

Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood parameters in rockfish

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Objective—To compare values of blood parameters in rockfish obtained by use of a point-of-care portable blood analyzer with values determined by a veterinary diagnostic laboratory, calculate reference ranges for various blood parameters in black rockfish, and compare values of blood parameters in clinically normal fish with those of fish with clinical abnormalities.

Design—Prospective study.

Animals—41 captive adult black rockfish (*Sebastes melanops*) and 4 captive adult blue rockfish (*Sebastes mystinus*).

Procedure—Rockfish were anesthetized with tricaine methanesulfonate for collection of blood samples. Heparinized blood samples were immediately analyzed with a point-of-care analyzer. Blood sodium, potassium, chloride, urea nitrogen, and glucose concentrations; Hct; pH; partial pressure of carbon dioxide; total carbon dioxide concentration; bicarbonate concentration; base excess; and hemoglobin concentration were determined. A microhematocrit technique was used to determine PCV, and a refractometer was used to estimate total plasma protein concentration. Paired heparinized blood samples were transported to a veterinary diagnostic laboratory for analyses.

Results—Data obtained with the point-of-care analyzer were reproducible; however, values for most blood parameters were significantly different from those obtained by the veterinary diagnostic laboratory. Fish with poor body condition had several blood parameter values that were lower than corresponding values in clinically normal fish.

Conclusions and Clinical Relevance—Point-of-care blood analyses may prove useful in rockfish. Point-of-care data for a large number of clinically normal fish must be obtained for reference ranges to be calculated, and further assessments of clinically abnormal fish are necessary to determine the relevance of the data. (*J Am Vet Med Assoc* 2005;226:255–265)

The antemortem diagnosis of diseases in fish has become more important as the economic, emotional, and exhibit values of fish have increased. Analyses

of blood, serum, or plasma from fish can be useful for determining health status; diagnosing and monitoring disease; and examining responses to stress, environmental conditions, and dietary changes.¹⁻⁶ However, few diagnostic methods are available to aquatic veterinarians, and most samples submitted for testing are collected from individual anesthetized fish. Repeated physical or chemical restraint of fish for diagnostic procedures and treatments induces stress,⁷ which increases blood cortisol concentration and possibly affects recovery from disease.

Point-of-care blood analyzers have been used in human and veterinary medicine and are valuable adjuncts to reference laboratory testing in many situations.⁸⁻¹⁵ These analyzers provide basic, patient-side health and metabolic status information rapidly⁸ with acceptable reliability, compared with traditional laboratory methods.^{13,16-20} Results of tank-side blood analyses in fish are potentially valuable because treatment could be instituted immediately, reducing the number of times the fish are handled and thereby decreasing stress. Changes in temperature of blood samples and delays in processing can affect the results determined by reference laboratories^{7,21}; tank-side testing can minimize the effects of these potentially confounding factors.

A point-of-care analyzer^a has been used to determine values of blood parameters in a small number of fish of various species²²; however, its use in substantial numbers of fish of the same species under controlled conditions has not been described. Results obtained with this point-of-care analyzer in fish have also not been compared with those determined by veterinary reference laboratories.

Determination of plasma sodium, chloride, glucose, albumin, globulin, creatinine, and total protein concentrations; activities of sorbitol dehydrogenase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK); and total and differential WBC counts and other hematologic indices is recommended for assessment of health status of fish.^{1,2} Sodium, chloride, and glucose concentrations and Hct are measured with a specific cartridge^b in the point-of-care analyzer,^a and hemoglobin concentration is calcu-

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Supported in part by Sea Grant No. NA084B and the Oregon Coast Aquarium, Oregon Zoo, and Oregon State University. One of the point-of-care analyzers used in the study was provided by Heska Corporation.

The authors thank Kelli Harvison, Philip Fensterer, Beverly Basham, Jaime Ueda, James Cederstrom, Joy Flachsbar, Nancy Hollingshead, and Dr. Katrina Baden for technical assistance.

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lated. Blood urea nitrogen concentration can also be measured by use of this cartridge. Historically, uric acid and urea nitrogen concentrations have been considered minimally useful in teleosts²; however, severe branchitis has been associated with high urea nitrogen concentration in goldfish⁵ and, in our experience, with hyperuricemia in rockfish, likely as a result of gill excretory dysfunction. Concentrations of sodium, chloride, glucose, urea nitrogen, and hemoglobin and Hct can also be determined by use of a similar cartridge.^c

Black rockfish (*Sebastes melanops*) and blue rockfish (*Sebastes mystinus*) are marine cold-water species found along the Pacific coast of North America. Rockfish may weigh up to 4.8 kg (10.5 lb) and be up to 63 cm (25 inches) long; rockfish may live to 40 to 50 years of age.²³ Rockfish are often fished for human consumption, and their populations in the wild are affected by overfishing and degradation of coastal habitat. Because of their economic importance in the region and marine conservation education value, they are commonly exhibited in public aquariums.

The purposes of the study reported here were to compare values of blood parameters in rockfish obtained by use of a point-of-care portable analyzer with values determined by a veterinary diagnostic laboratory, calculate reference ranges for various blood parameters in black rockfish, and compare values of blood parameters in clinically normal fish with those in fish with clinical abnormalities.

Materials and Methods

Fish—The study was approved by the Institutional Animal Care and Use Committee of Oregon State University. Forty-one adult black rockfish and 4 blue rockfish from a captive population housed at the Oregon Coast Aquarium were used. Fish had been individually line-caught along the Pacific coast of Oregon at least 3 years prior to the start of the study and housed in a group of 133 rockfish in a 102,000-L closed-circulation tank since the time of capture. Rockfish were fed vitamin-supplemented prey (shrimp, herring, capelin, anchovies, and squid) 3 times a week. Body weights ranged from 0.86 to 2.36 kg (1.89 to 5.19 lb). Fish were moved into a closed-circulation, aerated, 85,000-L holding tank at the Oregon Coast Aquarium 3 days prior to commencement of the study. Fish were maintained on a 12-hour light–12-hour dark photoperiod cycle. Water-quality parameters were within standard ranges for coldwater marine systems (temperature, 11.5°C; dissolved oxygen concentration, 8.0 mg/L; pH, 8.0; ammonia concentration, 0 mg/L; nitrite concentration, 0.019 mg/L; nitrate concentration, 0.8 mg/L; and salinity, 34 parts per thousand). Fish were not fed for 2 days prior to commencement of the study. Twenty fish had grossly visible abnormalities that included a mild degree of ectoparasitism (eg, copepod infestation and leeches; n = 4), exophthalmos (12), corneal ulceration (3), small skin ulcers or abrasions (3), and poor body condition (4); 5 fish had > 1 abnormality.

Techniques of fish handling and blood handling can affect results of blood analyses²⁴; therefore, fish handling, blood collection, sample storage procedures, and laboratory analyses were performed under conditions that closely matched those under which samples would be collected and analyzed by aquatic veterinarians.

Chemical restraint and handling of fish—Blood samples were collected from all fish on the same day. Each fish was gently corralled into a net, lifted out of the holding tank,

and placed in a solid-walled, insulated, 340-L plastic tub containing tricaine methanesulfonate^d (to a final concentration of 100 ppm) and an appropriate amount of sodium bicarbonate powder^e (pH buffer). Water quality parameters for the anesthetic induction tank were within standard ranges for cold-water marine systems (temperature, 11.5°C; ammonia concentration, 0 mg/L; nitrite concentration, 0.002 mg/L; nitrate concentration, 0.5 mg/L; and salinity, 34 parts per thousand). Oxygen was bubbled into the water in the tank through a ceramic diffuser to maintain dissolved oxygen at saturation. Water pH was 8.0 before and 7.6 after addition of tricaine methanesulfonate and sodium bicarbonate. Mean \pm SD time of exposure to tricaine methanesulfonate until fish were sufficiently anesthetized for venipuncture, as assessed by dysequilibrium, lateral recumbency, minimal response to touch, and maintenance of opercular movements, was 2.8 \pm 0.7 minutes (range, 1 to 5 minutes). Fish were kept in the tricaine methanesulfonate tank during venipuncture; mean \pm SD venipuncture time was 1.9 \pm 1.7 minutes (range, 1 to 9 minutes). The gills were kept underwater during venipuncture but were exposed briefly to air when fish were transferred to a weighing net and recovery tank after venipuncture. Fish regained normal body position within approximately 3 minutes of placement in the recovery tank. All fish were observed for 4 hours after blood collection; no anesthesia-related behavioral abnormalities were detected.

Blood collection and handling—Up to 2.8 mL of blood was collected from each fish with a 3-mL syringe that did not contain any heparin and a 22-gauge, 1.5-inch needle. The caudal vessels located on the ventral aspect of the vertebral column were accessed at the base of the tail (caudal peduncle) via a ventral midline approach. Blood was successfully collected from 44 of 45 rockfish.

The needle was removed from the syringe, and blood was aliquoted as follows: 0.4 mL was placed into each of 4 tubes containing lithium heparin,^f and 0.6 mL was placed into each of 2 gel-separator tubes containing lithium heparin.^g The resultant concentration of heparin in these tubes was 14 to 16 U/mL of blood.

Within 15 minutes after sample collection, heparinized blood was used for measurement and calculation of blood parameters with the point-of-care analyzer^a and cartridge^b; PCV and total plasma protein concentration were also determined. Two microhematocrit tubes that did not contain heparin were filled with heparinized blood from 1 of the 4 tubes containing lithium heparin and centrifuged at 13,000 \times g for 5 minutes, and the PCV was determined. The microhematocrit technique for determination of PCV with a centrifugal force > 6,500 \times g has been validated for use with fish blood.²⁵ Plasma removed from microhematocrit tubes was used to determine total plasma protein concentration via refractometry. Remaining heparinized blood samples were placed in plastic bags in an ice bath and transported within 4 hours of collection to the Veterinary Diagnostic Laboratory (VDL) at the College of Veterinary Medicine, Oregon State University, for immediate analyses of blood gas parameters, PCV, and hemoglobin and total plasma protein concentrations. Within 15 minutes after sample collection, blood in gel-separator tubes containing lithium heparin was centrifuged for 5 minutes at 1,315 \times g. Plasma was collected and placed in a separate vial in a plastic bag in an ice bath. All samples were transported to the VDL within 4 hours after collection. Immediately on arrival at the VDL, biochemical analyses were performed. At the VDL, analyses were performed in duplicate, except that blood gas analyses were performed only once. Analyses were performed with the point-of-care analyzer in triplicate; 3 separate instruments and cartridges were used.

Analyses of blood parameters with the point-of-care analyzer—All analyses were performed by veterinary personnel. The point-of-care analyzer was precalibrated to correspond with values obtained via diagnostic laboratory methods that use human blood at 37°C (98.6°F). Direct ion-selective electrode potentiometry was used for measurement of sodium, potassium, and chloride concentrations. For measurement of urea nitrogen concentration, urea was hydrolyzed to ammonium ions in a reaction catalyzed by urease, and the ammonium ion concentration was measured via an ion-selective electrode. The urea nitrogen concentration was then calculated from the measured potential. Blood pH and partial pressure of carbon dioxide (PCO₂) were measured via potentiometry. Glucose concentration was measured amperometrically; glucose oxidase acting on glucose resulted in the production of hydrogen peroxide, which was oxidized at an electrode to induce an electric current proportional to the glucose concentration in the sample. Hematocrit was determined via measurement of conductivity.

Hemoglobin and bicarbonate (HCO₃) concentrations, total carbon dioxide concentration (TCO₂), base excess, and anion gap were calculated by use of the point-of-care analyzer with formulas derived for use with human blood. Hemoglobin was calculated by multiplying Hct by 34, a conversion factor derived from reference values for RBC counts and mean corpuscular hemoglobin concentration in human blood. Bicarbonate concentration was calculated by use of a formula that incorporated the measured pH and PCO₂ values ($\log \text{HCO}_3 = \text{pH} + \log \text{PCO}_2 - 7.608$). Total carbon dioxide concentration was calculated by use of a formula that incorporated the PCO₂ and HCO₃ values ($\text{TCO}_2 = \text{HCO}_3 + 0.03 [\text{PCO}_2]$). Base excess was calculated by use of a formula that incorporated HCO₃ and pH values ($\text{base excess} = \text{HCO}_3 - 24.8 + 16.2 [\text{pH} - 7.4]$). Anion gap was calculated by subtracting measured chloride concentration plus calculated HCO₃ concentration from the measured sodium concentration plus potassium concentration.

Quality-control and calibration procedures for the point-of-care analyzer were performed each time a new blood-filled cartridge was inserted. Calibration fluid was contained within each cartridge and was analyzed before the blood sample was accessed for analysis. Integrated electronic, mechanical, thermal, and pressure sensors were also activated, and appropriate conditions were verified when each cartridge was inserted. Software associated with the point-of-care analyzers was updated every 6 months, and calibration fluid specifications corresponded to software updates. Two assayed quality-control samples (with normal and abnormal values with respect to reference ranges in humans) were analyzed by use of the cartridges used in the study, and values obtained were within the ranges determined by the manufacturer of the quality-control material.

Analyses of blood parameters at the VDL—Packed cell volume and total plasma protein concentration were determined at the VDL by use of the same microhematocrit tube and refractometer methods used tank-side. Hemoglobin concentration was measured spectrophotometrically.

Plasma concentrations of urea nitrogen, glucose, cholesterol, total protein, albumin, calcium, and phosphorus and activities of alkaline phosphatase (ALP), CK, AST, and ALT were assayed with a biochemical analyzer^h and commercial reagents.ⁱ The assay for urea nitrogen concentration measured the formation of oxidized nicotinamide adenosine dinucleotide from urea catalyzed by urease and glutamate dehydrogenase. Glucose concentration was measured by use of a standard hexokinase assay that coupled the phosphorylation of glucose with oxidation of glucose-6-phosphate; the product, reduced nicotinamide adenosine dinucleotide phosphate (NADPH), was assayed. The cholesterol assay used chole-

sterol esterase and cholesterol oxidase to form a colored product proportional to the cholesterol concentration in the sample. Total protein concentration was determined by use of the biuret method, and albumin concentration was determined by use of bromocresol green binding. The assay for ALP activity measured the amount of *p*-nitrophenol released when *p*-nitrophenylphosphate was cleaved by ALP. The assay for CK activity measured the rate of formation of NADPH, which was proportional to the CK activity in the reaction of creatine phosphate with ADP. The assay for AST activity measured the decrease in nicotinamide adenosine dinucleotide phosphate (NADH) directly proportional to the rate of formation of oxaloacetate from α -ketoglutarate and L-aspartate. The ALT activity assay measured the decrease in NADH directly proportional to the rate of formation of pyruvate from α -ketoglutarate and L-alanine. Calcium concentration was measured via the reaction of calcium with *o*-cresolphthalein complexone to form a colored compound. Phosphorus concentration was measured by formation of phosphomolybdate complex in acidic solution. Sodium, potassium, and chloride concentrations were measured by use of indirect ion-selective electrode potentiometry. All assays at the VDL were performed by trained laboratory personnel.

Blood gas analyses were performed with a fully automated temperature-controlled (37.2°C) blood gas analyzer.^j Assays were performed with analyte-specific sensors and potentiometry. One- and 2-point calibrations were performed every 30 minutes.

A comprehensive quality assurance program was in place for the clinical pathology laboratory at the VDL. Standard procedures including assay of multiple control materials at least every 8 hours and analysis of results by means of the Westgard multirule to assure accurate error detection were used. Regular maintenance procedures for the chemistry analyzer were performed, and the laboratory subscribed to an external peer proficiency testing program. Validation studies were performed when changes in reagents or procedures were made, and regression analysis comparisons were completed before new reagents were used.

Comparison of values of blood parameters—Data for all 44 blood samples (from 40 black rockfish and 4 blue rockfish) were used for comparison of values of blood parameters among replicate analyses and the 2 analysis methods (point-of-care analyzer and VDL). Data obtained from each blood sample were used regardless of the species or health status of the fish. The number of data points for each blood parameter differed (range, 32 to 41), subject to particular technical circumstances (eg, cartridge failure, analyzer battery failure, or clotted blood sample).

Coefficients of variation (CVs) for replicate values obtained with the point-of-care analyzer were calculated ($100 \times \text{SD}/\text{mean}$) for each of 7 measured blood parameters (sodium, potassium, urea nitrogen, and glucose concentrations; Hct; pH; and PCO₂),^k and CVs for a subset of these 7 blood parameters that had > 1 replicate performed at the VDL were also determined (sodium, potassium, urea nitrogen, and glucose concentrations and PCV). These data were used to assess and compare repeatability (precision) of measurements performed with the point-of-care analyzer and instruments at the VDL.

Mean values of blood parameters for each blood sample were calculated by use of the replicate determinations obtained with the point-of-care analyzer and instruments at the VDL for 10 blood parameters (sodium, potassium, urea nitrogen, glucose, HCO₃, and hemoglobin concentrations; Hct or PCV; pH; PCO₂; and TCO₂).^k Blood gas measurements at the VDL were performed only once; therefore, single values for pH, PCO₂, HCO₃ concentration, and TCO₂ were used as the means. The Bland-Altman method was used to plot the

percentage difference between the 2 mean values (mean value obtained with the point-of-care analyzer minus the mean value obtained at the VDL) versus the overall mean value calculated by use of all values determined with both methods.^{26-28,k}

Bias (the mean percentage difference between values obtained with the 2 methods) and variability (± 2 SD of the mean percentage difference) were determined for all 10 blood parameters. These calculations were used to assess the degree of discrepancy between measurements obtained with the point-of-care analyzer and by the VDL. Student *t* tests (2-tailed, assuming unequal variances) and correlation coefficients (*r* values) were also used to compare values determined for all fish with the point-of-care analyzer with those determined by the VDL.^k

Reference ranges for blood parameters in black rockfish were calculated with values determined by the VDL; values from 20 clinically normal black rockfish were used. When data were normally distributed, the parametric reference range was determined (mean ± 2 SD). When data did not have a normal (Gaussian) distribution, data were either transformed by use of the natural logarithm to achieve normal distribution or reference ranges were determined by means of nonparametric methods. Nonparametric reference ranges were defined as the values between the 2.5th and 97.5th percentiles. Outlier data were identified by use of a statistical analysis program,^l and these data were not used. Values of blood parameters in black rockfish with abnormalities (exophthalmia; focal skin ulceration, ectoparasitism, or both; or poor body condition) that were apparent on physical examination were compared with those of clinically normal fish by use of Student *t* tests (2-tailed, assuming unequal variances).^k Reference ranges for blood parameters were also calculated by use of values determined with the point-of-care analyzer for the 20 clinically normal fish. Values for the clinically normal fish obtained with the point-of-care analyzer were compared with those obtained by the VDL by means of Student *t* tests (2-tailed, assuming unequal variances). Values of *P* < 0.05 were considered significant.

Results

Repeatability of measurements—Repeatability (precision) of the point-of-care analyzer and VDL methods was acceptable. Coefficients of variation were < 6% for blood parameters measured with the point-of-care analyzer (sodium, potassium, urea nitrogen, and glucose concentrations; Hct; pH; and PCO_2) and < 7% for blood parameters measured by the VDL for which > 1 replicate was measured (sodium, potassium, urea nitrogen, and glucose concentrations and PCV). Assayed control values were within predetermined limits for the point-of-care analyzer and the VDL.

Reliability of the point-of-care analyzer—One hundred thirty-two cartridges were used, all of which were from 1 production lot. No results were available for 17 analyses (1.0% of the total number of analyses performed with the point-of-care analyzer) that involved 5 cartridges (3.8% of the total number of cartridges used). In addition, 3 blood-filled cartridges did not yield data as a result of analyzer battery failure (*n* = 2) or a clot in the sample (1). Test failures were most frequent for determinations of Hct and hemoglobin concentration (8 failures, 4 cartridges); 1 of these 4 cartridges also failed to measure sodium, potassium, and urea nitrogen concentrations. Hematocrit and hemoglobin concentrations were successfully mea-

sured with 1 cartridge; however, measurement of pH and PCO_2 failed and therefore HCO_3^- concentration, TCO_2 , and base excess could not be calculated. Test failures appeared to be unrelated to sample quality; repeated analyses of the same blood sample with a new cartridge yielded a complete set of values.

Chloride concentrations were consistently > 140 mEq/L (greater than the reported limit of measurement of the instrument²⁹); therefore, anion gap could not be calculated for any blood sample. Seven fish had urea nitrogen concentrations < 3 mg/dL (lower than the reported limit of measurement of the instrument²⁹); therefore, their urea nitrogen concentrations were not included in summary comparisons.

Comparison of values for blood parameters determined by use of the 2 methods—The degrees of discrepancy in sodium, glucose, urea nitrogen, potassium,

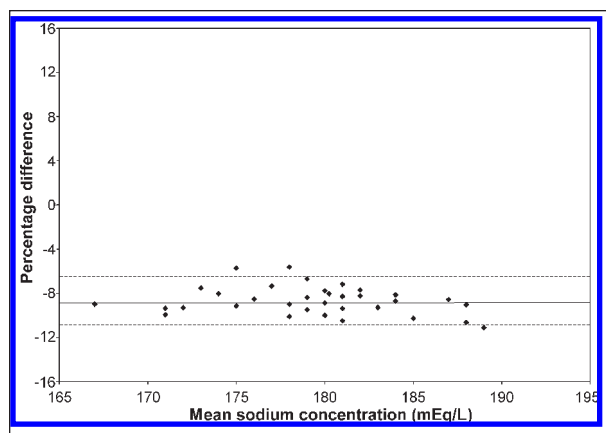


Figure 1—Bland-Altman plot of percentage difference between mean sodium concentrations determined with a point-of-care analyzer and a veterinary diagnostic laboratory (VDL) versus overall mean sodium concentration calculated from all values determined with both methods. The solid line indicates the mean percentage difference between values determined with the 2 methods (bias), and the dashed lines indicate the variability (± 2 SD) of the mean difference. The percentage difference was calculated by subtracting mean values determined by the VDL from mean values determined with the point-of-care analyzer. Each point represents the value for an individual fish.

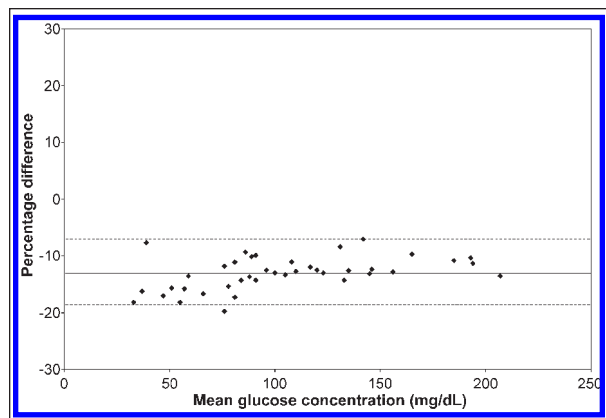


Figure 2—Bland-Altman plot of percentage difference between mean glucose concentrations determined with a point-of-care analyzer and a VDL versus overall mean glucose concentration calculated from all values determined with both methods. See Figure 1 for key.

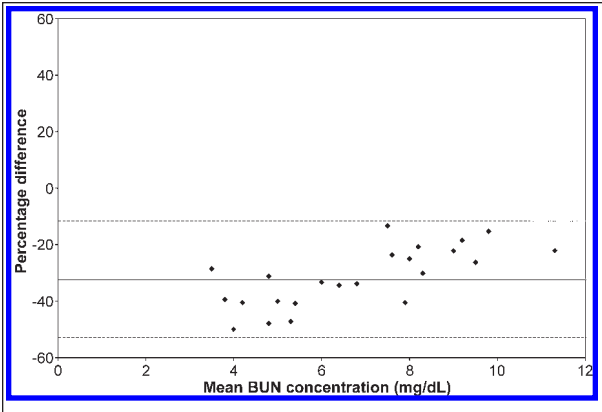


Figure 3—Bland-Altman plot of percentage difference between mean urea nitrogen concentrations determined with a point-of-care analyzer and a VDL versus overall mean urea nitrogen concentration calculated from all values determined with both methods. See Figure 1 for key.

Table 1—Mean ± SD values of blood parameters in anesthetized captive adult black rockfish (*Sebastes melanops*) and blue rockfish (*Sebastes mystinus*) determined by use of a point-of-care analyzer and by veterinary diagnostic laboratory (VDL) instruments and correlation coefficient (*r*) for values of blood parameters determined with both methods.

Blood parameter	No. of samples	Point-of-care analyzer	VDL	<i>r</i>
Urea nitrogen (mg/dL)*	32	5 ± 2	7 ± 2	0.97
Glucose (mg/dL)	41	99 ± 43	112 ± 47	> 0.99
Sodium (mEq/L)*	39	172 ± 5	187 ± 5	0.92
Potassium (mEq/L)	34	2.6 ± 0.3	2.8 ± 0.4	0.45
Chloride (mEq/L)*	41	> 140	152 ± 4	NA
pH*	40	6.93 ± 0.07	7.29 ± 0.09	0.81
Partial pressure of CO ₂ (mm Hg)*	40	37.5 ± 7.5	18.7 ± 4.4	0.87
Total CO ₂ (mm Hg)*	40	9 ± 2	15 ± 2	0.73
Bicarbonate (mEq/L)*	40	8 ± 2	13 ± 2	0.72
Base excess (mEq/L)*	40	-24 ± 2	-17 ± 2	0.68
PCV (%) or Hct*	37	27 ± 4	35 ± 5	0.90
Hemoglobin (g/dL)*	37	9 ± 2	15 ± 2	0.89

*Significant (*P*, 0.05) difference in values determined by the 2 methods.
 NA = Not available (could not be calculated).
 The number of blood samples used in calculations for each parameter varied because of technical problems with the point-of-care analyzer, including cartridge failure, analyzer battery failure, and clotted blood samples.

HCO₃, and hemoglobin concentrations; Hct and PCV; pH; PCO₂; and TCO₂ determined with the 2 methods of analysis were calculated. Bias (mean percentage difference between values determined with the 2 methods) was -5% for potassium concentration, -49% for HCO₃ concentration, -50% for hemoglobin concentration, -30% for Hct and PCV, -5% for pH, 67% for PCO₂, and -48% for TCO₂. Variability (± 2 SD of the mean percentage difference) was ± 29% for potassium concentration, ± 26% for HCO₃ concentration, ± 17% for hemoglobin concentration, ± 14% for Hct and PCV, ± 1% for pH, ± 20% for PCO₂, and ± 22% for TCO₂. Bias and variability were determined for sodium, glucose, and urea nitrogen concentrations (Figures 1–3). Values of most blood parameters determined with the point-of-care analyzer were significantly different than values determined by the VDL; only potassium and glu-

Table 2—Reference ranges (and mean ± SD) for blood parameters in anesthetized captive adult black rockfish (*S melanops*) calculated from values obtained for 20 clinically normal black rockfish by use of a point-of-care analyzer and VDL instruments. Heparinized blood was used for determinations performed with the point-of-care analyzer, and heparinized blood and plasma were used for determinations performed at the VDL.

Blood parameter	Point-of-care analyzer	VDL
Urea nitrogen (mg/dL)	0–9 (5.0 ± 2.0)	2.3–11.4 (6.9 ± 2.2)
Glucose (mg/dL)	20–188 (104 ± 42)	27–208 (118 ± 45)
Cholesterol (mg/dL)	—	98–349 (224 ± 63)
Total plasma protein (g/dL), refractometer method	—	4.9–8.4 (6.7 ± 0.9)
Total plasma protein (g/dL), biuret method	—	3.5–6.4 (5.0 ± 0.7)
Albumin (g/dL)	—	1.2–2.1 (1.7 ± 0.2)
Alkaline phosphatase (U/L)	—	5–86 (46 ± 20)
Creatine kinase (U/L)	—	0–51 (26 ± 13)
Aspartate aminotransferase (U/L)	—	0–12 (6 ± 3)
Alanine aminotransferase (U/L)	—	0–51 (26 ± 13)
Sodium (mEq/L)*	165–180 (173 ± 4)	179–198 (189 ± 5)
Potassium (mEq/L)	2.0–3.0 (2.5 ± 0.3)	1.9–3.5 (2.7 ± 0.4)
Chloride (mEq/L)	> 140 (NA)	145–158 (152 ± 3)
Calcium (mg/dL)	—	12.2–15.4 (13.8 ± 0.8)
Phosphorus (mg/dL)	—	7.2–13.4 (10.3 ± 1.6)
pH*	6.83–7.07 (6.95 ± 0.06)	7.15–7.45 (7.31 ± 0.07)
Partial pressure of CO ₂ (mm Hg)*	23.8–49.4 (36.6 ± 6.4)	10.2–26.2 (18.2 ± 4.0)
Total CO ₂ (mm Hg)*	6–12 (9 ± 2)	11.2–18.0 (14.6 ± 1.7)
Bicarbonate (mEq/L)*	5–11 (8 ± 1)	10.1–15.7 (12.9 ± 1.4)
Base excess (mEq/L)*	-28 to -20 (-24 ± 2)	-19.4 to -14.3 (-16.9 ± 1.3)
PCV (%) or Hct*	18–35 (27 ± 4)	25–45 (35 ± 5)
Hemoglobin (g/dL)*	6–12 (9 ± 1)	12.0–18.0 (15.0 ± 1.5)

*Significant (*P* < 0.05) difference in mean values between methods.
 NA = Not available (could not be calculated).
 — = Not done.

cose concentrations were not significantly different between the 2 methods (Table 1). Values of most blood parameters in individual fish determined with the point-of-care analyzer were lower than values determined by the VDL; exceptions included potassium concentration (values were distributed around the mean difference value on the Bland-Altman plot) and PCO₂ (values determined with the point-of-care analyzer were consistently higher than those determined by the VDL). Correlation coefficients were high for urea nitrogen and glucose concentrations.

Reference ranges and comparison of values for fish with clinical abnormalities with those for clinically normal fish—Twenty black rockfish were considered to be clinically normal and of similar age (adult)

Table 3—Mean ± SD values of blood parameters determined in 17 anesthetized captive adult black rockfish (*S melanops*) with grossly visible abnormalities and in clinically normal black rockfish by use of VDL instruments.

Blood parameter	Clinical abnormality			
	Exophthalmia (No. of samples = 10)	Ectoparasitism, skin ulcers, or both (6)	Poor body condition (4)	Clinically normal (20)
Urea nitrogen (mg/dL)	7 ± 3	5 ± 1	5 ± 2	6.9 ± 2.2
Glucose (mg/dL)	92 ± 44	116 ± 57	55 ± 15*	118 ± 45
Cholesterol (mg/dL)	185 ± 55	211 ± 48	165 ± 18*	224 ± 63
Total plasma protein (g/dL), refractometer method	4.4 ± 1.0*	5.1 ± 0.7	3.9 ± 0.5*	6.7 ± 0.9
Total plasma protein (g/dL), biuret method	4.3 ± 0.7*	4.7 ± 0.4	3.8 ± 0.5*	5.0 ± 0.7
Albumin (g/dL)	1.4 ± 0.3*	1.5 ± 0.2	1.2 ± 0.2*	1.7 ± 0.2
Alkaline phosphatase (U/L)	40 ± 21	35 ± 6	35 ± 10	46 ± 20
Creatine kinase (U/L)	ND	ND	11 ± 4	26 ± 13
Aspartate aminotransferase (U/L)	ND	ND	ND	6 ± 3
Alanine aminotransferase (U/L)	ND	ND	ND	26 ± 13
Sodium (mEq/L)	183 ± 6*	185 ± 7	181 ± 6	189 ± 5
Potassium (mEq/L)	2.8 ± 0.2	2.7 ± 0.3	2.8 ± 0.2	2.7 ± 0.4
Chloride (mEq/L)	151 ± 5	150 ± 5	150 ± 4	152 ± 3
Calcium (mg/dL)	12.1 ± 0.9*	13.2 ± 1.1	12.2 ± 1.1	13.8 ± 0.8
Phosphorus (mg/dL)	9.4 ± 1.7	10.6 ± 2.6	8.5 ± 0.7*	10.3 ± 1.6
pH	7.22 ± 0.07*	7.26 ± 0.10	7.21 ± 0.07	7.31 ± 0.07
Partial pressure of CO ₂ (mm Hg)	19.4 ± 4.1	20.2 ± 6.8	21.9 ± 3.9	18.2 ± 4.0
Total CO ₂ (mm Hg)	13.4 ± 2.1	14.8 ± 3.2	15.2 ± 3.1	14.6 ± 1.7
Bicarbonate (mEq/L)	11.9 ± 1.8	13.0 ± 2.6	13.2 ± 2.8	12.9 ± 1.4
Base excess (mEq/L)	-19 ± 2*	-17 ± 2	-18 ± 4	-16.9 ± 1.3
PCV (%)	32 ± 7	34 ± 6	30 ± 7	35 ± 5
Hemoglobin (g/dL)	14.0 ± 1.9	14.9 ± 1.8	13.5 ± 2.4	15.0 ± 1.5

*Significantly ($P < 0.05$) different from value for clinically normal fish.
 ND = Not done (several values were below the limit of detection of the assay; therefore, mean values could not be calculated).
 Three fish had > 1 abnormality. Heparinized blood and plasma samples were used for analyses.

Table 4—Mean ± SD values of blood parameters determined in 17 anesthetized captive adult black rockfish (*S melanops*) with grossly visible abnormalities and in clinically normal black rockfish by use of a point-of-care analyzer.

Blood parameter	Clinical abnormality			
	Exophthalmia (No. of samples)	Ectoparasitism, skin ulcers, or both (No. of samples)	Poor body condition (No. of samples)	Clinically normal (20)
Urea nitrogen (mg/dL)	6 ± 2 (8)	4 ± 1 (4)	5 ± 0 (1)	5 ± 2
Glucose (mg/dL)	92 ± 38 (8)	109 ± 54 (5)	50 ± 14 (3)*	104 ± 42
Sodium (mEq/L)	169 ± 6 (8)	170 ± 7 (5)	168 ± 8 (3)	173 ± 4
Potassium (mEq/L)	2.6 ± 0.2 (8)	2.6 ± 0.4 (5)	2.7 ± 0.3 (3)	2.5 ± 0.3
Chloride (mEq/L)	> 140 (8)	> 140 (5)	> 140 (3)	> 140
pH	6.87 ± 0.04 (8)*	6.90 ± 0.07 (5)	6.87 ± 0.07 (3)	6.95 ± 0.06
Partial pressure of CO ₂ (mm Hg)	39 ± 7 (8)	42 ± 14 (5)	46 ± 9 (3)	36.6 ± 6.4
Total CO ₂ (mm Hg)	8 ± 2 (8)	9 ± 2 (5)	10 ± 2 (3)	9 ± 2
Bicarbonate (mEq/L)	7 ± 1 (8)	8 ± 2 (5)	8 ± 1 (3)	8 ± 1
Base excess (mEq/L)	-26 ± 2 (8)	-25 ± 2 (5)	-25 ± 2 (3)	-24 ± 2
Hct	24 ± 6 (8)	25 ± 5 (5)	21 ± 6 (3)	27 ± 4
Hemoglobin (g/dL)	8 ± 2 (8)	8 ± 2 (5)	7 ± 2 (3)	9 ± 1

*Significantly ($P < 0.05$) different from value for clinically normal fish.
 Three fish had > 1 abnormality. Some blood parameters could not be determined in all fish. Heparinized blood samples were used for analyses.

and to have had consistent venipuncture conditions. Values for blood parameters determined by the VDL and the point-of-care analyzer for these 20 black rockfish were used to calculate reference ranges (Table 2). In general, data were normally distributed; therefore, data were interpreted to be appropriately reflective of the hypothetical reference population. Wide variations in glucose concentrations were detected; therefore, the

reference ranges calculated for glucose concentration were wide. Significant differences in mean values for various blood parameters in the 20 clinically normal fish were found between the 2 methods of analysis.

Values of blood parameters determined with both methods in 17 black rockfish with grossly visible abnormalities were compared with corresponding values in clinically normal fish (Tables 3 and 4). Fish

with exophthalmia had significantly lower mean total protein, albumin, sodium, and calcium concentrations; pH; and base excess than did clinically normal fish, when values for blood parameters determined by the VDL were used. Fish with exophthalmia had a significantly lower mean pH than did clinically normal fish when blood parameters obtained with the point-of-care analyzer were used. No significant differences in values of blood parameters obtained with either of the 2 methods were found among fish with minor skin ulcers, mild ectoparasitism, or both and clinically normal fish. Fish with poor body condition had significantly lower mean total protein, albumin, glucose, cholesterol, and phosphorus concentrations than did clinically normal fish when values determined by the VDL were used; fish with poor body condition had significantly lower mean glucose concentration than did clinically normal fish when glucose concentration was measured with the point-of-care analyzer. Of the abnormal values found in fish with poor body condition, glucose concentration was the only parameter measured by means of both methods.

Discussion

The range of values for most hematologic and biochemical parameters reported in most species of clinically normal fish is wide, and values vary depending on the age, physiologic status, and state of nutrition of the fish. The stress of net capture and anesthesia is also likely to cause changes in hematologic and biochemical parameters.^{7,30,31} Anesthesia is used in fish to facilitate venipuncture, lessen stress-induced changes, and minimize tissue trauma and clotting activation that can be associated with venipuncture in fish that are only physically restrained.^{2,32-34} The transfer of fish between holding tanks is a stressor; however, the effects of transfer should be sufficiently small by 24 hours after transfer to be clinically irrelevant.³⁵ Anesthesia with tricaine methanesulfonate induces physiologic changes that may affect values of blood parameters^{34,36}; however, these changes are minimized if tricaine methanesulfonate is buffered,³⁷ as in our study. Stress-induced changes in blood parameters may occur if venipuncture requires > 30 seconds or if fish are captured one by one from the same holding tank.^{7,38} Many of these stressors were present in our study, but we believe that the collection of blood samples in clinical practice would take place under similar conditions.

Heparin at concentrations ranging from 50 to 100 U/mL of blood or from 0.25 to 4 mg/mL of blood^{2,33,39} is the recommended anticoagulant for fish blood. We used 14 to 16 U of lithium heparin/mL of blood, the standard provided for commercial tubes containing lithium heparin, and blood samples remained visibly unclotted at this concentration. Sodium heparin is often used as an anticoagulant in blood collection syringes for fish; however, use of sodium heparin has the potential to result in artifactually high plasma sodium concentrations. Ammonium heparin is used occasionally; however, use of ammonium heparin can result in artifactually high potassium concentrations when a point-of-care analyzer is used for blood analyses.²⁹

Because of differences in body temperature, blood pH, and osmolality between cold-water marine fish and mammals, values of blood parameters determined with methods designed for human and other mammalian blood samples are unlikely to reflect true values for fish.^{2,7,40,41} For example, the optimal pH range for measurement of ALT and AST activities in English sole (a cold-water marine species) is 6.8 to 7.0⁴²; however, assays used for these analyses are calibrated to pH ranges found in mammals that are typically higher than those of fish. Heparin causes activation of lipases in blood in vivo and in vitro; therefore, cholesterol reference ranges determined in heparinized blood may not reflect true blood concentrations. The high concentration of ammonia in fish blood, compared with that in mammalian blood, may result in artifactually high activities of ALT and AST.⁴¹ Blood samples from cold-water species of fish are subjected to temperature increases during centrifugation and routine analyses performed at 37°C (standard temperature for human blood analyses), which may result in artifactual changes in blood parameters, especially in enzyme activities.⁴⁰ The importance of abnormalities of blood parameters in fish, as for other species, should be assessed within the context of changes in the individual's blood parameters, clinical signs, and reference ranges calculated for these blood parameters under the same sampling and analysis conditions.

In our study, values of blood parameters determined with the point-of-care analyzer were reproducible among the 3 analyzers used to analyze each blood sample. This finding agrees with results of previous studies^{17,18} in which human blood was tested. Cartridge failure and inaccuracy of certain measurements have been reported in studies^{43,44} performed in birds and humans, respectively. The incidence of test failure in our study was considered acceptable.

Most values of blood parameters obtained with the point-of-care analyzer in the present study were significantly different from those determined by the VDL, especially those for blood gas analyses. Although tubes containing aliquots of blood were capped, entrapped air was not detected prior to transport to the VDL, and blood gas parameters in chilled blood samples are reportedly stable for several hours after collection, small quantities of entrapped air, processing delays, and interassay variability may have been responsible for some of the discrepancies between values determined with the point-of-care analyzer and those determined at the VDL. Rapid and gentle separation of plasma from cells and refrigeration during transport are used to decrease artifactual changes in blood samples over time.³³ We followed these recommendations as much as possible; however, sample handling artifacts may have contributed to some of the observed discrepancies.

Conversion factors have been applied to blood gas parameters (pH, PCO₂, and HCO₃ concentration) in loggerhead sea turtles (*Caretta caretta*) determined with the same point-of-care analyzer to reflect the fact that a sea turtle's body temperature is < 37°C.^{45,46} After conversion factors are applied to blood gas values in sea turtles, corrected pH values are typically 0.11 to 0.14 pH

units higher and corrected PCO_2 values are 18% to 33% lower than values measured at 37°C. To the authors' knowledge, temperature-based correction factors for blood gas values have not been determined for fish.

Although the reference ranges for pH in rockfish appear to be wide for both of the analysis methods used in the present study, observed variability was not judged to be sufficiently great to be clinically relevant. A large degree of bias between the 2 methods was detected in values for PCO_2 ; however, the degree of bias was fairly consistent throughout the analytical range. Therefore, PCO_2 may be more useful for evaluation of respiratory efficiency (gill function) than other blood gas parameters in rockfish.

Heparinized blood was used for determinations of blood parameters with the point-of-care analyzer and for blood gas analyses at the VDL in the present study, and plasma was used for refractometric measurement of total plasma protein and for measurement of biochemical parameters at the VDL. Gross hemolysis was not detected at any stage of sample handling; however, lesser degrees of hemolysis may have been present. Hemolysis as a result of RBC trauma during collection of blood samples would have caused changes in blood parameters (ie, high phosphorus and potassium concentrations and high AST activity) regardless of the method of analysis^{2,47} because hemolysis would have been induced before the sample was aliquoted or centrifuged. Hemolysis as a result of inappropriate sample handling may have resulted in discrepancies in values of blood parameters that may have been recognizable in blood (point-of-care analyzer) and plasma (VDL) samples. In our study, potassium concentration was measured with both methods, and mean concentrations were not significantly different.

Packed cell volumes measured with the microhematocrit tube method and Hct determined by use of the point-of-care analyzer were significantly different. The point-of-care analyzer is calibrated to match Hct values obtained in human blood samples anticoagulated with tripotassium EDTA.²⁶ Red blood cell shrinkage occurs in tripotassium EDTA; therefore, use of tripotassium EDTA anticoagulated blood in the point-of-care analyzer will result in Hct values that underestimate true values by 4% to 5% in mammals.²⁹ Hematocrit values in birds are approximately 25% lower when determined with a point-of-care analyzer, compared with PCV determined by use of a microhematocrit tube technique.¹¹ Hematocrit determinations in rainbow trout^m and rockfish in our study revealed a similar pattern. The discrepancy between methods may be the result of the fact that avian and fish RBCs are elliptical; therefore, their passage through the conductivity sensors of a point-of-care analyzer may artifactually decrease the Hct. Centrifugation of blood samples for measurement of PCV is therefore recommended for fish blood. In anemic humans, Hct values determined by use of the point-of-care analyzer are artifactually decreased if plasma protein concentrations are < 6.5 g/dL^{18,26}; this artifact could occur frequently with fish blood because fish plasma protein concentrations are often < 6.5 g/dL and Hcts are often < 40%. Tricaine methanesulfonate can cause fish RBCs to swell,³⁵ fur-

ther complicating the estimation of Hct and PCV in fish. Reported ranges of Hct and PCV in fish of other species are wide, possibly as a result of the factors described or physiologic factors such as age, reproductive status, or temperature of acclimation. Hemoglobin concentration calculated by the point-of-care analyzer was significantly lower than hemoglobin concentration measured spectrophotometrically at the VDL, likely partly the result of lower Hct values. Use of internal formulas for calculation of hemoglobin concentration in human blood has also resulted in discrepancies between hemoglobin concentrations determined with point-of-care analyzers and those determined by use of diagnostic laboratory instruments.¹⁷ The RBC indices and oxygen-carrying capacity of an individual fish should ideally be evaluated with reference to data generated in fish of the same species with similar signalment that were sampled via similar techniques.

Total protein concentrations are variable depending on the method of measurement used. Refractometry and methods that use optical density measurements result in concentrations of total protein in trout sera that are approximately 1.5 times those obtained by use of copper sulfate specific gravity, biuret, and phenol reagent methods⁴⁸; a similar pattern was detected in total protein concentration determined via refractometry versus that determined via the biuret method in our study.

With increasing urea nitrogen concentration, the degree of bias between values obtained with the point-of-care analyzer and those measured by the VDL decreased. **Clinical Laboratory Improvement Act (CLIA)** guidelines state that discrepancies in urea nitrogen concentrations > 9% between traditional and new methods should be cause for concern when evaluating new instruments for human blood analysis; the urea nitrogen concentrations of blood samples used in our study had high variability. However, goldfish with branchitis induced by copper exposure had urea nitrogen concentrations 2 to 3 times higher than urea nitrogen concentrations prior to copper exposure,⁵ suggesting that a measurement variability of 20% may still be acceptable when urea nitrogen concentrations are used to diagnose gill disease.

In our study, the ranges of glucose concentrations obtained with both methods were wide; this finding suggests that fish have naturally wide ranges of glucose concentrations and that measurement of glucose concentration as an indicator of disease or metabolic perturbation in fish may not be clinically useful. According to the CLIA guidelines, a variability in glucose concentration < 10% is acceptable for instrument comparison; glucose concentrations in our study had a variability of 5.8%, suggesting acceptable agreement between the point-of-care analyzer and VDL analytic methods.

Low bias but high variability in potassium concentrations was evident between the 2 methods of analysis in the present study. The low correlation coefficient for the 2 methods ($r = 0.45$) was consistent with these findings. Potassium concentration is not considered a valuable diagnostic parameter in fish, and the unpredictable nature of our data supports this conclusion.

No simple explanation can be offered for the discrepancies in values of blood parameters between the point-of-care analyzer and the VDL instruments that were found in the present study. Sodium, potassium, and chloride concentrations; pH; and PCO_2 were measured by use of the same techniques in both instruments (except that the point-of-care analyzer used direct ion-selective electrode potentiometry and the instrument at the VDL used indirect ion-selective electrode potentiometry for measurements of sodium, potassium, and chloride concentrations), yet sodium concentration, pH, and PCO_2 differed significantly between instruments. The use of formulas developed for calculation of human blood parameters may have resulted in hemoglobin and HCO_3 concentrations, TCO_2 , and base excess values determined with the point-of-care analyzer that were different from those determined by the VDL. Additional studies that use larger numbers of fish of various species for determination of blood parameters with the point-of-care analyzer are necessary before recommendations regarding assay methodology can be offered.

A limited number of clinically normal fish were available for calculation of reference ranges; however, some patterns of change could be identified in values of blood parameters in fish with clinical abnormalities. Skin disease (dermatitis or ulceration) may cause changes in electrolyte concentrations or hematologic parameters as a result of loss of integrity of the barrier between body fluids and the environment in marine fish. However, fish with mild skin ulcers or ectoparasitism in the present study did not have significantly different values for blood parameters, compared with those for clinically normal fish, suggesting that their lesions were mild and physiologically unimportant. Exophthalmia in fish in the present study may have been caused by the rapid transition from deep-water pressure to surface pressure during capture several years prior to our study or may have been the result of a spontaneous occurrence of idiopathic gaseous exophthalmia, a condition seen frequently in large-eyed predatory marine fish.⁴⁹⁻⁵¹ Regardless of etiology, exophthalmia did not appear to be associated with blood gas abnormalities in the fish of our study, despite the presence of up to 21 mL of gas posterior to the eye. Fish with exophthalmia had lower total protein and albumin concentrations than did clinically normal fish, which may have been a result of chronic low-level malnutrition (because of buoyancy or vision impairment that interferes with food acquisition or undiagnosed gastrointestinal tract pathology), although the exophthalmic fish of our study were not noticed to have poor body condition. Total protein and albumin concentrations cannot be measured with the point-of-care analyzer; therefore, refractometry or other techniques must be used. Fish with exophthalmia had lower sodium and calcium concentrations (determined by the VDL) than did clinically normal fish; these changes are difficult to explain because unhealthy marine fish often develop concurrent gill dysfunction that results in sodium accumulation in the blood. Poor body condition was associated with lower concentrations of total protein, albumin, glucose, and cholesterol (determined

by the VDL), compared with clinically normal fish, which are expected findings given the reflection of overall nutritional status. Glucose concentration determined with the point-of-care analyzer was also lower in fish with poor body condition than in clinically normal fish. Activity of CK was not significantly different in fish with poor body condition, compared with clinically normal fish, likely as a result of chronicity of muscle mass loss.

Reasons for the variability in values of blood parameters include sampling artifact as a result of fish manipulation (eg, use of anesthetic agents) or blood-sample handling techniques (eg, hemolysis); artifact as a result of within-instrument imprecision; and true patient-origin physiologic changes that may or may not be clinically important. Blood sample collection and handling procedures used in our study were routine and accepted within the aquatic veterinary community and minimized the possibility of sampling artifact. The point-of-care analyzer and instruments used at the VDL had degrees of variation between replicates that were considered to be within acceptable limits. However, discrepancies between values of blood parameters determined by these instruments were commonly detected.

The point-of-care analyzer and cartridge used in our study may prove useful for measurement of sodium, urea nitrogen, and hemoglobin concentrations; Hct; pH; and PCO_2 in rockfish. However, reference ranges must be calculated from point-of-care data collected from a large number of clinically normal fish, and assessment of clinically abnormal fish is necessary to determine the relevance of the data. Measurement of other blood parameters with the point-of-care analyzer is not recommended because the reported upper or lower limits of measurement of the analyzer were incompatible with typical values in fish (eg, chloride concentration), wide reference ranges were detected (eg, glucose concentration), or variability resulting from unknown factors in fish or instrument inconsistency was identified (eg, potassium concentration, TCO_2 , and HCO_3 concentration). In our study, none of the assays provided by the point-of-care analyzer and cartridge could be used to detect underlying systemic illness in fish with clinical abnormalities. This may be because of a true lack of systemic effects associated with external clinical signs or a lack of sensitivity of analysis to detect systemic disease. However, it is possible that several important conditions in fish, including branchitis, respiratory dysfunction, and anemia, could be detected by use of a point-of-care analyzer in the absence of obvious clinical signs of disease.

The limitations in interpretation of blood parameters measured with the point-of-care analyzer in rockfish are evident, and there is a need for further studies to explain the discrepancies between values obtained with the 2 methods. We hope that as tank-side technology becomes available to aquatic veterinarians, the clinical relevance of relatively novel parameters such as blood gas values (and their association with particular diseases, many of which may be subclinical or difficult to diagnose on physical examination) will be better described.

- a. i-STAT portable clinical analyzer, Heska Corp, Fort Collins, Colo.
- b. EC8+ portable clinical analyzer cartridge, lot #A02340, Heska Corp, Fort Collins, Colo.
- c. 6+ portable clinical analyzer cartridge, Heska Corp, Fort Collins, Colo.
- d. Finquel MS-222, Argent Chemical Laboratories, Redmond, Wash.
- e. Arm & Hammer sodium bicarbonate powder, Church & Dwight Co, Princeton, NJ.
- f. Microtainer nongel lithium heparin blood tubes, Becton-Dickinson, Franklin Lakes, NJ.
- g. PST Microtainer gel separator lithium heparin blood tubes, Becton-Dickinson, Franklin Lakes, NJ.
- h. Hitachi 717 biochemical analyzer, Roche Diagnostics, Indianapolis, Ind.
- i. Roche Diagnostics, Indianapolis, Ind.
- j. RapidLab 865, Chiron Diagnostics, Bayer Corp, Tarrytown, NY.
- k. Excel 2000, Microsoft Corp, Redmond, Wash.
- l. Analyze-It, Microsoft Corp, Redmond, Wash.
- m. Mylniczenco N, John G, Shedd Aquarium, Chicago, Ill: Personal communication, 2004.

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