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EVALUATION OF HAND-HELD METERS FOR DETERMINATION OF HEMOLYMPH LACTATE AND PROTEIN CONCENTRATIONS IN RED SWAMP CRAYFISH *PROCAMBARUS CLARKII*

Christopher P. Bonvillain [1,*], D. Allen Rutherford [1], William E. Kelso [1], and Christopher C. Green [2]

[1] School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
[2] Louisiana State University Agricultural Center, Aquaculture Research Station, 2410 Ben Hur Road, Baton Rouge, LA 70820, USA

ABSTRACT

We evaluated the accuracy of an Accutrend® lactate meter and a Reichert® Vet 360 temperature compensated refractometer for determining red swamp crayfish *Procambarus clarkii* (Girard, 1852) hemolymph lactate and protein concentrations, with results compared to measurements obtained from spectrophotometric analyses. Results from the lactate meter ($R^2 = 0.926$) and refractometer ($R^2 = 0.830$) both demonstrated strong relationships with spectrophotometer measurements, indicating that these instruments provide rapid, reliable, and economical methods for determining crayfish hemolymph lactate and protein concentrations. Both meters can provide on-site information to commercial crayfish farmers and processors to assess crayfish health and stress levels, and can also provide important biomonitoring data to field biologists as they evaluate the success of water management activities designed to reduce hypoxia and improve habitat conditions for aquatic biota.

KEY WORDS: Cambaridae, lactate meter, Louisiana, metabolite, refractive index, refractometer

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INTRODUCTION

The crayfish industry in Louisiana is the largest in the United States (McClain et al., 2007) with a total value of more than $209 million (LSUAC, 2012). Crayfish are harvested either from managed ponds (farmed), or natural habitats (wild): the majority of landings consisting of the red swamp crayfish *Procambarus clarkii* (Girard, 1852). Water quality parameters fluctuate both spatially and temporally in both farmed and wild crayfish habitats and hypoxia (dissolved oxygen $\leqslant$ 2.0 mg/l) is common in these shallow, often stagnant systems (Avault et al., 1975; McClain, 1999; Sabo et al., 1999; Rutherford et al., 2001; Kaller et al., 2011; Bonvillain et al., 2012). Although *P. clarkii* are tolerant of relatively low dissolved oxygen concentrations (Avault et al., 1975; McClain, 1999; Bonvillain et al., 2012), prolonged exposure to sub-optimal dissolved oxygen conditions can lead to reduced survival (Avault et al., 1975; Melancon and Avault, 1977; McClain, 1999; Sladkova and Kholodkevich, 2011), growth (Jussila and Evans, 1997; McClain, 1999; Reynolds, 2002; McClain et al., 2007) and size at maturity (Huner and Romaire, 1978). Moreover, physiologically stressed crayfish harvested from hypoxic waters can suffer increased mortality rates during transport and storage. Crayfish are typically shipped and stored in 16 to 20 kg plastic mesh sacks without water for several days (McClain et al., 2007). Although these methods are economical and typically allow high survival rates, they can still increase stress levels and mortality in crayfish removed from poor water quality habitats (McClain et al., 2007).

Many physiological parameters, such as metabolite concentrations and immunological responses, have been used to assess overall stress levels in a diversity of crustaceans (Fotedar and Evans, 2011). In crayfish, biomarkers such as hemolymph lactate and protein concentrations have been assessed as indices of physiological health (Jussila et al., 1999; Silva-Castiglioni et al., 2010, 2011; Sladkova and Kholodkevich, 2011; Bonvillain et al., 2012), but conventional analytical methods for analyzing hemolymph metabolites are expensive and time consuming, and do not provide real-time data of use to commercial fishers or aquatic resource managers. Portable meters offer a potential solution to this problem, and can provide easy to use, economical alternatives for determination of real-time physiological parameters in the field. For example, refractometric methods have been used to assess physiological health in lobsters (Oliver and MacDiarmid, 2001; Ozbay and Riley, 2002; Bolton et al., 2009; Chandrapavan et al., 2009, 2011), prawns and shrimp (Smith and Dall, 1982; Moore et al., 2000; Anuta et al., 2011), and crabs (Cormier et al., 1999; Nolan and Smith, 2009; Uhlmann et al., 2009; Coffey et al., 2012). During an extensive study of *P. clarkii* ecology and physiology in the Atchafalaya River Basin in south-central Louisiana, it became clear that field assessments of hemolymph characteristics would be particularly useful for assessing real-time stress responses of crayfish to hypoxia. Consequently, we

* Corresponding author, current address: Department of Biological Sciences, Nicholls State University, Box 2012, Thibodaux, LA 70310, USA. E-mail: cbonvillain@hotmail.com

initiated a study to evaluate the accuracy of a hand-held refractometer and lactate meter for determination of *P. clarkii* hemolymph protein and lactate concentrations by comparing results obtained from the hand-held meters against those obtained by spectrophotometry.

Materials and Methods

Hemolymph Collection and Processing

Adult intermolt *P. clarkii* were collected monthly from April to July 2010 at five locations in the southeastern Atchafalaya River Basin. Five pillow traps per site were baited with 150 g of Purina® manufactured bait and fished overnight. Crayfish hemolymph collected by pericardial cavity puncture (20-gauge needle) at the time of capture was transferred via capillary tube into a microcentrifuge tube, and was then placed immediately on ice, allowed to clot, and stored at −4°C in the laboratory. Because of limited hemolymph volume in some samples, available quantities were used for either lactate ($n = 81$) or protein ($n = 85$) determinations. We also conducted identical analyses on hemolymph samples obtained from *P. clarkii* (lactate $n = 45$, protein $n = 51$) exposed in laboratory experiments to three levels of dissolved oxygen (>7.5, 2.0 and 1.0 mg/l) for periods of 12, 24 and 48 hours (Bonvillain et al., 2012). Hemolymph samples were collected with the same methods used in the field, with the clotted samples stored at −4°C. We collected hemolymph from physiologically stressed (i.e., individuals exposed to environmental hypoxia) and unstressed (i.e., individuals exposed to normoxic conditions) *P. clarkii* in both field and laboratory samples.

Hemolymph Metabolite Determinations with Spectrophotometry

We first analyzed hemolymph samples of *P. clarkii* with spectrophotometry to establish reference lactate and protein levels for later comparisons with the hand-held meters. To accomplish this, clotted hemolymph samples were thawed and broken up, the sample was centrifuged at 2415 *g* for 15 minutes at 4°C, and the supernatant serum was extracted. Hemolymph lactate concentrations were enzymatically determined with an L-lactate assay kit (Eton Bioscience, San Diego, CA, USA). After a 50-fold dilution of each sample with deionized water, 20 μl of the diluted samples and lactate standards were added to 96-well microplates. Fifty μl of Lactate Assay Solution was added to each well, mixed by gentle agitation, and incubated at 37°C for 30 minutes. The reaction was then stopped by adding 50 μl of 0.5-M acetic acid to each well. Standard curves were prepared at concentrations of 0, 39, 78, 156, 312.5, 625, 1250 and 2500 μM L-lactate as per the manufacturer's instructions. Total protein concentration of crayfish serum was determined with the Pierce Coomassie Plus assay (Thermo Fisher Scientific, Rockford, IL, USA) modified from the Bradford method (Bradford, 1976). A 100-fold dilution of each sample was prepared with deionized water, and standard curves were prepared at concentrations of 0, 25, 125, 250, 500, 750, 1000, 1500 and 2000 μg/ml bovine serum albumin per manufacturer's instructions. Standard curves for both assays had R^2 values of 0.99 or greater. After sample preparation, triplicate spectrophotometric assays of lactate (read at 490 nm) and protein (read at 595 nm) concentrations were obtained with a BioTek® Synergy 2 Multi-Purpose Microplate Reader (BioTek®, Winooski, VT, USA) and interpreted with Gen5™ analysis software (BioTek®).

Hemolymph Metabolite Determinations with Hand-Held Meters

A 25 μl aliquot of hemolymph sample was applied to a Cobas® BM-Lactate test strip (Roche Diagnostics, Mannheim, Germany) and an Accutrend® Lactate meter (Roche Diagnostics) was then used to determine lactate concentrations in *P. clarkii* hemolymph samples. Because the measurement range for the lactate meter was 0.7-26.0 mmol/l, we excluded data from 29 crayfish collected in the field that exhibited lactate concentrations below 0.7 mmol/l. Hemolymph protein concentrations were determined from a 50 μl aliquot of hemolymph viewed with a Reichert® Vet 360 temperature compensated hand-held refractometer (Reichert, Depew, NY, USA).

Data Analysis

Analysis of covariance (PROC MIXED; SAS version 9.3, SAS, Cary, NC, USA) indicated that field and laboratory data yielded similar relationships between spectrophotometer and hand-held measurements of hemolymph lactate and protein concentrations. Consequently, we pooled all of the data and used simple linear regression (PROC GLM; SAS version 9.3, SAS) to examine differences in hemolymph lactate ($n = 126$) and protein ($n = 136$) concentrations determined with the hand-held meters and the spectrophotometer.

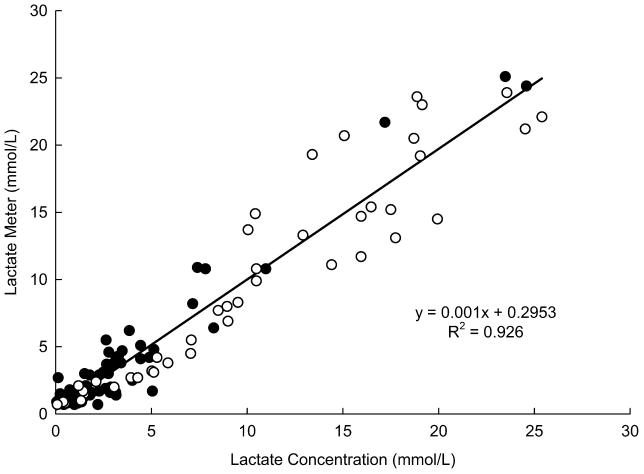

Fig. 1. Relationship between hemolymph lactate concentrations determined by lactate meter and spectrophotometric measurements from field (closed circles) and laboratory (open circles) sampled *Procambarus clarkii* ($n = 126$).

Results and Discussion

Hemolymph lactate concentrations for *P. clarkii* determined with the spectrophotometer and lactate meter exhibited a strong positive relationship ($P < 0.0001$; $R^2 = 0.926$; Fig. 1), as did measurements of hemolymph protein taken with the spectrophotometer and refractometer ($P < 0.0001$; $R^2 = 0.830$; Fig. 2). These results demonstrate that the hand-held lactate meter and refractometer are reliable methods for determining hemolymph lactate and protein concentrations, respectively. Both of these hemolymph parameters were found to be indicative of hypoxia-induced stress in *P. clarkii* (Bonvillain et al., 2012), making these hand-held instruments easy to use, low-cost alternatives for assessing crayfish physiological condition. Traditional laboratory-

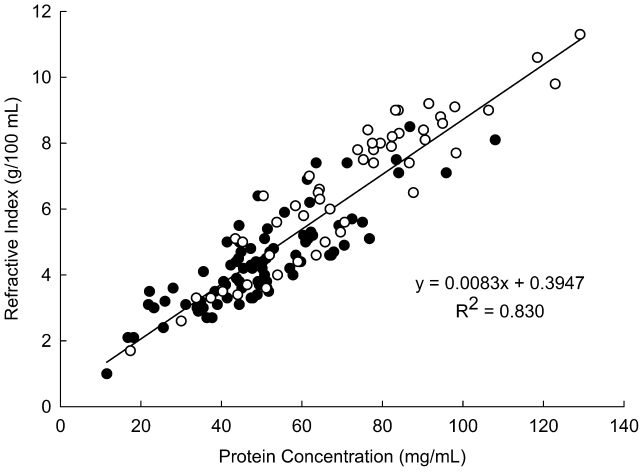

Fig. 2. Relationship between hemolymph protein concentrations determined by refractometer and spectrophotometric measurements from field (closed circles) and laboratory (open circles) sampled *Procambarus clarkii* ($n = 136$).

based analyses of hemolymph metabolite characteristics are time-consuming and require thousands of dollars in laboratory equipment, e.g., spectrophotometer and associated supplies, and metabolite assay kits (several hundred dollars per kit). In contrast, non-digital refractometers are typically less than US$ 200 with no additional associated supply costs, with lactate meters ranging from US$ 300-400 plus the purchase of individual test strips.

The greatest benefit of these meters is their ability to provide immediate, real-time information about crayfish physiological condition, which can be integrated with environmental data collected prior to and at the time of capture. This information would be valuable to commercial crayfish farmers for monitoring population responses to biotic and abiotic parameters (water quality, stocking density) during production, and could provide processors with information on the physical condition of crayfish stocks coming from producers and going to consumers. These data could also be integrated into field and laboratory biomonitoring programs developed by resource agencies not only to monitor wild crayfish health and stress levels, but also to evaluate the success of ecosystem restoration and water management activities. Although additional research is needed to apply these methods to the assessment of physiological changes and survival of other crayfish species exposed to additional environmental stressors (e.g., low pH, heavy metals, pesticides), the potential of these instruments to contribute to an environmental monitoring program in both aquaculture and natural settings is significant.

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