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Point-of-care blood analyzers measure the nutritional state of eighteen free-living bird species

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ABSTRACT

Point-of-care devices offer the potential to democratize a suite of physiological endpoints and assess the nutritional state of wild animals through plasma metabolite profiling. Measurements of plasma metabolites typically occur on frozen tissue in the laboratory, thus dissociating measurements from field observations. Point-of-care devices, widely used in veterinary and human medicine, provide rapid results (seconds or minutes) allowing in situ measurements of wild animals in remote areas without the need for access to freezers. Using point-of-care devices, we measured glucose, triglyceride, cholesterol and β-hydroxybutyrate levels in plasma from 18 wild bird species spanning nine families and three orders. The values from six different point-of-care devices correlated strongly with one another, and with traditional laboratory measurements from stored plasma (R^2 = 0.70–0.90). Although POC devices provided accurate relative values in wild birds, absolute values varied from laboratory measurements by up to 50% illustrating the need for calibration equations. Furthermore, three case studies showed the potential for point-of-care devices at research stations where participants do not have access to a lab and sample preservation is difficult: (i) at a remote seabird colony, birds that were provided with supplemental food had higher levels of glucose and lower β-hydroxybutyrate and cholesterol levels than unfed birds, suggesting they were in a better nutritional state; (ii) at a migration monitoring station, levels of triglycerides of two migratory songbirds increased with time of day, implying that they were fattening during stopover; and (iii) for diving seabirds, individuals that worked harder (shorter surface intervals) had higher glucose and lower β-hydroxybutyrate implying that nutritional state is an index of foraging effort and success. We demonstrate that point-of-care devices, once validated, can provide accurate measurements of the nutritional state of wild birds. Such real-time measurements can aid in ecological research and monitoring, care of wildlife at rehabilitation centres, and in veterinary medicine of exotics.

1. Introduction

Energy is a fundamental currency in ecology, linking animal behaviour to individual fitness and together to population dynamics (Halsey et al., 2011; Speakman, 1997). However, measuring energy intake and expenditure in wild animals is notoriously difficult (Elliott, 2016; Halsey et al., 2011). Measures of plasma metabolites provide a non-destructive means of estimating the nutritional state of an animal, providing insight into net energy intake (Alonso-Alvarez and Ferrer, 2001; Gerson and Guglielmo, 2013; Guglielmo et al., 2005; Jenni-Eiermann et al., 2011; McGuire et al., 2009).

In wild birds, high glucose and triglyceride concentrations in plasma typically represent recent energy intake, while high cholesterol and low β-hydroxybutyrate concentrations represent longer-term net energy intake. Thus, the plasma composition of these four different metabolites could be used to determine the nutritional state of a bird. (Alonso-Alvarez and Ferrer, 2001). For instance, when enduring food restriction, birds go through different phases of fasting characterized by changes in mass and plasma metabolite composition (Fig. 1). These different phases are followed by continuous fasting until reaching phase 3, or the “deterioration” period, which is characterized by a sharp decrease in all four metabolites, reaching a critically depleted level that may result in death. The main purpose of these metabolites is to signal the body (prior to death) that a new feeding event is necessary

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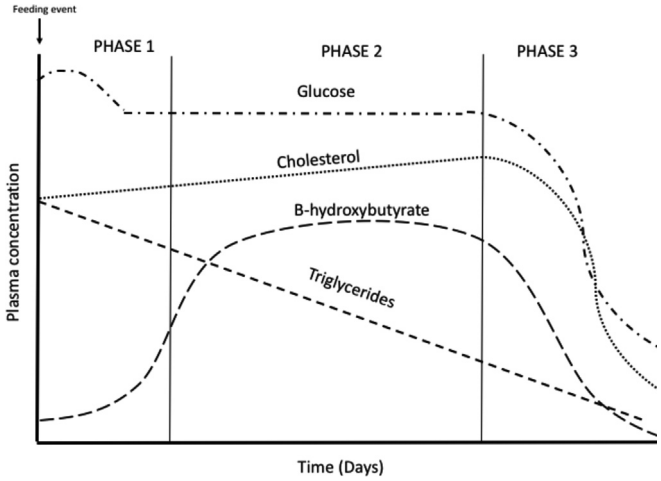

Fig. 1. Predicted changes of plasma composition of four different metabolites immediately following a feeding event and subsequently during fasting in birds. Modified from Alonso-Alvarez and Ferrer (2001). The scale on the x-axis is for illustrative purposes only.

(Friedman, 1995).

In the context of wild animals, plasma metabolite profiles are blood tests that can provide us with important information for physiological and ecological studies (Guglielmo et al., 2005; Lindholm and Altimiras, 2016). Results from blood analyses provide valuable information regarding a wide spectrum of compounds such as metabolites, proteins, hormones and electrolytes, which are reflective of the health status of individuals (Alonso-Alvarez and Ferrer, 2001; Scanes, 2015a). For example, in migratory birds, plasma concentrations of triglycerides and β-hydroxybutyrate are used to estimate fuel deposition rates at stopovers (Guglielmo et al., 2005; Schaub and Jenni, 2001). Fuel deposition rates can then be used to interpret different migration strategies and behavioural patterns, and to assess the quality of stopover habitat (Guglielmo et al., 2005; Schaub and Jenni, 2001). Blood biochemistry analyses have also been used to infer impacts of ectoparasite load (e.g. Quillfeldt et al., 2004), causes of body mass changes (e.g. Albano et al., 2016; Azeredo et al., 2016), causes of population declines (e.g. Artacho et al., 2007) and the proximate mechanisms underlying moult in birds (Frelin, 1974; Podlaszczuk et al., 2017).

Point-of-care (POC) devices have the potential to be applied in wildlife research and veterinary medicine of exotic animals. Traditional lab assays require freezing and transporting blood to a laboratory. Freeze-thaw cycles and extended periods on ice prior to freezing may alter metabolites in blood samples (Cuhadar et al., 2013; Morris et al., 2002). In contrast, POC devices provide rapid on-site measurements from small blood samples, reducing logistical, permitting or financial challenges with transporting frozen samples from remote locations, reducing response time and risks of sample deterioration, and increasing the potential for physiological measurements to impact ecological research and monitoring. Beyond the application of POC use in research, nutritional state is one of the best predictors of the success of both incoming and outgoing animals undergoing rehabilitation. Plasma metabolite measurements can help with (i) determining whether an animal should be placed in a rehabilitation centre, (ii) triaging for financially limited rehabilitation centres; and (iii) determining whether and when an animal should be released following rehabilitation. Lastly, POC devices are widely used in veterinary medicine, but the lack of data for wild or exotic animals often hampers their use on these animals (Lindholm and Altimiras, 2016).

Few POC devices have been validated for wild or exotic animals (Lindholm et al., 2019; Lindholm and Altimiras, 2016; Sommers et al., 2017). Several POC devices are capable of producing analytical results with the use of very small blood samples, rendering them applicable for use in small bird species without compromising the welfare of the animal. Although the selection of POC devices calibrated for avian species is very limited, many of the biochemical principles are universal in homeotherms, and consequently such devices may be co-opted for birds. Due to biochemical differences among species of different taxa (Lindholm and Altimiras, 2016), applications of POC devices designed for use on avian species require validation to determine accuracy of the blood biochemistry results. This validation necessitates comparison of POC values to values obtained using conventional laboratory tests (Stoot et al., 2014). For example, previous studies have demonstrated that measurements from POC devices calibrated for humans underestimate glucose levels in birds by up to 33%, yet were significantly correlated with laboratory tests (Acierno et al., 2012; Lieske et al., 2002; Mohsenzadeh et al., 2015).

The main objective of this study was to validate and examine the potential use of POC devices across a broad range of wild bird species. We aimed to validate six POC devices for use in the field as alternatives to laboratory tests for measuring a metabolite panel (cholesterol, triglycerides, β-hydroxybutyrate, glucose) in up to 18 species of wild birds from nine families. Taken together, measurements of blood chemistry in birds can indicate short-term foraging success (glucose), medium-term foraging success (triglycerides and β-hydroxybutyrate) and long-term foraging success (cholesterol) (Fig. 1). We investigated whether POC devices provide accurate, linear, and precise measurements of the metabolite panel. We also tested whether relationships are device-specific or family-specific (among the nine avian families examined), with the assumption that if relationships are constant across a diversity of species and devices, then POC devices are likely broadly accurate for many species and devices. While previous studies have assessed the use of other handheld human and veterinary devices on birds (Acierno et al., 2012; Lieske et al., 2002; Lindholm et al., 2019; Mohsenzadeh et al., 2015; Stoot et al., 2014), our suite of devices had yet to be validated. As reference ranges of the target species have yet to be established, the blood biochemistry results in this study also contribute as baseline values for interpretation of diagnostic tests and future health evaluations of these species (See appendix).

To further show the potential of these devices for field biologists in remote field locations and researchers with no lab access, we presented three case studies at different study sites using POC devices. At a remote seabird colony, we predicted that birds that were supplemented with extra food during the breeding season will have higher triglycerides, glucose and cholesterol, and lower β-hydroxybutyrate levels, than non-supplemented individuals. Furthermore, at a migration monitoring station, we predicted that triglyceride levels of two species of migratory songbirds will increase with time of day due to the replenishment of fat storages during stopover. Finally, at a second seabird colony, we hypothesized that diving birds that work harder (spend more time underwater) will subsequently have higher glucose, triglycerides and cholesterol and lower β-hydroxybutyrate levels due to an increase in foraging effort and foraging success.

2. Materials and methods

2.1. Field methods

Birds species sampled for the validation of the devices were part of six separate ongoing monitoring programs: wintering passerines, snow buntings, migrating passerines, kittiwakes, murres and mergansers (Table 1). In kittiwakes, only blood samples from chicks were used in the validation. Blood sampling was completed under animal use protocols 2007–5446 and 2015–7599 from McGill University, and under respective federal banding permits.

Blood was collected in capillary tubes from the brachial vein of each murre, kittiwake and passerine bird, or from the tarsal vein of ducks. For each individual, two to three capillary tubes containing 50 μL or 70 μL blood were collected. One of the capillaries was sealed with

Table 1
Study location, dates, sample size, capture methods and species sampled for each monitoring project used in the validation of the devices.

Project	Location	Coordinates	Date	Sample size	Capture method	Species
Winter passerines	McGill Bird Observatory, Montreal, Quebec	45.43 N, 73.94 W	November 2016, 2018	97	Mist-nets	*Carduelis tristis, Spizella arborea, Poecile atricapillus, Zonotrichia albicollis, Junco hyemalis, Haemorhous mexicanus*
Snow buntings	Saint-Clet, Quebec	45.35 N, 74.16 W	January 2017	19	Ground traps	*Plectrophenax nivalis*
Migrating passerines	McGill Bird Observatory, Montreal, Quebec	45.43 N, 73.94 W	Spring and Fall of 2017	47	Mist-nets	*Turdus migratorius, Icterus galbula, Bombycilla cedrorum, Catharus minimus, Dumetella carolinensis, Agelaius phoeniceus, Catharus ustulatus, Melospiza georgiana*
Kittiwakes	Middleton Island, Alaska	57.43 N, 146.33 W	May–June 2018	24	By hand at nest	*Rissa tridactyla*
Murres	Coats Island, Nunavut	62.44 N, −83.10 W	July 2017	45	Noose pole at nest	*Uria lomvia*
Mergansers	Kouchibouguac National Park, New Brunswick	46.81 N, 64.93 W	July 2017	13	By hand at nest	*Mergus serrator*

Fisherbrand Hemato-Seal capillary tube sealant and stored for subsequent plasma extraction and laboratory analysis, while the remaining ones were emptied onto the POC devices in situ. The stored samples were kept in a cooler with ice packs until processed within 5 h after sampling. Capillary tubes were spun for 10 min in a hematocrit centrifuge to separate the cells and plasma in the blood. The plasma from each capillary tube was extracted using a Hamilton 25 μL syringe, which was rinsed at least three times with MilliQ water before and after each use to prevent contamination between samples. The extracted plasma from each sample was then transferred to a microcentrifuge tube. The tubes were labeled with the corresponding birds' unique identifier and kept in a freezer at −80 °C for a few weeks in passerines and kittiwakes and up to 6 months in mergansers and murres. In winter passerines and snow buntings, we used the extracted plasma to test for plasma glucose concentrations with the Contour device in addition to the whole blood measurements.

2.2. POC devices

We tested six POC devices: Bayer Contour®, Cardiochek®, Abaxis VetScan VS2®, Precision XTra®, Nova Max Plus ® and CentriVet™. Not all of the POC devices were used in every project (See Table 2 for details).

Bayer Contour is a battery powered handheld POC device designed for human use and which measures blood glucose (detection range = 1–600 mg/dL) using reagent strips (Bayer Inc, 2011). The device measures the electrical current produced by the reaction between the blood glucose and FAD glucose dehydrogenase enzyme in the strip, and the current is correlated with the glucose concentration in the sample. The measurement is done by applying the tip of the test strip directly to the blood sample. Results in mg/dL are displayed within 5 s.

CardioChek is a battery powered handheld POC device designed for human use which measures a wide range of blood biochemistry parameters including glucose (detection range = 40–600 mg/dL), cholesterol (detection range = 100–400 mg/dL) and triglycerides (detection range = 50–500 mg/dL) using reagent strips (Polymer Technology Systems Inc, 2010). The device employs light reflectance technology to measure an enzymatic chemical reaction. Applying blood onto the test strip results in a chemical reaction that produces a colour change on the strip and the device takes a reading of the light reflected off the underside of the application window. The intensity of the colour change is correlated with the concentration of glucose, cholesterol or triglycerides in the sample. An incomplete colour change on the underside of the blood application window is an indication of an insufficient sample, which can lead to an inaccurate and underestimated result. We discarded samples with incomplete colour change.

The Abaxis VetScan VS2 is a bench-top POC device designed for measuring different blood biochemistry profiles in companion and domesticated animals, using species-specific reagent rotors that require a sample size of 100 μL (Abaxis Inc, 2009). We used the T4/Cholesterol Profile rotor (detection range = 20–520 mg/dL), which is designed for dogs and cats. We created a 1:2 dilution of whole blood with phosphate-buffered saline (PBS) in order to be able to use a smaller amount of blood than the one required by the device. Therefore, the correlation between VetScan results using diluted samples and laboratory results using undiluted samples are examined in this study. The rotors were kept in a cooler with ice packs until ready for the blood analysis and were handled by the sides to prevent damages to the bar code located on top that can result in errors during analysis. The diluted mix of 50 μL of blood and 50 μL of PBS was loaded into the rotor with a fixed 100 μL pipette. The filled rotor was then placed inside the VetScan VS2 for analysis. Inside the device, the rotor is spun to distribute the blood sample into multiple wells located along the periphery of the rotor, which contain different dry reagents to determine T4 and cholesterol levels (Abaxis Inc, 2003). In case of an insufficient sample size, the analysis process was prematurely terminated and yielded no results.

Table 2
Specifications for POC devices used, including volume of blood required per strip, systems used, results display time, cost per strip and units displayed. Duplicate measurements require two strips.

Device and metabolite	Volume (μl)	Systems used	Sample size	Time (s)	Cost ($)	Units
Bayer contour(Glucose)	0.6	Winter passerines, buntings, murres	70	5	$0.50	mg/dL
CardioChek (Cholesterol)	15	Mergansers, winter passerines, buntings, migrating passerines, kittiwakes	89	60	$4.00	mg/dL
CardioChek (Triglycerides)	15	All	137	60	$4.00	mg/dL
Abaxis VetScan VS2(Cholesterol)	100 (diluted 1:2)	Winter passerines	34	720	$24.00	mmol/L
Precision XTra (Ketones)	1.5	Migrating passerines, murres	66	10	$2.00	mg/dL
Precision XTra (Glucose	1.5	Migrating passerines	23	10	$2.00	mg/dL
Nova Max Plus (Ketones)	1.5	Migrating passerines	19	10	$2.00	mg/dL
Nova Max Plus (Glucose)	1.5	Migrating passerines	17	10	$2.00	mg/dL
CentriVet (Glucose)	1.5	Migrating passerines	19	10	$2.00	mg/dL
CentriVet (Ketones)	1.5	Migrating passerines	20	10	$2.00	mg/dL

Because all T4 measurements yielded no results (samples were too dilute), we do not report T4 values. Values were converted to mg/dL for comparison with laboratory values.

Precision XTra and Nova Max Plus are handheld POC devices that are used to measure ketone (range = 0.1–8 mmol/L) and glucose (Precision Xtra detection range = 50–300 mg/dL; Nova Max detection range = 20–600 mg/dL) parameters in humans (Abbott Inc, 2008; Nova Biomedical, 2012), while the CentriVet meter is used to measure the same parameters but in various companion and domesticated animals using species specific reagent strips (Glucose detection range = 10–600 mg/dL; Ketone detection range = 0–8 mmol/L) (CentriVet, 2016). We used the CentriVet bovine blood glucose and ketone test strips, as there are no avian versions available. The test strips for these three devices are placed into the meter and the tip of the strip is applied directly to the blood sample. These devices measure the electrical current produced by the reaction between the enzymes in the strips and the blood glucose or β-hydroxybutyrate, which is correlated to their concentrations in the sample.

2.3. Case studies

2.3.1. Case study 1: Stress response to additional food provisioning in black-legged kittiwakes through plasma metabolite measurements

Black-legged kittiwakes (*Rissa tridactyla*) in our case study are part of an ongoing monitoring project of a breeding colony in Middleton Island, Alaska. The birds are divided into two experimental groups: fed and unfed. The unfed birds rely on their daily foraging trips in order to obtain their food, whereas the fed group is provided with additional food three times per day throughout the duration of the breeding season. A total of 122 nesting adult kittiwakes (42 fed, 80 unfed) were captured by hand at their nests and sampled once for glucose, triglycerides and β-hydroxybutyrate using the Bayer Contour, CardioChek and Centrivet devices respectively. Cholesterol was measured using both the VetScan and CardioChek devices.

2.3.2. Case study 2: Effects of time of day on triglyceride concentrations of two species of migratory songbirds

We sampled 31 Tennessee warblers (*Leiothlypis peregrina*) and 63 Swainson's thrushes (*Catharus ustulatus*) caught by mist-nets during the fall migration monitoring period at the McGill Bird Observatory (MBO), located in St-Anne de Bellevue, Quebec. Blood was collected from the brachial vein of each bird and analyzed in situ for triglycerides using the CardioChek POC device. Even though only relative values were needed for the purpose of this study, the POC values were corrected using the CardioChek correlation equation once the device was successfully validated.

2.3.3. Case study 3: Association between nutritional status and foraging effort in thick-billed murres

We attached time-depth recorders (TDRs) (16 g; Technosmart, Italy) to the back of thick-billed murres (*Uria lomvia*) at Coats Island. Following Elliott et al. (2008), we used the sequential differences method to sub-divide diving periods into dive bouts, and recorded the surface interval within each dive bout. Because there is an exponential relationship between surface interval duration and dive duration, we calculated the residual of ln(surface intervals) on dive duration. Following deployment of TDRs, birds were captured in the colony after their foraging trip and blood metabolite levels were measured with CardioChek, Precision and Bayer POCs.

2.4. Laboratory analysis

Frozen plasma samples were thawed and vortexed prior to being analyzed in the laboratory. Laboratory kits: Cayman Chemical Glucose Colourimetric Assay Kit, Cell Biolabs Total Cholesterol Assay Kit (Colourimetric), Wako LabAssay Triglyceride Kit and AAT Bioquest Colourimetric β-hydroxybutyrate Assay Kit were used for measuring glucose, cholesterol, triglycerides and β-hydroxybutyrate respectively. In black-legged kittiwake chicks, the Fujifilm L-type triglyceride kit was used. This kit measures triglycerides without taking free glycerol into account, unlike the Wako LabAssay Kit which does. We tested both kits to determine if there were significant differences in the results between the two. To do this, we analyzed winter passerine plasma with both the Wako LabAssay Kit and the glycerol-blanking Fujifilm L-Type triglyceride kit. All of the kits were performed following the manufacturer's protocol (Cayman Chemical Company, 2019; Cell Biolabs Inc., 2012; Wako Chemicals USA, 2017; AAT Bioquest, 2017; Fujifilm Wako Pure Chemical Corporation, 2018) using a BioTek Epoch 2 Microplate Spectrophotometer (Biotek Instruments Inc., 2014). Replicates of the measurements were done for all five of the laboratory kits performed.

2.5. Statistical analysis

To determine the accuracy of and validate the POC devices, results obtained from the laboratory kits were used as references for comparison with the corresponding results from POC devices. Paired two-tailed *t*-tests were performed for each biochemical parameter (glucose, cholesterol, triglycerides and β–hydroxybutyrate) to assess the measurement differences between each set of POC device and laboratory results. Correlations between POC device and laboratory measurements were also examined using linear regression. Regression results with an R^2 higher than 0.7 were considered strongly correlated. To test whether relationships varied among families, we used an ANOVA to determine whether the POC-lab value residuals varied among families.

Regression and paired t-tests are not good measures of reproducibility because they conflate between-sample variability with technique accuracy (Altman and Bland, 1983; Lin, 1989). Thus, as recommended by Altman and Bland (1983), we also report coefficient of variation. Concordance correlation coefficients (CCC) were also calculated to assess concordance between lab and device values (Lin, 1989). However, in our study, we do not expect 1:1 agreement between lab and POC values; rather, we expect to have reproducibility in relative values and

develop calibration equations that allow conversion of POC values into lab-equivalent values. In contrast to the main example of Altman and Bland (1983) where absolute accuracy is important (determining the likely birth date of a child), most physiological applications will be interested in the relative ranking and magnitude of values. In this case, it is the accuracy of a device relative to the inter-individual variability that is important, and so both t-tests and regressions are helpful tests.

Case study 1 examined whether metabolites were related to nutritional status (feeding rates). Thus, to examine whether values varied between fed and unfed kittiwakes, we used a t-test for each metabolite after testing for normality. Case study 2 examined whether metabolites were related to daily fattening rates at migratory stopovers, where birds lose mass each night and gain mass through the day. Thus, to examine whether triglycerides varied with time of day in migrants, we used a regression between hours since sunrise and triglyceride levels. This regression was calculated separately for each species. Case study 3 examined whether metabolites were related to foraging behavior, under the assumption that longer surface intervals are associated with less time spent foraging in diving birds. Thus, to examine whether metabolites were associated with residual surface interval on time spent diving (assuming an exponential relationship between time spent diving and surface intervals), we used a regression of metabolites against residual surface interval on dive duration.

3. Results

3.1. Validation of the devices

Most POC devices correlated with measurements from the laboratory ($R^2 > 0.7$), with the exception being glucose levels measured with the CardioChek device (Table 3). Although relative levels of blood parameters were correlated among many of the POC devices, absolute levels were often different (Figs. 2-6). See Appendix for absolute values.

β-hydroxybutyrate levels measured with the Nova Max Plus, CentriVet and Precision XTra POC devices all strongly correlated with measurements from the laboratory ($R^2 > 0.8$) (Fig. 2). However, absolute levels differed significantly from laboratory values. Laboratory values were quite high and fell out of the typical range for birds, which could be due to a dilution error during the laboratory assay. However, all the values were strongly correlated with the POCs and therefore, without another validation attempt, these devices could only currently be used if relative values are needed. A new validation attempt would be needed to determine if these devices can be used for more than relative values. Furthermore, levels measured with the Precision Xtra correlated with measurements from the CentriVet and Nova Max Plus, and levels measured with the CentriVet correlated with levels measured with the Nova Max Plus (Table 3). The relationship was significant and with $R^2 > 0.8$ for all families with a sample size greater than five, except for thick-billed murres, which had the smallest range of β-hydroxybutyrate values of all birds sampled. The confidence interval for slope and intercept overlapped among all families tested with a sample size greater than five. The coefficient of variation (CV) or variability of the devices averaged 3.3% to 5.6% (Table 4).

Glucose levels measured with the Contour POC device correlated with measurements from the laboratory ($R^2 > 0.8$) (Fig. 3a). The relationship had an $R^2 > 0.5$ for all families with a sample size greater than five. Concordance between values was significant (CCC = 0.86). The confidence interval for slope and intercept overlapped among all families tested with a sample size greater than five. Levels measured using the Contour on plasma correlated with levels measured using the Contour on whole blood ($R^2 = 0.76$) and in the lab using plasma ($R^2 = 0.81$) (Table 3). Also, glucose levels measured with the Contour device strongly correlated with levels in the Precision Xtra ($R^2 = 0.76$; CCC = 0.78) and Centrivet ($R^2 = 0.90$; CCC = 0.92) (Fig. 3b, Table 3), but were weakly correlated to the Nova Max ($R^2 = 0.47$; CCC = 0.51). Glucose levels measured with the CardioChek meter were only weakly correlated ($R^2 = 0.18$) to levels measured in the laboratory (Table 3). The CV of the devices averaged 2.8% to 7.9% and that of the lab assay was 2.9%.

Cholesterol levels from the lab correlated with the CardioChek ($R^2 > 0.76$) and VetScan VS2 ($R^2 > 0.83$) POC field measurements (Fig. 4). The CardioChek and VetScan VS2 also correlated with one another ($R^2 > 0.78$). The relationship was significant and with $R^2 > 0.7$ for all families with a sample size greater than five, except for thrushes, which had the smallest range of cholesterol values of any group. The confidence interval for slope and intercept overlapped among all families tested with a sample size greater than five. CCC values however were quite different, with the CardioChek device showing higher concordance (CCC = 0.79) between POC and lab values than the VetScan (CCC = 0.32). CV for the laboratory assay was of 2.25%.

Triglycerides measured from the CardioChek correlated with

Table 3
Regression values of each metabolite between all POC measurements and laboratory results. Comparisons are shown for each POC device and its corresponding value measured in the laboratory, and in some cases, between other devices. Correction equations are also presented, except for β -hydroxybutyrate for which accurate metabolite values were not obtained from the lab assay and for correlations with an R^2 lower than 0.7.

Device	Comparison	Metabolite	Correction equation	R2	T value	df	P value
CardioChek	Laboratory	Cholesterol	Lab = 1.04 * CardioChek – 18.4	0.76	3.99	74	< 0.001
CardioChek	Laboratory	Glucose	–	0.18	2.56	30	0.02
CardioChek	Laboratory	Triglycerides	Lab = 1.72 * CardioChek – 21.1	0.79	7.59	78	< 0.0001
Contour	Laboratory	Glucose	Lab = 0.80 * Contour (blood) + 72.7	0.82	5.5	69	< 0.0001
Contour (Plasma)	Laboratory	Glucose	Lab = 0.74 * Contour (plasma) – 6.14	0.81	25.7	68	< 0.0001
Contour (Plasma)	Contour (whole blood)	Glucose	Contour(blood) = 0.83 * Contour(plasma) – 62.1	0.76	14.6	66	< 0.0001
Contour	Precision Xtra	Glucose	Contour = 0.90 * Precision – 8.48	0.76	8.21	21	< 0.0001
Contour	CentriVet	Glucose	Contour = 1.06 * CentriVet – 32.2	0.90	12.4	17	< 0.0001
Contour	Nova Max Plus	Glucose	–	0.47	3.67	15	< 0.001
CentriVet	Laboratory	β -hydroxybutyrate	–	0.84	5.37	18	< 0.0001
CentriVet	Nova Max Plus	β -hydroxybutyrate	Nova Max = 1.04 * Centrivet – 0.63	0.96	19.5	18	< 0.0001
CentriVet	Nova Max Plus	Glucose	–	0.51	3.99	15	< 0.0001
VetScan	Laboratory	Cholesterol	Lab = 1.79 * VetScan – 46.7	0.83	12.4	33	< 0.0001
VetScan	CardioChek	Cholesterol	CardioChek = 1.49 * VetScan +17	0.78	10.4	33	< 0.0001
Nova Max Plus	Laboratory	β -hydroxybutyrate	Lab = 4.00 * Nova Max +0.2398	0.83	6.09	17	< 0.0001
Precision Xtra	Laboratory	β -hydroxybutyrate	–	0.83	8.43	55	< 0.0001
Precision Xtra	CentriVet	β -hydroxybutyrate	CentriVet = 0.85 * Precision – 0.115	0.97	25.7	19	< 0.0001
Precision Xtra	Nova Max Plus	β -hydroxybutyrate	Nova Max = 0.90 * Precision – 0.801	0.94	16.6	18	< 0.0001
Precision Xtra	CentriVet	Glucose	Precision = 0.89 * CentriVet +43.8	0.77	7.40	16	< 0.0001
Precision Xtra	Nova Max Plus	Glucose	–	0.60	4.54	14	< 0.0001

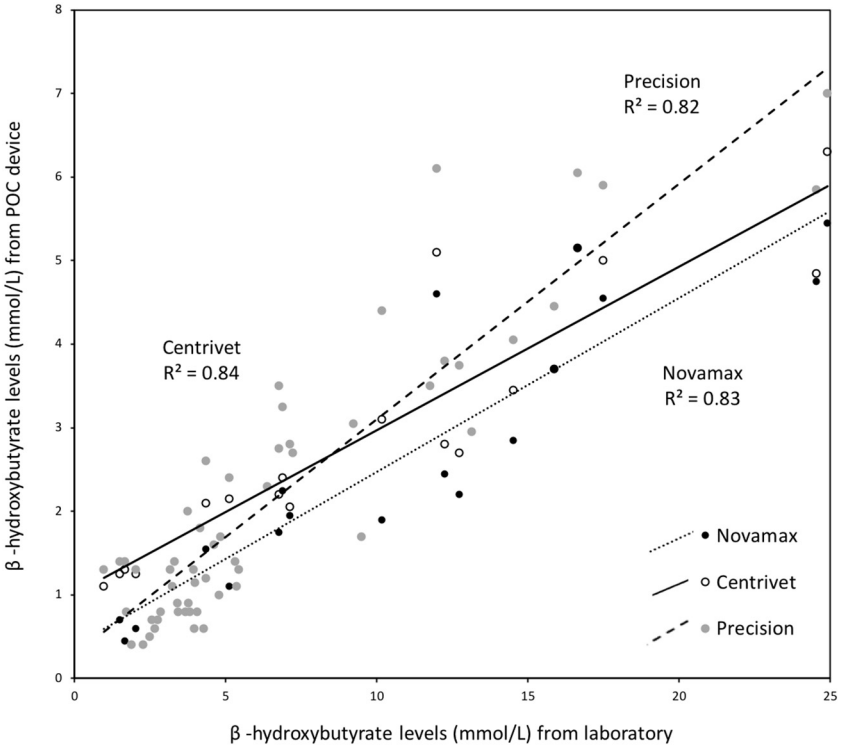

Fig. 2. β-hydroxybutyrate levels measured using Nova Max Plus®, CentriVet and Precision XTra POC device on several bird species correlated with β-hydroxybutyrate levels measured in the laboratory. However, absolute levels differed significantly from laboratory values. Sample sizes per taxa are shown in the Appendix. POC device values are shown in the y-axis in a regression relative to laboratory values.

measurements from the lab ($R^2 > 0.78$) (Fig. 5). If we remove the apparent outlier value, the relationship is still significant with $R^2 = 0.7$. However, we believe this is an actual valid point that was exceptionally high for that individual and should therefore still be included. The relationship was also significant and with $R^2 > 0.7$ for all families with a sample size greater than five. The confidence interval for slope and intercept overlapped among all groups tested with a sample size greater than five. Black-legged kittiwake triglyceride values also correlated significantly with measurements from the lab with $R^2 = 0.84$, however this correlation was not the same as for other families. For this reason, kittiwake chick values and validation equations are included separately on Fig. 6. There was no significant difference between the Wako LabAssay kit and the glycerol-blanking Fujifilm L-type Triglyceride kit values when tested in winter passerines, suggesting that this different correlation in kittiwakes may be due to other unknown factors related to the different blood biochemical parameters of this species. The CCC between POC and lab values for all families was of 0.6. The CV for the lab assay was of 2.28%.

3.2. Case studies

3.2.1. Kittiwakes

In both fed and unfed groups of adult kittiwakes, triglyceride levels were often above the limit of detection of the device (> 500 mg/dL). Therefore, no analysis was performed on triglycerides. However, glucose levels were significantly higher on fed birds, while β-hydroxybutyrate and cholesterol levels were higher in unfed birds (t-test; $p < .01$ for all three metabolites) (Fig. 7).

3.2.2. Migrants

Triglyceride levels of the two migratory passerine species sampled with a CardioChek device at a banding station increased with time of day ($p < .01$) (Figure 8a). When both species were analyzed separately, this correlation was only apparent for Tennessee warblers ($p < .03$).

3.2.3. Murres

Triglyceride levels were often below the detection limit of the device. Therefore, no analysis was performed on triglycerides. Glucose levels decreased and β-hydroxybutyrate levels increased with surface interval duration, but there was no relationship with cholesterol (Fig. 8b).

4. Discussion

POC devices, once validated, accurately measured the nutritional state of a wide range of wild bird species. The ease of use of these devices allows assays to be done by almost any researcher under most field conditions— provided they have the appropriate blood sampling and ethical training. Many 'citizen science' initiatives have revolutionized our understanding of population trends for many taxa. However, measures of individual physiology—which may be early warning signals for population declines—are typically completed by specialized researchers. POC devices provide the opportunity to expand the number of researchers who can easily perform such measurements. Indeed, a variety of POC devices accurately measured relative levels of four plasma metabolites, representing a 'nutritional panel' that indicated the nutritional status of wild birds. The relative variability of the devices (< 10% CV for all devices) matched the level typically found acceptable in the lab. Nonetheless, absolute levels of various blood parameters were often inaccurate (until corrected by calibration equations), and values could not be used to test whether individual birds fell within a certain reference range, unless a particular device was validated and a calibration equation obtained. Coupling the development of correction formulae in the laboratory with measurements in duplicate, and taking a triplicate measurement when duplicates vary by > 15%, reliable measurements are obtainable in wild birds. We recommend the use of these POC devices for the measurement of nutritional state in wild birds.

The case studies also demonstrated the potential of POC devices in field studies. Food-supplemented birds had a different metabolite panel than non-supplemented ones, providing direct evidence that plasma metabolites represent the nutritional state of individual birds.

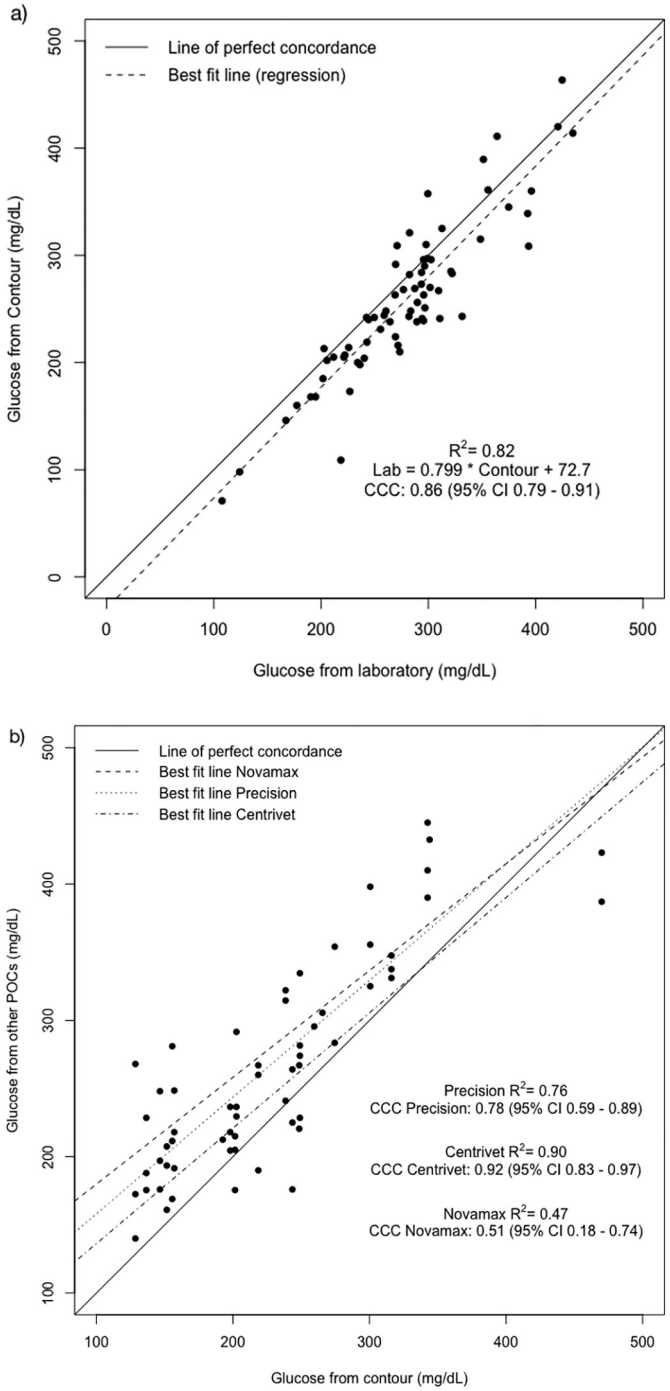

Fig. 3. (a) Glucose levels measured with a Contour POC device correlated with levels measured in the laboratory for five species from two families (b) Glucose levels measured with a Contour point-of-care device correlated with levels measured using the CentriVet and Precision XTra devices for seven species from five families. Sample sizes per taxa are shown in the Appendix.

Measurements of metabolites at remote sites without electricity currently require blood cards, preservation of samples in ethanol, or the use of a dry shipper—the use of POC devices obviates those needs and allows for measurement in real-time. Furthermore, indigenous groups increasingly wish to be empowered to conduct science themselves, and such devices could allow, for instance, Inuit to measure the health of wildlife they harvest or monitor. In our second case study, we showed that migration monitoring sites could use POC devices to potentially estimate daily fattening rates during stopover. Given that hundreds of thousands of songbirds are handled annually by bird-banding sites, we could potentially learn more about their health via a small blood sample. With a larger sample size, we may uncover important trends in glucose, triglycerides, β-hydroxybutyrate and cholesterol that could not only be used to assess the overall health and stopover refueling performance of our migratory bird populations but also to determine differences in habitat quality between stopover sites (Guglielmo et al., 2005). In our final case study, we used tracking devices to monitor foraging effort. Those individuals with high foraging effort (short surface intervals) had higher glucose and lower β-hydroxybutyrate, implying that we can use POC devices to obtain estimates of foraging success.

The use of whole blood versus plasma did not create significant variability. All POC measurements used for validation were on whole blood while all lab measurements were on plasma, yet the values were strongly correlated. Indeed, when we used Contour POC devices to measure glucose levels in both plasma and whole blood, the values were highly correlated. As such, we argue that levels of all four metabolites in whole blood are representative of levels in plasma. By conducting measurements in whole blood, field logistics are greatly facilitated by removing the need for centrifugation. Moreover, levels of cholesterol in diluted blood were strongly correlated with those in whole blood. Thus, if previously validated, measurements in diluted blood can be acceptable, facilitating sampling from small birds where blood volume is limited or in birds where certain metabolites are higher than the measuring limit of the device, such as triglycerides in kittiwakes. One exception was when measuring triglyceride levels in murres, as the levels in whole blood were too low to be reliably measured with the CardioChek device.

4.1. Validations between POC devices and laboratory results were similar among most species and between the majority of devices

Validations derived similar relationships for all species groups (except black-legged kittiwakes). Similarly, a variety of different POC devices were all correlated with one another. Relationships were stronger for those devices measuring levels electrically (most devices) than those relying on colourimetric determination (e.g., CardioChek). Nonetheless, all devices were accurate enough to provide biologically-relevant information useful in combination with field observations for insight into the behaviour of the individual being sampled.

4.1.1. Triglycerides

Triglycerides are used by the muscles as a source of energy and are also taken up by adipose tissue for storage (Buyse and Decuypere, 2015), which are very important for migratory birds as a major metabolic fuel during sustained flights. Variations in triglyceride levels among individuals can be due to differences in body mass and migratory distance (Braun, 2015). For example, high lipid stores of snow buntings, needed to migrate through regions where access to food may be limited by snow, could explain the high triglycerides compared to other species sampled. In our case, the differences in triglyceride trends throughout the day between our two songbird species could be due to a difference in their foraging behaviour or diet. Triglyceride levels measured using the CardioChek were mostly within the expected range (90–197 mg/dL) (Stanford, 2006). The device underestimated blood triglyceride concentrations by approximately 18%. As CardioChek values were more variable than other POC devices (for other parameters), we recommend measuring values in duplicate.

4.1.2. Glucose

Blood glucose concentrations are significantly higher in birds than in mammals of similar body mass (Scanes, 2015b). Variations in blood glucose levels among individuals may be due to differences in the time of day when sampled, amount of food intake, stress levels and ambient temperatures. Blood glucose concentrations can be lower early in the

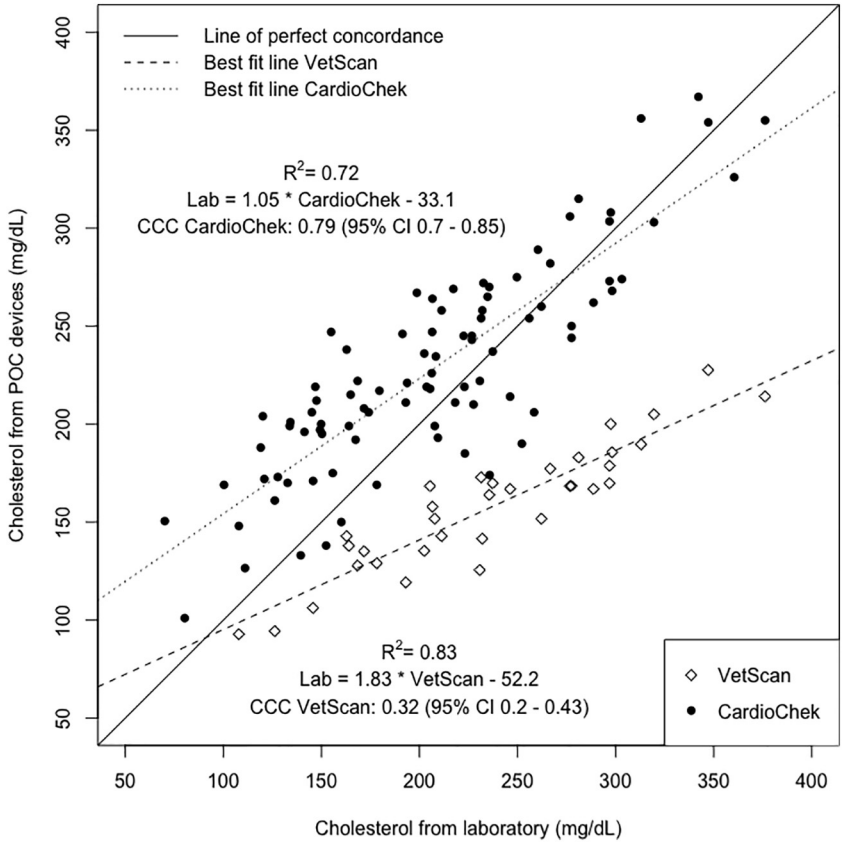

Fig. 4. Cholesterol levels measured using a CardioChek and VetScan VS2 POC device correlated with cholesterol levels measured in the laboratory. The CardioChek device ($N = 75$) was tested on 14 species from six families. The VetScan VS2 device ($N = 34$) was tested on 29 individuals from two families.

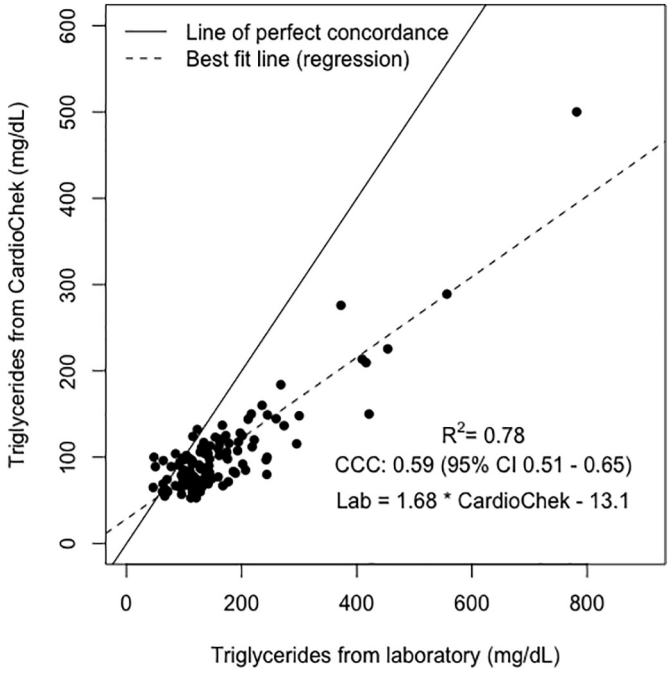

Fig. 5. Triglyceride levels measured using a CardioChek POC device in 113 birds from six families (11 species) correlated with triglyceride levels measured using a laboratory kit.

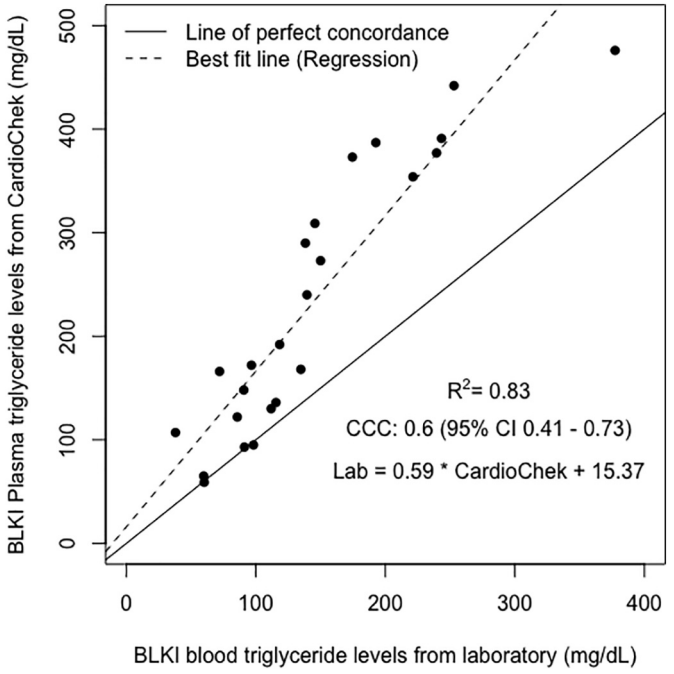

Fig. 6. Triglyceride levels of black-legged kittiwake (BLKI) chicks measured using a CardioChek POC device correlated with triglyceride levels measured using a laboratory kit.

Table 4
Coefficient of variation (CV) and range for all POC devices. The devices were tested on 18 species of birds (N = 190) spanning nine families from six different projects (Winter passerines, buntings, passerine migration, murres, mergansers and kittiwakes).

Device	Metabolite	CV	Range
CardioChek	Cholesterol	9.1%	0–29.9%
CardioChek	Triglycerides	8.3%	0–30.3%
CardioChek	Glucose	7.5%	0–26.5%
Contour	Glucose	7.9%	0–28.8%
CentriVet	β -hydroxybutyrate	3.3%	0–12.9%
CentriVet	Glucose	2.8%	0–12.2%
VetScan	Cholesterol	2.6%	0–10.1%
Nova Max Plus	β -hydroxybutyrate	5.6%	0–20.2%
Nova Max Plus	Glucose	6.3%	1.6–22.7%
Precision Xtra	β -hydroxybutyrate	3.9%	0–13.2%
Precision Xtra	Glucose	5.4%	0–19.8%

morning after overnight fasting (Downs et al., 2010; Frelin, 1974), as both the amount of prior food intake (Savory, 1987) and elapsed time since recent feeding (Lill, 2011), can contribute to variation in blood glucose concentrations. In addition, the elapsed time between capture and blood sampling can influence blood glucose concentrations, as a result of a stress response that increases concentrations during capture and handling (Lynn and Porter, 2008; Romero and Romero, 2002). Therefore, longer handling times may result in elevated blood glucose measures that are an inaccurate representation of the normal levels. In addition, blood glucose concentrations are significantly higher at lower ambient temperatures due to increased requirements for maintaining body temperature (Downs et al., 2010). This latter influence may explain why snow buntings have the highest average blood glucose levels, as samples were collected at lower temperatures. Blood glucose concentrations in birds range between 126 and 456 mg/dL (Harr, 2002), with an overall average of 244 mg/dL (Scanes, 2015b). However, there are significant differences across and within orders (Lill, 2011; Scanes, 2015b). The blood glucose levels of the species in our study were within the expected range.

4.1.3. β-hydroxybutyrate

Ketones, such as β-hydroxybutyrate, are representative of lipid utilization and fasting (Castellini and Rea, 1992). During fasting, fatty acids are converted to ketone bodies (β-hydroxybutyrate) in the liver to spare carbohydrates and protein from being used as glucose precursors. Ketones are subsequently metabolized to provide energy to peripheral tissues in need, especially those unable to catabolize fatty acids (e.g. the brain) (Castellini and Rea, 1992; Robinson and Williamson, 1980). Thus, levels of β-hydroxybutyrate are positively correlated with time since the last meal and total mass loss of the bird (Alonso-Alvarez and Ferrer, 2001).Variation in levels of β-hydroxybutyrate between species could be due to differences in time of sampling, foraging efficiency, refueling, and whether or not the bird had just arrived from a migratory flight (Guglielmo et al., 2005; Jenni-Eiermann et al., 2002; Jenni-Eiermann and Jenni, 1991). In passerines, β-hydroxybutyrate levels are often higher early in the morning, suggesting a correlation with food intake (Azeredo et al., 2016). In migratory birds, β-hydroxybutyrate levels are known to be an indicator of stopover-refueling efficiency, with birds stopping at low quality sites having higher β-hydroxybutyrate levels than birds at higher quality sites (Guglielmo et al., 2005). Blood β-hydroxybutyrate levels of the 10 species sampled with the POC devices were all within the expected ranges, with thick-billed murres and red-breasted mergansers showing lower concentrations than migratory passerines, likely because seabirds are able to obtain food more readily compared to migrating birds.

4.1.4. Cholesterol

Blood cholesterol concentrations are proportional to the long-term dietary intake of individuals and could be reflective of their feeding success and nutritional state (Whiteman et al., 2013). Variation in cholesterol concentrations between individuals may be due to differences in food intake and diet (Stanford, 2006). The average blood cholesterol levels measured using both the VetScan VS2 and CardioChek were within the expected range (90–320 mg/dL) (Harr, 2002). For the VetScan VS2, blood cholesterol levels were underestimated by approximately 31% on average. However, despite using diluted samples, VetScan VS2 measurements were still strongly correlated with laboratory results, which accurately predict the laboratory results with little error. On the other hand, the CardioChek overestimated blood cholesterol levels by approximately 5%. CardioChek measurements were more variable and less correlated with laboratory results than the VetScan VS2, and we recommend the use of VetScan VS2—or CardioChek in duplicate.

4.2. Recommendations for use of POC devices on wild birds

The POC devices assessed are validated for adaptive use in the field to measure blood biochemistry levels. We recommend use of all six POC device values in wild birds in association with calibration equations if

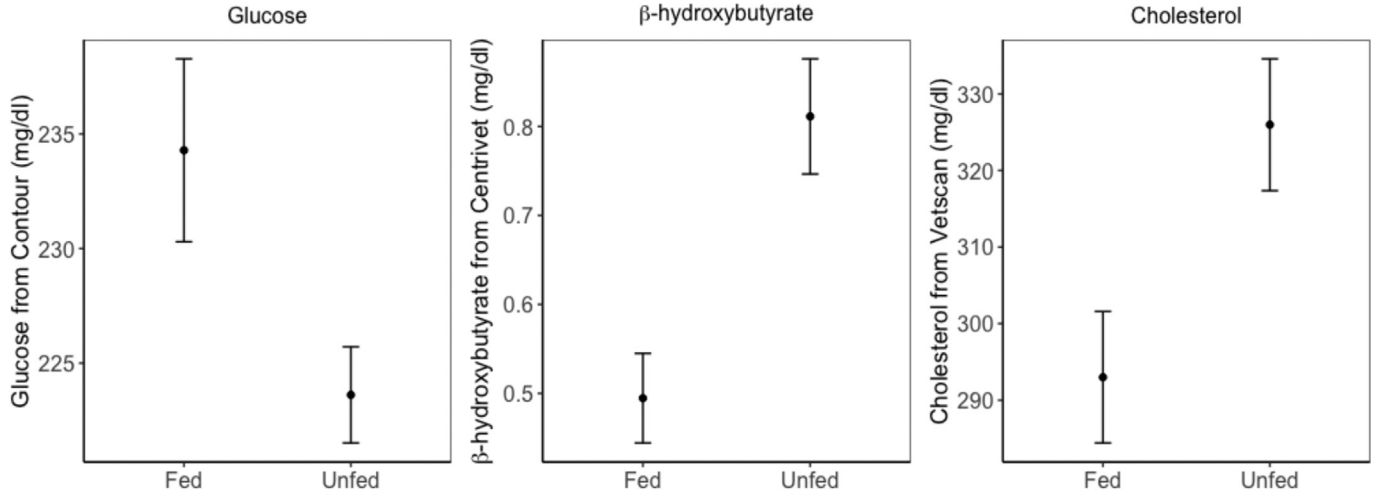

Fig. 7. Comparison of glucose, β-hydroxybutyrate and cholesterol levels between fed and unfed black-legged kittiwakes measured at a remote subarctic island using three different POC devices (Contour, CentriVet and VetScan). The differences between both experimental groups were significant for all three metabolites tested ($p < .01$).

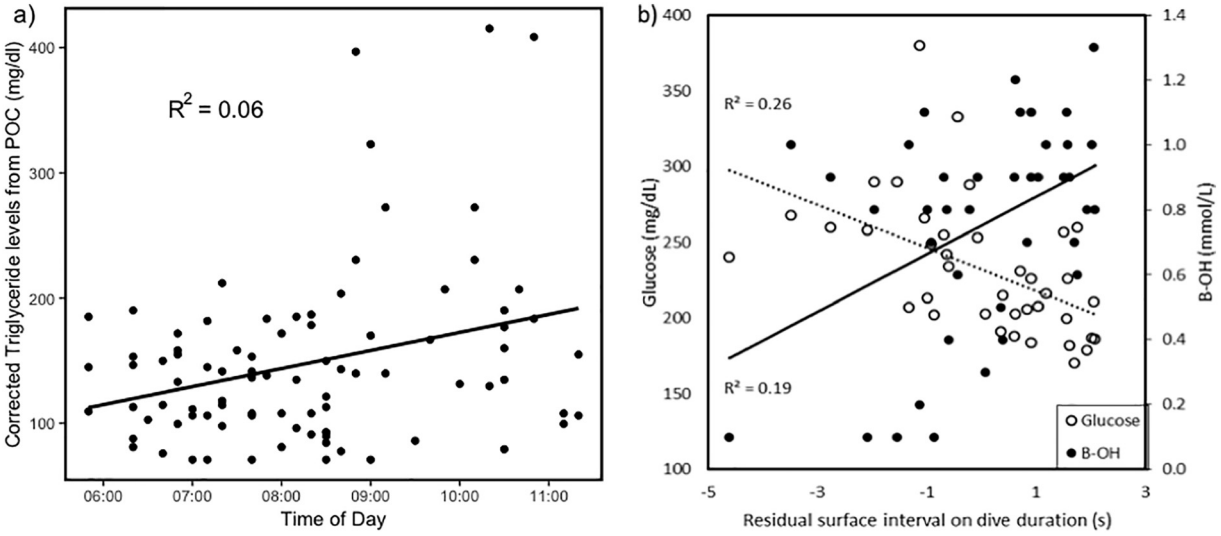

Fig. 8. (a) Triglyceride levels increased with time of day in Swainson's thrushes and Tennessee warblers sampled at a migration monitoring station using a CardioChek device ($p < .01$). (b) Glucose decreased and β-hydroxybutyrate increased with surface interval duration in thick-billed murres.

possible (except the CardioChek for glucose). Our suite of POC devices can likely be used for most species within the families we validated, and are likely accurate for most Neoaves, especially passerines. However, we encourage validation of these devices for other species, including other non-passerine families. Devices using electronic measurements rather than colorimetric measurements are preferable. The VetScan VS2 was accurate at predicting blood cholesterol concentrations. However, its relatively larger size, required storage (rotors need to be refrigerated) and considerable power requirements may limit its use in remote field conditions. For CardioChek (which uses a colorimetric assay), checking colour development within the test strips is important, as well as discarding any samples where colour development is not uniform.

With the exception of the VetScan VS2, the different POC devices tested require a relatively small volume of blood (Table 2). The total volume of blood on a 7-g bird is thus below the maximum recommended volume corresponding to 1% of its total body weight (McGuill and Rowan, 1989). Indeed, a total of 64.6 μL for all four measurements in duplicate (excluding the VetScan) allows that any bird over 7 g in weight (> 99% of all bird species) can be sampled in duplicate with the entire panel.

The costs varied from $0.50 to $20 per strip (Table 2) while the assay kits assessed cost $200–$400 each, and typically measure 200 samples. The only exception was the triglyceride kit, which can measure 800 samples. With standards and duplicates, the kits typically cost $2.50 to $5.00 per sample, or $0.60 for triglycerides. Thus, with the exception of the VetScan VS2, the POC meters ($21 per individual for the entire panel in duplicate) were comparable in price to lab assays ($15.60 per individual for the entire panel in duplicate). Additionally, the costs of laboratory equipment for analyzing samples in the lab (e.g. Microplate spectrophotometer, > $25,000) are considerable for small projects with limited funding, in comparison to the affordability of POC devices (less than $100 per device for all ketone meters, glucose meters and CardioChek meters used in this study; with the exception of the VetScan VS2 which costs over $10000). Furthermore, lab assays require a significant amount of time to prepare and run, and samples must be properly stored and transported from the field to the lab. Consequently, the total costs of lab assays can be much higher due to storage, transportation and labor costs. In addition, risks for sample loss and mix-up during storage and transportation are avoided.

In conclusion, the success of plasma metabolite analyses and their increased popularity for studies of free-living wildlife and veterinary medicine, make the potential use of POC devices in remote field studies more relevant than ever. Metabolite profiling can be of great importance in field physiology by allowing the rapid pairing of physiological measurements with behavioural and ecological data in the field. For example, the ability to match metabolite values with in-field observations can lead to better understanding of behavioural observations, morphological measurements or an individual's current physical appearance and condition. It can also assist interpretation of climatic/habitat relationships associated with blood chemistry measurements that affect starvation or otherwise low fitness. Moreover, given the financial and time costs associated with lab assays, especially if samples must be transported, we argue that POC devices show strong promise for use in field biology.

Authors' contributions

C.L., B.F. and K.E. initially conceived the study, with all authors contributing to designing the conceptual framework. A.M, C.L., Z.B., S.W., K.E., and R.T. collected the data; A.M., C.L. and K.E. conducted the analysis; A.M, C.L and K.E. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for the publication.

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Data accessibility

Data available as a supplementary materials file.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.110594.

References

AAT Bioquest, 2017. Colorimetric β-Hydroxybutyrate (Ketone Body) Assay Kit. AAT Bioquest®, Sunnyvale, CA.

Abaxis Inc, 2003. VetScan ® Avian Reptilian Profile Plus. (Union City, CA).

Abaxis Inc, 2009. VetScan VS2® Operator's Manual. Abaxis, Inc, Union City, CA.

Abbott Inc, 2008. Precision Xtra Blood Glucose & Ketone Monitoring System Users' Manual. Abbott Diabetes Care Inc, Alameda, CA.

Acierno, M.J., Schnellbacher, R., Tully, T.N., 2012. Measuring the level of agreement between a veterinary and a human point-of-care glucometer and a laboratory blood analyzer in Hispaniolan amazon parrots (*Amazona ventralis*). J. Avian Med. Surg. 26, 221–224. https://doi.org/10.1647/2011-038R1.1.

Albano, N., Santiago-Quesada, F., Villegas, A., Sánchez-Guzmán, J.M., Masero, J.A., 2016. Plasma metabolites correlate with weekly body mass changes in migrating black-tailed godwits *Limosa limosa* feeding on different diets. J. Ornithol. 157, 201–207. https://doi.org/10.1007/s10336-015-1268-4.

Alonso-Alvarez, C., Ferrer, M., 2001. A biochemical study of fasting, subfeeding, and recovery processes in yellow-legged gulls. Physiol. Biochem. Zool. 74, 703–713. https://doi.org/10.1086/322932.

Altman, D.G., Bland, J.M., 1983. Measurement in medicine: the analysis of method comparison studies. J. Roy. Stat. Soc. 32, 307–317. https://doi.org/10.2307/2987937.

Artacho, P., Soto-Gamboa, M., Verdugo, C., Nespolo, R.F., 2007. Blood biochemistry reveals malnutrition in black-necked swans (*Cygnus melanocoryphus*) living in a conservation priority area. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 146, 283–290. https://doi.org/10.1016/j.cbpa.2006.10.031.

Azeredo, L.M.M., Oliveira, T.C., Lopez, L.C.S., Azeredo, L.M.M., Oliveira, T.C., Lopez, L.C.S., 2016. Blood metabolites as predictors to evaluate the body condition of *Neopelma pallescens* (Passeriformes: Pipridae) in northeastern Brazil. Zoologia (Curitiba) 33. https://doi.org/10.1590/s1984-4689zool-20160043.

Bayer Inc, 2011. Bayer Contour® Blood Glucose Monitoring System User Guide. Bayer Inc, Toronto, ON.

Biotek Instruments Inc Inc, 2014. Epoch 2 Microplate Spectrophotometer. Biotek Instruments, Inc, Winooski, VT.

Braun, E.J., 2015. Chapter 40 - physiological challenges of migration. In: Scanes, C.G. (Ed.), Sturkie's Avian Physiology, 6th ed. Academic Press, San Diego, pp. 975–977. https://doi.org/10.1016/B978-0-12-407160-5.00040-3.

Buyse, J., Decuypere, E., 2015. Chapter 19 - adipose tissue and lipid metabolism. In: Scanes, C.G. (Ed.), Sturkie's Avian Physiology, 6th ed. Academic Press, San Diego, pp. 443–453. https://doi.org/10.1016/B978-0-12-407160-5.00019-1.

Castellini, M.A., Rea, L.D., 1992. The biochemistry of natural fasting at its limits. Experientia 48, 575–582. https://doi.org/10.1007/BF01920242.

Cayman Chemical Company, 2017. Glucose Colourimetric Assay Kit. Cayman Chemical Company, Ann Arbor, MI.

Cell Biolabs Inc, 2012. Total Cholesterol Assay Kit (Colourimetric). Cell Biolabs, Inc, San Diego, CA.

CentriVet, G.K., 2016. CentriVet GK Blood Glucose & Ketone Meter User's Manual. ACON Laboratories, Inc, San Diego, CA.

Cuhadar, S., Koseoglu, M., Atay, A., Dirican, A., 2013. The effect of storage time and freeze-thaw cycles on the stability of serum samples. Biochem. Med (Zagreb) 23, 70–77.

Downs, C.T., Wellmann, A.E., Brown, M., 2010. Diel variations in plasma glucose concentrations of malachite sunbirds < emphasis type = "italic" > Nectarinia famosa < /emphasis > J. Ornithol. 151, 235. https://doi.org/10.1007/s10336-009-0439-6.

Elliott, K.H., 2016. Measurement of flying and diving metabolic rate in wild animals: review and recommendations. Comp. Biochem. Physiol. A Mol. Integr Physiol. 202, 63–77. https://doi.org/10.1016/j.cbpa.2016.05.025.

Elliott, K.H., Davoren, G.K., Gaston, A.J., 2008. Increasing energy expenditure for a deep-diving bird alters time allocation during the dive cycle. Anim. Behav. 75, 1311–1317. https://doi.org/10.1016/j.anbehav.2007.10.026.

Frelin, C., 1974. Daily variations of serum glucose levels in moulting redpolls *Acanthis flammea* and in migrating robins *Erithacus rubecula*. Ornis Scandinavica (Scandinavian). J. Ornithol. 5, 83–86. https://doi.org/10.2307/3676052.

Friedman, M.I., 1995. Control of energy intake by energy metabolism. Am. J. Clin. Nutr. 62, 1096S–1100S. https://doi.org/10.1093/ajcn/62.5.1096S.

Fujifilm Wako Pure Chemical Corporation, 2018. L-Type Triglyceride M Assay. Fujifilm Wako Pure Chemical Corporation, Osaka, Japan.

Gerson, A.R., Guglielmo, C.G., 2013. Energetics and metabolite profiles during early flight in American robins (*Turdus Migratorius*). J. Comp. Physiol. B. 183, 983–991. https://doi.org/10.1007/s00360-013-0767-y.

Guglielmo, C.G., Cerasale, D.J., Eldermire, C., 2005. A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. Physiol. Biochem. Zool. 78, 116–125. https://doi.org/10.1086/425198.

Halsey, L.G., Shepard, E.L.C., Wilson, R.P., 2011. Assessing the development and application of the accelerometry technique for estimating energy expenditure. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 158, 305–314. https://doi.org/10.1016/j.cbpa.2010.09.002.

Harr, K.E., 2002. Clinical chemistry of companion avian species: a review. Vet. Clin. Pathol. 31, 140–151. https://doi.org/10.1111/j.1939-165X.2002.tb00295.x.

Jenni-Eiermann, S., Jenni, L., 1991. Metabolic responses to flight and fasting in night-migrating passerines. J. Comp. Physiol. B. 161, 465–474. https://doi.org/10.1007/BF00257901.

Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindström, Å., Piersma, T., Visser, G.H., 2002. Fuel use and metabolic response to endurance exercise: a wind tunnel study of a long-distance migrant shorebird. J. Exp. Biol. 205, 2453–2460.

Jenni-Eiermann, S., Almasi, B., Maggini, I., Salewski, V., Bruderer, B., Liechti, F., Jenni, L., 2011. Numbers, foraging and refuelling of passerine migrants at a stopover site in the western Sahara: diverse strategies to cross a desert. J. Ornithol. 152, 113–128. https://doi.org/10.1007/s10336-010-0572-2.

Lieske, C.L., Ziccardi, M.H., Mazet, J.A.K., Newman, S.H., Gardner, I.A., 2002. Evaluation of 4 handheld blood glucose monitors for use in seabird rehabilitation. J. Avian Med. Surg. 16, 277–285. https://doi.org/10.1647/1082-6742(2002)016[0277:EOHBGM]2.0.CO;2.

Lill, A., 2011. Sources of Variation in Blood Glucose Concentrations of Free-Living Birds [WWW document]. https://doi.org/10.3184/175815511X13073729328092.

Lin, L.I.-K., 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45, 255–268. https://doi.org/10.2307/2532051.

Lindholm, C., Altimiras, J., 2016. Point-of-care devices for physiological measurements in field conditions. A smorgasbord of instruments and validation procedures. Comp. Biochem. Physiol. Part A 202, 99–111. https://doi.org/10.1016/j.cbpa.2016.04.009.

Lindholm, C., Altimiras, J., Lees, J., 2019. Measuring ketones in the field: rapid and reliable measures of β-hydroxybutyrate in birds. Ibis 161, 205–210. https://doi.org/10.1111/ibi.12643.

Lynn, S.E., Porter, A.J., 2008. Trapping initiates stress response in breeding and non-breeding house sparrows *Passer domesticus*: implications for using unmonitored traps in field studies. J. Avian Biol. 39, 87–94. https://doi.org/10.1111/j.0908-8857.2008.04204.x.

McGuill, M.W., Rowan, A.N., 1989. Biological effects of blood loss: implications for sampling volumes and techniques. ILAR J. 31, 5–20. https://doi.org/10.1093/ilar.31.4.5.

McGuire, L.P., Fenton, M.B., Faure, P.A., Guglielmo, C.G., 2009. Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. Physiol. Biochem. Zool. 82, 812–818. https://doi.org/10.1086/605951.

Mohsenzadeh, M.S., Zaeemi, M., Razmyar, J., Azizzadeh, M., 2015. Comparison of a point-of-care glucometer and a laboratory Autoanalyzer for measurement of blood glucose concentrations in domestic pigeons (*Columba livia* domestica). J. Avian Med. Surg. 29, 181–186. https://doi.org/10.1647/2014-020.

Morris, J.D., Fernandez, J.M., Chapa, A.M., Gentry, L.R., Thorn, K.E., Weick, T.M., 2002. Effects of sample handling, processing, storage, and hemolysis on measurements of key energy metabolites in ovine blood. Small Rumin. Res. 43, 157–166. https://doi.org/10.1016/S0921-4488(01)00266-8.

Nova Biomedical, 2012. Nova Max Plus Owner's Guide. Nova Biomedical, Waltham, MA.

Podlaszczuk, P., Włodarczyk, R., Janiszewski, T., Kaczmarek, K., Minias, P., 2017. When moult overlaps migration: moult-related changes in plasma biochemistry of migrating common snipe. PeerJ 5, e3057. https://doi.org/10.7717/peerj.3057.

Polymer Technology Systems Inc, 2010. CardioChek® Brand Analyzers Portable Whole Blood Test System User Guide. Polymer Technology Systems, Inc, Indianapolis, IN.

Quillfeldt, P., Masello, J.F., Möstl, E., 2004. Blood chemistry in relation to nutrition and ectoparasite load in Wilson's storm-petrels *Oceanites oceanicus*. Polar Biol. 27, 168–176. https://doi.org/10.1007/s00300-003-0572-2.

Robinson, A.M., Williamson, D.H., 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev. 60, 143–187. https://doi.org/10.1152/physrev.1980.60.1.143.

Romero, L.M., Romero, R.C., 2002. Corticosterone Responses in Wild Birds: The Importance of Rapid Initial Sampling. vol. 104. The Condor, Waco, pp. 129–135.

Savory, C.J., 1987. How closely do circulating blood glucose levels reflect feeding state in fowls? Comp. Biochem. Physiol. A Comp. Physiol. 88, 101–106. https://doi.org/10.1016/0300-9629(87)90106-X.

Scanes, C.G., 2015a. Chapter 10 - blood. In: Scanes, C.G. (Ed.), Sturkie's Avian Physiology, 6th ed. Academic Press, San Diego, pp. 167–191. https://doi.org/10.1016/B978-0-12-407160-5.00010-5.

Scanes, C.G., 2015b. Chapter 18 - Carbohydrate metabolism. In: Sturkie's Avian Physiology, 6th ed. Academic Press, San Diego, pp. 421–441. https://doi.org/10.1016/B978-0-12-407160-5.00018-X.

Schaub, M., Jenni, L., 2001. Variation of fuelling rates among sites, days and individuals in migrating passerine birds. Funct. Ecol. 15, 584–594. https://doi.org/10.1046/j.0269-8463.2001.00568.x.

Sommers, A.S., Boyle, W.A., McGuire, L.P., 2017. Validation of a field-ready handheld meter for plasma β-hydroxybutyrate analysis. J. Field Ornithol. 88, 399–404. https://doi.org/10.1111/jofo.12233.

Speakman, J., 1997. Doubly Labelled Water: Theory and Practice. Springer, US.

Stanford, M., 2006. Significance of Cholesterol Assays in the Investigation of Hepatic Lipidosis and Atherosclerosis in Psittacine Birds.

Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. Conserv. Physiol. 2. https://doi.org/10.1093/conphys/cou011.

Wako Chemicals USA, 2017. LabAssay ™ Triglyceride. Wako Chemicals USA, Richmond, VA.

Whiteman, J.P., Frank, N., Greller, K.A., Harlow, H.J., Ben-David, M., 2013. Characterization of blood lipoproteins and validation of cholesterol and triacylglycerol assays for free-ranging polar bears (Ursus maritimus). J. Vet. Diagn. Investig. 25, 423–427. https://doi.org/10.1177/1040638713486114.