear rate of NADH disappearance at 340 nm for 10 min was measured. The linear rate from 2–10 min was determined for each duplicate (after stabilization of NADH reduction of 1–2 min), and Na^+-K^+-ATPase activity was calculated as the difference in ATP hydrolysis in the absence/presence of ouabain (expressed as μM ADP mg^{-1} of protein h^{-1}). A standard curve was run with each plate (0–20 nmol ADP per well).

Statistical analysis

All data were analysed using the program R (R Development Core Team 2010). To incorporate categorical and continuous explanatory variables and non-normally distributed data, generalized linear models (GLM) with a gamma error distribution and inverse link function were used to analyse the alkaline phosphatase and the flow cytometry eosinophilic and neutrophilic granulocyte data. The flow cytometry lymphocyte and Na^+-K^+-ATPase activity GLM's used a normal error distribution and identity link function. The Na^+-K^+-ATPase data were square-root transformed prior to analysis to normalize the data and stabilize the variance. The 'full' model contains all the variables for which there were data. The 'full' model for the alkaline phosphatase data consisted of the following explanatory variables: number of adult female copepods (AFE), AFE^2, AFE^3, number of adult male and juvenile copepods (OC), OC^2, OC^3 along with the location, month and year the fish were caught and the fish weight, fork length and condition factor. Year was not included in the 'full' model for the Na^+-K^+-ATPase and leucocyte data, because both were only sampled in 1 year. Month was excluded from the 'full' model of the leucocyte data because the sites were only sampled in 1 month (September for Jones Beach and November for Rockaway Beach and Sandy Hook). Location was excluded from the 'full' model of the Na^+-K^+-ATPase data because only two sites were sampled and one of them, Jones Beach, had a small sample size (n = 4). The variables AFE, AFE^2, AFE^3 and OC, OC^2, OC^3 were standardized about their means to remove the increased influence that higher order terms would have on a model and to facilitate the direct comparison of the terms of any order. Model simplification and selection for the 'best' model was accomplished using the 'step' function in R to minimize Akaike's information criteria (AIC), which is equal to $-2 \times$ log-likelihood + $2 \times$ parameters (Akaike 1974). Akaike's information criteria is used to compare nested models, which takes into account the number of parameters in the models, so if two models fit the data equally well, the one with less parameters will have a lower AIC score and the more parsimonious model would be selected (Faraway 2006). To assess how well the 'best' model fits the data, the 'best' model was compared with the 'null' model, which only has an intercept term and no predictor variables (Faraway 2006). This was accomplished using the 'anova.glm' function in R, which was also used to compare the 'best' and 'full' models. It should be noted that in using the 'anova.glm' function in R, this function is calculating a F-statistic to assess any significant differences and the F-statistic has an exact F distribution for the normally distributed data, but the F-statistic is only approximately F distributed for the gamma distributed data (Faraway 2006). The correlation coefficient (r) was used to compare the WBC differential counts of the flow cytometry data to the blood smear data, and significance was assessed using normal linear regression. In the text,

the following terms were used to denote the level of statistical significance: significant ($P \leq 0.05$), very significant ($P \leq 0.01$) and highly significant ($P \leq 0.001$).

Results

Gill Na^+-K^+-ATPase activity

The overall mean and standard error of the mean for Na^+-K^+-ATPase activity was 3.31 ± 0.36 μM ADP mg^{-1} of protein h^{-1}. The 'best' model for Na^+-K^+-ATPase activity was one that included OC, OC^2, OC^3 and AFE^2 with only OC being significant (Table 1). The sum of the OC terms and the AFE^2 term are both positive, indicating that as the number of copepods (juveniles, adults and both sexes) increases so does Na^+-K^+-ATPase activity. The 'best' model is not significantly different from either the 'null' or the 'full' model.

Serum alkaline phosphatase

The overall mean and standard deviation for serum alkaline phosphatase (AP) was 21.21 ± 7.93 UL^{-1}. The AP 'best' model included AFE, AFE^2, AFE^3, month, year and weight with all but AFE^3 showing some level of statistical significance (Table 1). The weight of the fish affected AP serum levels the most with AP decreasing as the weight of the fish increased. The total effect of the summed AFE terms shows AP increasing as AFE increased. The years were highly significantly different from one another with AP higher on average in 2007 than in 2008. The months of October and November were highly significantly different from September but not from each other, with September being highest in AP and November being lowest. The 'best' model fitted the data well and was highly significantly different when compared with the 'null' model, but not compared with the 'full' model.

Flow cytometry analysis

All of the white blood cells seem to be affected by the presence of *D. oblongum* (Table 2). Eosinophilic granulocytes 'best' model included OC^2, AFE^2, AFE^3 and fork length. Fork length was the only term that was significant and as fork length increases so do the eosinophilic granulocytes. None of the copepod terms in the model are significant, but their effects are contradictory with the sum of AFE terms increasing eosinophilic granulocytes and OC^2 decreasing the cells. The 'best' model fitted the data well and was very significantly different from the 'null' model but not from the 'full' model.

Neutrophilic granulocytes 'best' model only included AFE^3 and location (Table 2). The effects of AFE^3 were very significant and positively related to the neutrophilic granulocytes. Rockaway Beach, NY,

Table 1 Gill Na^+-K^+-ATPase activity (a) and serum alkaline phosphatase (b) 'best' generalized linear model (GLM) results. The Gill Na^+-K^+-ATPase activity GLM used a normal error distribution with an identity link function, while the serum alkaline phosphatase model used a gamma error distribution with an inverse link function. In addition to standard model results and summary statistics, the table includes the 'best' models Akaike information criteria (AIC) and residual deviance (degrees of freedom). To assess model fit, the residual deviances (degrees of freedom) are given for the 'null' and 'full' models along with the *P*-values (degrees of freedom) for the model comparisons between the 'best' and 'null' models and the 'best' and 'full' models (see methods for details)

Coefficients	Estimate	Std. Error	*t* value	Pr(>|*t*|)	AIC	'Best' residual deviance	'Null' deviance	'Full' residual deviance	'Best' vs. 'null' Pr(>F)	'Best' vs. 'full' Pr(>F)
(a) GILL Na^+-K^+-ATPase 'best' GLM										
Intercept	1.732	0.102	16.910	<0.001***	41.39	4.781 (19)	7.444 (23)	3.936 (13)	0.066 (4)	0.822 (6)
OC	−1.883	0.862	−2.185	0.042*						
OC^2	4.520	2.205	2.050	0.055						
OC^3	−2.601	1.457	−1.786	0.09						
AFE^2	0.230	0.111	2.071	0.052						
(b) Serum alkaline phosphatase 'best' GLM										
Intercept	0.030	0.004	7.456	<0.001***	443.05	3.883 (64)	9.507 (71)	3.701 (57)	<0.001*** (7)	0.899 (7)
AFE	−0.022	0.007	−2.998	0.004**						
AFE^2	0.040	0.018	2.302	0.025*						
AFE^3	−0.020	0.012	−1.702	0.094						
October	0.029	0.006	5.187	<0.001***						
November	0.034	0.004	7.895	<0.001***						
2008	0.024	0.004	5.640	<0.001***						
Weight	0.001	0.000	2.197	0.032*						

Statistical significance levels (Pr(>|*t*|) or Pr(>F)) are denoted at the 0.05(*), 0.01(**) and 0.001(***) levels, respectively.

Table 2 Eosinophilic granulocyte (a), neutrophilic granulocyte (b) and lymphocyte (c) 'best' generalized linear model (GLM) results. The eosinophilic granulocyte and neutrophilic granulocyte GLM's used a gamma error distribution with an inverse link function, while the lymphocyte GLM used a normal error distribution with an identity link function. The results are presented in the same manner as Table 1

Coefficients:	Estimate	Std. Error	*t* value	Pr(>|*t*|)	AIC	'Best' residual deviance	'Null' deviance	'Full' residual deviance	'Best' vs. 'null' Pr(>F)	'Best' vs. 'full' Pr(>F)
(a) Eosinophilic granulocyte 'best' GLM										
Intercept	0.239	0.049	4.841	<0.001***	181.51	3.006 (31)	4.799 (35)	2.631 (24)	0.005** (4)	0.842 (7)
OC^2	0.009	0.007	1.434	0.162						
AFE^2	−0.051	0.025	−2.000	0.054						
AFE^3	0.041	0.024	1.701	0.099						
Fork Length	−0.001	0.001	−2.680	0.012*						
(b) Neutrophilic granulocyte 'best' GLM										
Intercept	0.047	0.002	20.923	<0.001***	192.74	0.934 (32)	1.530 (35)	0.872 (24)	0.001*** (3)	0.985 (8)
AFE^3	−0.004	0.001	−3.561	0.001***						
Jones Beach	0.008	0.004	2.280	0.029*						
Rockaway Beach	0.010	0.004	2.749	0.009**						
(c) Lymphocyte 'best' GLM										
Intercept	72.433	2.311	31.342	<0.001***	221.43	708.5 (31)	1347.7 (35)	638.49 (24)	0.0004*** (4)	0.907 (7)
AFE^3	−3.243	0.906	−3.579	0.001***						
Jones Beach	3.057	2.078	1.471	0.151						
Rockaway Beach	3.580	1.920	1.864	0.072						
Weight	−0.466	0.295	−1.581	0.124						

was very significantly different and Jones Beach, NY, was significantly different from Sandy Hook, NJ. Sandy Hook, NJ has the highest proportion of neutrophilic granulocytes and Rockaway Beach, NY, had the lowest. The 'best' model fitted the data well and was highly significantly different from the 'null' model but not from the 'full' model.

The lymphocyte data suffered from being skewed to the left and any attempts to normalize the data increased the violation of equal variances. As such the 'best' model does not fit the data well and any conclusions from the model would be spurious at best (Table 2). With that said the 'best' model included AFE^3, which was highly significant, and location and weight, neither of which were significant. The best model was highly significantly different from the 'null' model, but not the 'full' model. Neither the 'null' nor the 'full' model fitted the data well.

Blood smear and flow cytometry data comparison

White blood cell (WBC) differential counts, obtained from the triplicate blood smear counts, were highly variable for the eosinophilic and neutrophilic granulocytes with a mean coefficient of variation (MCV) of 28.2% (±0.144 SD) and 24.7% (±0.164 SD), respectively, but not for the lymphocytes (MCV = 6.3%, ±0.032 SD). Even though the WBC differential counts from the same slide were highly variable, the overall averages between the microscope and flow cytometry WBC differential counts for the samples were not statistically significantly different (Table 3). Comparing the smear and flow cytometry WBC differential counts (Table 4), there was a highly significant strong correlation for the eosinophilic granulocytes, a significant moderate correlation for the lymphocytes and no significant correlation for the neutrophilic granulocytes (Table 4). Pair-wise comparison of all the smear and flow cytometry WBC differential counts shows there was a very significant moderate negative correlation between the flow cytometry lymphocytes compared with the microscope eosinophilic granulocytes and vice-versa. The microscopic confirmation on the sorted cell fractions rarely found any lymphocytes in either of the granulocyte fractions or either granulocyte in the lymphocytic fraction suggesting that these negative correlations are just an artefact of comparing two proportional data sets. To varying degrees within the samples, there was some amount of unlysed and 'ghost' (cells with the cell membrane and no nucleus) red blood cells that were found in the lymphocyte sorted fraction. When the samples with the most red blood cell contamination were removed and the analysis was rerun (Table 4, $n = 12$), the lymphocyte comparison became very significant with a strong correlation and the eosinophilic granulocyte comparison had a very significant and still strong

Table 3 Mean (±standard deviation) flow cytometry and microscope white blood cell differential counts as a percentage of total white blood cells

	Percentage lymphocyte	Percentage neutrophilic granulocyte	Percentage eosinophilic granulocyte
Flow cytometry	72.31 ± 7.11	18.29 ± 3.88	9.41 ± 4.07
Microscope	72.71 ± 5.33	16.43 ± 4.07	10.85 ± 3.76

correlation, whereas the neutrophilic granulocyte comparison remained non-significant. Similar to before, there was a very significant but now strongly negative correlation between the flow cytometry eosinophilic granulocytes/microscope lymphocytes and a significant moderate negative correlation between the flow cytometry lymphocytes/microscope eosinophilic granulocytes.

Finally, it should be noted that all thrombocytes appear to have been lysed by the red blood cell lysis solution. There were no intact or 'ghost' thrombocytes seen in the samples after processing, and thus, thrombocytes were not included in the flow cytometry or microscopic count analysis.

Sturgeon white blood cell and thrombocyte comparison

Atlantic sturgeon white blood cells and thrombocytes are shown in Fig. 2. In terms of total cell size, as measured by area, the eosinophilic granulocytes were the largest followed by neutrophilic granulocytes, thrombocytes and lymphocytes (Table 5). All pair-wise comparisons (Wilcoxon two-sample test with Bonferroni correction) between cell types were statistically significantly different ($P < 0.05$) except the lengths between eosinophilic granulocytes and thrombocytes and the widths between lymphocytes and thrombocytes (data not shown).

Discussion

To our knowledge, this is the first study to look at the levels of gill Na^+-K^+-ATPase activity, serum alkaline phosphatase and leucocyte differentials in marine stage *A. o. oxyrinchus*. The overall mean of *A. o. oxyrinchus* gill Na^+-K^+-ATPase activity was slightly lower than the shortnose sturgeon, *Acipenser brevirostrum* (Lesueur), at 20‰ (Jarvis & Ballantyne 2003) and well below the green sturgeon, *Acipenser medirostris* (Ayres), at 33‰ (Allen, Cech & Kültz 2009), two saltwater diadromous sturgeon for which there are data. In this study, gill Na^+-K^+-ATPase activity was shown to increase with an increasing number of *D. oblongum*. Post-smolt Atlantic salmon, *Salmo salar* L., have similarly shown significant increases in gill Na^+-K^+-ATPase activity when infected with *Lepeophtheirus salmonis*, with ten lice/fish at 5 and 10 days post-infection (Nolan, Reilly & Bonga 1999). Juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), exposed to, but not infected with, *L. salmonis* had gill Na^+-K^+-ATPase activity that was not significantly different from the unexposed group but did show a significant increase in activity after being stressed by

Table 4 Correlation coefficients (r) and their associated P-values (P) for flow cytometry-microscope white blood cell (WBC) differential counts (a) and flow cytometry-microscope WBC differential counts with red blood cell-contaminated samples removed (b); see results for details. Statistical significance levels are denoted at the $P < 0.05$(*), $P < 0.01$(**) and $P < 0.001$(***) levels, respectively

	Microscope eosinophilic granulocyte	Microscope neutrophilic granulocyte	Microscope lymphocyte
(a) Complete flow cytometry vs. WBC comparison (n = 18)			
Flow cytometry eosinophilic granulocyte	r = 0.801	r = 0.041	r = −0.597
	$P < 0.001$***	P = 0.870	P = 0.009**
Flow cytometry neutrophilic granulocyte	r = 0.388	r = 0.204	r = −0.429
	P = 0.111	P = 0.418	P = 0.075
Flow cytometry lymphocyte	r = −0.671	r = −0.135	r = 0.576
	P = 0.002**	P = 0.594	P = 0.012*
(b) Flow cytometry vs. WBC comparison with RBC contaminated samples removed (n = 12)			
Flow cytometry eosinophilic granulocyte	r = 0.782	r = 0.036	r = −0.797
	P = 0.003**	P = 0.911	P = 0.002**
Flow cytometry neutrophilic granulocyte	r = 0.313	r = 0.348	r = −0.562
	P = 0.322	P = 0.267	P = 0.057
Flow cytometry lymphocyte	r = −0.647	r = −0.183	r = 0.771
	P = 0.023*	P = 0.569	P = 0.003**

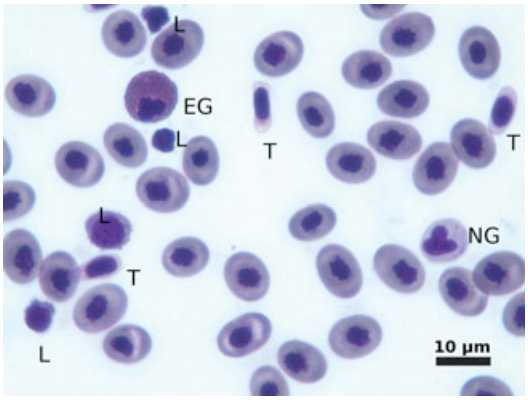

Figure 2 Sturgeon blood lymphocytes (L), neutrophilic granulocyte (NG), eosinophilic granulocyte (EG) and thrombocytes (T).

confinement (Nolan *et al.* 2000). Gill Na^+-K^+-ATPase activity can also be affected by changes in salinity as seen in the green sturgeon that had significant differences between < 3‰ and 10‰ and 33‰, with the lowest activity at < 3 ‰, highest at 10‰ and 33‰ being intermediate (Allen *et al.* 2009). In contrast, the shortnose sturgeon showed no significant differences in gill Na^+-K^+-ATPase activity at 0‰ and 20‰ (Jarvis & Ballantyne 2003). Krayushkina, Semenova & Vyushina (2006) showed in several sturgeon species that their gill Na^+-K^+-ATPase activity can fluctuate over several days when the sturgeon were moved from fresh water to brackish water (12.6‰). Unfortunately, it is not known how long the sturgeon that were caught were at their locations (bottom salinities 29.2–31.5‰) at the time of capture. The possibility cannot be excluded that some of the fish may have had a recent freshwater excursion, but as the sample variation in gill Na^+-K^+-ATPase activity was small, when compared to the studies listed earlier, this would not seem to be the case.

Alkaline phosphatase (AP) is used to evaluate liver function, specifically the flow of bile, but different isoforms are produced in bone, liver, kidney and intestinal tissues of vertebrates (Thrall *et al.* 2004) that are not differentiated by the AP test, and this needs to be kept in mind when interpreting results. Weight was the variable that affected AP levels the most with AP decreasing as weight increased.

A decrease in AP was seen from juvenile to adult female Persian sturgeon, *Acipenser persicus* (Borodin), but increased between juvenile to adult males (Asadi *et al.* 2006). In this study, weight was highly positively correlated with fork length (Spearman's rho = 0.9838, $P < 2.2 \times 10^{-16}$) suggesting that AP decreases with *A. o. oxyrinchus* age, but the possible contributions to the level of AP due specifically to intrinsic differences between sexes cannot be discounted as the sex of the fish was not assessed. The number of AFE's caused AP to increase suggesting that infection may be affecting liver function. However, the only liver enzyme (data from Fast *et al.* (2009)) to show a significant correlation with AP was aspartate aminotransferase (AST) (Spearman's rho = 0.472, $P = 2.887 \times 10^{-05}$), and AST is not specific to the liver but can also be released by damaged muscle tissue (Thrall *et al.* 2004). Furthermore, AST was significantly correlated with creatine kinase (Spearman's rho = 0.469, $P = 3.75 \times 10^{-05}$, data from Fast *et al.* (2009)), considered a leakage enzyme specific to muscles, making the increase in AP because of liver damage unlikely (Thrall *et al.* 2004). The reason that the AFE terms are retained in the 'best' model and causing an increase in AP may be due to the release of AP from the parasites as part of their feeding. For example, the cattle tick, *Boophilus microplus* (Canestrini), and the buffalo fly, *Haematobia irritans exigua* (de Meijere), produce AP in their salivary glands (Kerlin & Hughes 1992) and the cestode, *Echinococcus multilocularis* (Leuckart), produces a distinctly different AP than the hepatic enzyme (Sarciron *et al.* 1991; Lawton, Sarciron & Petavy 1995), and the parasite AP has been identified in their excretory ducts and tegument (Arsac *et al.* 1997). Furthermore, several studies on the increase in salmonid mucus AP associated with the parasitic copepod *L. salmonis* have suggested that some of the increase in AP may be due to the parasite itself (Ross *et al.* 2000; Fast *et al.* 2002, 2003) although no detailed study has been carried out to differentiate any different isozymes in the

Table 5 White blood cell and thrombocyte size comparison [mean ± standard deviation (coefficient of variation)]

	Eosinophilic granulocyte	Neutrophilic granulocyte	Lymphocyte	Thrombocyte
Length (µm)	11.755 ± 0.979 (8.33)	10.786 ± 1.095 (10.15)	6.755 ± 1.169 (17.30)	11.552 ± 1.716 (14.86)
Width (µm)	10.995 ± 0.947 (8.61)	9.853 ± 0.967 (9.81)	5.814 ± 0.992 (17.09)	5.655 ± 0.919 (16.27)
Area (μm^2)	96.123 ± 15.702 (16.34)	80.991 ± 15.469 (19.10)	29.819 ± 10.025 (33.62)	50.062 ± 11.655 (23.28)

mucus associated with the copepod infections. The temporal variation in AP is difficult to assess because months (nor sites) were sampled in each year (i.e. incomplete sample design), but the variation may result from the combination of variations in weights and number of AFE both of which fluctuate amongst months and between sites (data not shown). The overall AP from the Atlantic sturgeon was much lower than in juvenile shortnose sturgeon (Knowles *et al.* 2006), mature male and female starry sturgeon, *Acipenser stellatus* (Pallas), (Shahsavani, Mohri & Kanani 2010), juvenile and adult, male and female Persian sturgeon (Asadi *et al.* 2006) and 6-month-old Amur, *Acipenser schrenckii* (Brandt),and Chinese, *Acipenser sinensis* (Gray), (Shi *et al.* 2006) sturgeon.

In vertebrates, the role of lymphocytes, particularly T helper 2 cells, (Díaz & Allen 2007) and eosinophilic granulocytes (Klion & Nutman 2004; Cadman & Lawrence 2010) have long been known to be a part of the host immune system response to metazoan parasitic infections, whereas the role of neutrophilic granulocytes in metazoan parasitic infections is still emerging (Cadman & Lawrence 2010). In this study, percentage eosinophilic and percentage neutrophilic granulocytes increased with increasing copepods while percentage lymphocytes decreased relative to the other white blood cells. The eosinophilic granulocytes 'best' model was the only leucocyte GLM to retain one of the OC variables (OC^2), which includes the adult male and juvenile copepod stages, although this variable was non-significant. Fast *et al.* (2009) did not note any white blood cells in histological sections associated with juvenile stages of *D. oblongum*. This lack of leucocyte infiltration does not exclude the possibility of the juvenile copepods causing a cellular immune response. Nolan *et al.* (1999) showed, in Atlantic salmon, that heavy leucocyte infiltration can occur far away from the site of *L. salmonis* infections and that this leucocyte reaction was similar to stress responses in general for salmon. Although white blood cell counts were not evaluated by Nolan *et al.* (1999), the increase in leucocytes far away from the site of infection would presumably be in part due to the movement of leucocytes from the blood into target organs (i.e. skin) and a similar mechanism could be the reason why the OC^2 variable was retained in the eosinophilic granulocyte GLM and why this variable would be associated with a decrease in circulating eosinophilic granulocytes.

All three leucocyte GLM's retained at least one higher order term for the AFE variable with the neutrophilic granulocyte and lymphocyte AFE^3 variable being highly significant, while the eosinophilic granulocyte AFE^2 variable was barely insignificant. At the AFE site of infections, the back of the sturgeon opercular cavity, Fast *et al.* (2009) described multifocal petechial haemorrhaging associated with the AFE and also noted that these females lacked swimming appendages, suggesting restrictive movements. This, taken together with the current leucocyte results, suggests that petechial haemorrhaging is because of the abandoned feeding sites of the few adult females currently infecting the host rather than the remnants of a more intense infection, although indirect pathologies associated with AFE feeding cannot be ruled out. Eosinophilic granulocytes are typically found at the primary site of metazoan parasite infections (Klion & Nutman 2004; Cadman & Lawrence 2010), and the authors have also noted that eosinophilic granulocytes infiltrate areas of *A. o. oxyrinchus* gills associated with *Nitzschia sturionis* (Abildgaard) infections (unpublished data). If the eosinophilic granulocytes are infiltrating recent feeding sites, while the neutrophilic granulocytes are involved in repairing tissues at older sites of infection, and the lymphocytes are involved in some aspect of all sites (i.e. regulating eosinophils and neutrophils via Th2 and Th17 (Díaz & Allen 2007)), one would expect the later two leucocytes to have a greater non-linear relationship with the parasites than the eosinophils, which is what is seen in our model results. Histological studies that included T-cell differentiation (ie. Th2 or Th17) of current and abandoned infection sites would give us a greater understanding of how the host cellular immune system and the parasite's feeding biology interact.

Although there are numerous studies on the leucocyte infiltrates associated with parasitic infections of fish, there are few studies involving peripheral blood leucocytes associated with these infections and findings have often been variable between host and parasitic species (Mackinnon 1993; Densmore *et al.* 2001; Blanar, Curtis & Chan 2006; Sabri *et al.* 2009; Witeska, Kondera & Ługowska 2010). In studies in which multiple parasitic infections were accessed, such as in *Schizothorax* spp. and *Cyprinus* spp. (Shah *et al.* 2009), and common snook, *Centropomus undecimalis* (Bloch) (Fujimoto *et al.* 2009), all fish had non-significant changes in WBC differential counts in which eosinophilic and neutr-

ophilic granulocytes increased while lymphocytes decreased in infected fish when compared to healthy fish. These data are similar to the current study. White blood cell differential counts may also change as part of the natural fluctuation as sturgeon age, as has been seen in Persian and European, *Huso huso* (L.), sturgeon (Bahmani, Kazemi & Donskaya 2001). Although this is a subset of samples compared with AP (see methods), fork length and weight were again highly correlated (Spearman's rho = 0.987, $P < 2.2 \times 10^{-16}$) so the retention of one variable over the other in the leucocyte GLM's is again trivial as both variables are related to overall size. Another possible reason for their retention is that fork length and weight were moderately positively but very significantly correlated with AFE (fork length Spearman's rho = 0.462, $P = 0.0046$; weight Spearman's rho = 0.426, $P = 0.0095$) suggesting some covariation between increasing fish size and AFE infection intensities in this subset of data that were not seen in the overall data set used by Fast *et al.* (2009).

The increase in lymphocytes and decrease in neutrophilic granulocytes at Jones Beach and Rockaway Beach compared with Sandy Hook was not because of significant difference in infection level amongst sites in this data subset (data not shown). Fast *et al.* (2009) did find significant differences in infection level across sites of the whole data set, suggesting that the changes in neutrophilic granulocytes and lymphocytes are due specifically to site differences. Whether or not these differences in WBC differential counts are because of anthropogenic factors as suggested for some of the serum chemistry parameters studied by Fast *et al.* (2009) remains to be investigated.

The overall mean WBC differential counts from the blood smears and flow cytometry analyses were not significantly different while two of the three blood smear/flow cytometry comparisons showed a significant correlation in WBC differential counts. The correlation for the lymphocytes became stronger when the samples that showed incomplete removal of the red blood cells were removed from the analysis. This suggests that although the new method for preparing white blood cells in the field for future flow cytometry analysis is adequate, there is still a need to improve this method, like increasing the amount of red blood cell lysis solution compared with the total amount of blood taken to more completely lyse and remove the red blood cells.

The Atlantic sturgeon's blood cell sizes are substantially smaller, in terms of area, when compared to previously published studies of the Siberian surgeon, *Acipenser baerii* (Brandt), starry sturgeon and European sturgeon (Palíková, Mareš & Jirásek 1999) and the Chinese sturgeon (Gao *et al.* 2007), except for the thrombocytes that are comparable to most of the sturgeon listed earlier.

In summary, *Dichelesthium oblongum* has been shown to affect, in a non-linear manner, Atlantic sturgeon gill Na^+-K^+-ATPase activity and alkaline phosphatase level along with increasing the percentages of eosinophilic and neutrophilic granulocytes while decreasing lymphocyte percentages. Being able to take into account confounding variables, such as differences in fish size along with the location and time of their sampling, through the use of generalized linear models allows researchers to gain insight into host–parasite systems. The new method of leucocyte preservation in the field for flow cytometry analysis has proven itself adequate in the current study.

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References

Akaike H. (1974) New look at statistical-model identification. *Ieee Transactions on Automatic Control* **AC19,** 716–723.

Allen P.J., Cech J.J. & Kültz D. (2009) Mechanisms of seawater acclimation in a primitive, anadromous fish, the green sturgeon. *Journal of Comparative Physiology B: Biochemical Systemic and Environmental Physiology* **179,** 903–920.

Arsac C., Walbaum S., Sarciron M.E. & Petavy A.F. (1997) Histochemical observations of alkaline phosphatase activity of

Echinococcus multilocularis during *in vivo* development in golden hamsters, an alternative definitive host. *Experimental Animals* **46,** 25–30.

Asadi F., Masoudifard M., Vajhi A., Lee K., Pourkabir M. & Khazraeinia P. (2006) Serum biochemical parameters of *Acipenser persicus*. *Fish Physiology and Biochemistry* **32,** 43–47.

Bahmani M., Kazemi R. & Donskaya P. (2001) A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiology and Biochemistry* **24,** 135–140.

Blanar C.A., Curtis M.A. & Chan H.M. (2006) Growth, nutritional composition, and hematology of Arctic charr (*Salvelinus alpinus*) exposed to toxaphene and tapeworm (*Diphyllobothrium dendriticum*) larvae. *Archives of Environmental Contamination and Toxicology* **48,** 397–404.

Bonga S.E.W. (1997) The stress response in fish. *Physiological Reviews* **77,** 591–625.

Boreman J. (1997) Sensitivity of North American sturgeons and paddlefish to fishing mortality. *Environmental Biology of Fishes* **48,** 399–406.

Cadman E. & Lawrence R. (2010) Granulocytes: effector cells or immunomodulators in the immune response to helminth infection? *Parasite Immunology* **32,** 1–19.

Densmore C.L., Blazer V.S., Waldrop T.B. & Pooler P.S. (2001) Effects of whirling disease on selected hematological parameters in rainbow trout. *Journal of Wildlife Diseases* **37,** 375–378.

Díaz A. & Allen J.E. (2007) Mapping immune response profiles: the emerging scenario from helminth immunology. *European Journal of Immunology* **37,** 3319–3326.

Dunton K.J., Jordaan A., Mckown K.A., Conover D.O. & Frisk M.G. (2010) Abundance and distribution of Atlantic sturgeon (*Acipenser oxyrinchus*) within the Northwest Atlantic Ocean, determined from five fishery-independent surveys. *Fishery Bulletin* **108,** 450–465.

Faraway J.J. (2006) *Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models*. Chapman and Hall/CRC, Boca Raton.

Fast M.D., Ross N.W., Mustafa A., Sims D.E., Johnson S.C., Conboy G.A., Speare D.J., Johnson G. & Burka J.F. (2002) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of Aquatic Organisms* **52,** 57–68.

Fast M.D., Burka J.F., Johnson S.C. & Ross N.W. (2003) Enzymes released from *Lepeophtheirus salmonis* in response to mucus from different salmonids. *Journal of Parasitology* **89,** 7–13.

Fast M.D., Sokolowski M.S., Dunton K.J. & Bowser P.R. (2009) *Dichelesthium oblongum* (Copepoda: Dichelesthiidae) infestation in wild-caught Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*. *ICES Journal of Marine Science* **66,** 2141–2147.

Fujimoto R.Y., Santana C.A., De Carvalho W.L.C., Diniz D.G., De Barros Z.M.N., Varella J.E.D. & Guimarães M.D.F. (2009) Haematology and metazoan parasites of camurim (*Centropomus undecimalis*, Bloch, 1792) captured in Bragnanca, PA, Brazil. *Boletim Do Instituto De Pesca* **35,** 441–450.

Gao Z.X., Wang W.M., Yang Y., Abbas K., Li D.P., Zou G.W. & Diana J.S. (2007) Morphological studies of peripheral blood cells of the Chinese sturgeon, *Acipenser sinensis*. *Fish Physiology and Biochemistry* **33,** 213–222.

Grunwald C., Maceda L., Waldman J., Stabile J. & Wirgin I.I. (2008) Conservation of Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus*: delineation of stock structure and distinct population segments. *Conservation Genetics* **9,** 1111–1124.

Jarvis P.L. & Ballantyne J.S. (2003) Metabolic responses to salinity acclimation in juvenile shortnose sturgeon *Acipenser brevirostrum*. *Aquaculture* **219,** 891–909.

Kerlin R.L. & Hughes S. (1992) Enzymes in saliva from 4 parasitic arthropods. *Medical and Veterinary Entomology* **6,** 121–126.

Klion A. & Nutman T. (2004) The role of eosinophils in host defense against helminth parasites. *Journal of Allergy and Clinical Immunology* **113,** 8.

Knowles S., Hrubec T.C., Smith S.A. & Bakal R.S. (2006) Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). *Veterinary Clinical Pathology* **35,** 434–440.

Krayushkina L.S., Semenova O.G. & Vyushina A.V. (2006) Level of serum cortisol and Na+/K+ ATP-ase activity of gills and kidneys in different acipenserids. *Journal of Applied Ichthyology* **22,** 182–187.

Lawton P., Sarciron M.E. & Petavy A.F. (1995) *Echinococcus granulosus*, *E. multilocularis* and mammalian liver-type alkaline phosphatases: a comparative study. *Comparative Biochemistry and Physiology B: Biochemistry & Molecular Biology* **112,** 295–301.

Mackinnon B.M. (1993) Host response of Atlantic salmon (*Salmo salar*) to infection by sea lice (*Caligus elongatus*). *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 789–792.

McCormick S.D. (1993) Methods for the nonlethal gill biopsy and measurements of Na+, K+-ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 656–658.

Nolan D.T., Reilly P. & Bonga S.E.W. (1999) Infection with low numbers of the sea louse *Lepeophtheirus salmonis* induces stress-related effects in postsmolt Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **56,** 947–959.

Nolan D.T., Ruane N.M., Van Der Heijden Y., Quabius E.S., Costelloe J. & Bonga S.E.W. (2000) Juvenile *Lepeophtheirus salmonis* (Krøyer) affect the skin and gills of rainbow trout *Oncorhynchus mykiss* (Walbaum) and the host response to a handling procedure. *Aquaculture Research* **31,** 823–833.

Palíková M., Mareš J. & Jirásek J. (1999) Characteristics of leukocytes and thrombocytes of selected sturgeon species from intensive breeding. *Acta Veterinaria Brno* **68,** 259–264.

Pickering A.D. & Duston A.J. (1983) Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to saprolegnia infection and furunculosis. *Journal of Fish Biology* **23,** 163–175.

Pickering A.D. & Pottinger T.G. (1989) Stress response and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry* **4,** 253–258.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ross N.W., Firth K.J., Wang A.P., Burka J.F. & Johnson S.C. (2000) Changes in hydrolytic enzyme activities of naive Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. *Diseases of Aquatic Organisms* **41,** 43–51.

Sabri D.M., El-Danasoury M.A.E.-H., Eissa I.A.E.-M. & Khouraiba H.M. (2009) Impact of henneguyosis infestation on hematological parameters of catfish (*Clarias garipienus*). *International Journal of Agriculture & Biology* **11,** 228–230.

Sarciron M.E., Hamoud W., Azzar G. & Petavy A.F. (1991) Alkaline phosphatase from *Echinococcus multilocularis*: purification and characterization. *Comparative Biochemistry and Physiology B: Biochemistry & Molecular Biology* **100,** 253–258.

Shah A.W., Parveen M., Mir S.H., Sarwar S.G. & Yousuf A.R. (2009) Impact of helminth parasitism on fish haematology of Anchar Lake, Kashmir. *Pakistan Journal of Nutrition* **8,** 42–45.

Shahsavani D., Mohri M. & Kanani H.G. (2010) Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. *Fish Physiology and Biochemistry* **36,** 39–43.

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

Thrall M.A., Baker D.C., Campbell T.W., Denicola D., Fettman M.J., Lassen E.D., Rebar A. & Weiser G. (2004) *Veterinary Hematology and Clinical Chemistry*. Lippincott Williams & Wilkins, Philadelphia.

Van Eenennaam J.P. & Doroshov S.I. (1998) Effects of age and body size on gonadal development of Atlantic sturgeon. *Journal of Fish Biology* **53,** 624–637.

Waldman J.R., Hart J.T. & Wirgin I.I. (1996) Stock composition of the New York Bight Atlantic sturgeon fishery based on analysis of mitochondrial DNA. *Transactions of the American Fisheries Society* **125,** 364–371.

Witeska M., Kondera E. & Ługowska K. (2010) The effects of ichthyophthiriasis on some haematological parameters in common carp. *Turkish Journal of Veterinary and Animal Sciences* **34,** 267–271.

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