Poeciliid Research

Utilization of a portable glucometer for the measurement of tissue glucose as a stress indicator in ornamental fish

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Abstract. The stress response in vertebrates is determined by measuring cortisol production following acute or chronic exposure to various environmental stimuli. Cortisol assays as responses to stressful events are done on blood samples using ELISA or radio-immunoassays. However, these procedures require expensive reagents and special equipment that are not available to most fish growers or hobbyists. A portable glucometer, which is a point-of-care (POC) device to monitor blood glucose levels, was assessed in terms of its usefulness in assessing the stress response in vertebrates by quantitating whole body (tissue) glucose. Using ornamental fish as our model species, glucose levels from tissue homogenates were measured in swordtail (*Xiphophorus hellerii*) following handling stress by exposure to air for 3 min. Tissue glucose was measured before air exposure (control), immediately after air exposure for 3 min, and at 30 min post-air exposure (recovery). There was an increase in tissue glucose immediately after exposure of the fish to air for 3 min. At 30 min post-exposure, the levels of tissue glucose were still elevated, but may be moving towards returning to the pre-air exposure levels (control), which were measured prior to the application of the stressor. Our results have shown that a portable glucometer has good potential in monitoring stress response in vertebrates using ornamental fish as a model by quantifying tissue glucose in lieu of a more expensive cortisol assay.
Key Words: aquaculture, aquatic, cortisol, handling stress, ornamental fish, physiology, point of care.

Introduction. Stress in fish results from husbandry activities that the animals are subjected to, as well as any imbalances in the rearing environment. Responses to these stressors, in extreme cases, may have significant negative effects on the physiological status of the fish, including growth, reproduction, flesh quality, and susceptibility to disease (Wedemeyer 1996; Barton 1997; Pankhurst & van der Kraak 1997). Because of these consequences, fish biologists have used a variety of methods for evaluating the effects of stress on fish (Adams 1990; Wedemeyer et al 1990). These methods for monitoring metabolic indicators of stress in fish have the obvious potential for improving husbandry protocols and product quality of the fish upon harvest (Wells & Pankhurst 1999). However, most of these techniques have been designed and optimized for laboratory research work and require sophisticated and expensive procedures and equipment. Fish culturists and fisheries managers need reliable field methods that can accurately detect stress in fish and must be easy to use and low cost.

Iwama et al (1995) and Morgan & Iwama (1997) tested the possibility of detecting and monitoring stress conditions in fish under field conditions. They concluded that by analyzing blood glucose with a portable instrument, the data can provide a reliable measure of stress in fish, and thus, have practical uses in aquaculture and field

monitoring activities. Comparative studies done by Wells & Pankhurst (1999) on the efficiency of portable devices and laboratory-based assays in monitoring blood glucose and lactate in fish showed strong correlation between the two methods. Moreover, they pointed out that these portable devices offer advantages in terms of portability, simplicity of use, and the ability to use fresh, unprocessed blood in micro-volumes.

From the standpoint of aquaculture, the industry will greatly benefit from standardizing the methods in assessing stress conditions through the use of these devices. However, the aquaculture industry is not only composed of rearing food fish. This industry also encompasses the ornamental fish industry, which is a multibillion-dollar business with a demand equivalent of at least 10 billion USD (Dey 2016). Given the importance of the ornamental fish industry, the role of stressors in the husbandry of ornamental fish must not be overlooked because these can affect productivity. There is limited information on stress physiology in ornamental fish, more so on the use and impacts of these portable devices in assessing stress in ornamental fish. To answer these research gaps, this study aimed to determine the feasibility of using a portable glucometer in measuring glucose as an index of the stress response in ornamental fish using swordtail (*Xiphophorus hellerii*) as a model organism. In addition, this study aimed to determine the usefulness of the whole fish as the biological material for measuring glucose levels from small-sized fish.

Material and Method. A portable battery-operated blood glucose meter designed for personal use was evaluated for measuring whole body tissue glucose in fish. The portable glucometer (OneTouch Select Plus Simple®, LifeScan Europe GmbH, Switzerland) records glucose levels in the range 20–600 mg dL^{-1} (approximately 1–30 mmol L^{-1}). The disposable test strip works when glucose in the blood or homogenized tissue sample mixes with the enzyme glucose oxidase in the test strip and a small electric current is produced. The strength of this current is correlated with the amount of glucose in the sample. The glucometer measures the current, calculates the glucose level and displays the result. Approximately 1 μl of the sample may be analyzed in the temperature operating range of 10 to 40°C.

Mixed sex juvenile swordtail (*X. hellerii*, average weight: 0.34 g) were subjected to severe handling stressor by holding them out of water in a dipnet for 3 min (Caipang et al 2014) and then returned back to the rearing container for recovery and monitoring. 7 fish were sampled before (non-stressed fish, control), immediately after air exposure and 30 min after air exposure for whole tissue glucose. All procedures employed in the study followed the institutional and national guidelines on responsible handling and welfare of fish in research. Figure 1 shows the schematic diagram of the procedure that was employed in the study.

Data are presented as means ±1 SD where appropriate. Student's t-test for independent samples was used to compare the tissue glucose levels immediately after the application of the stressor and recovery with the pre-stress (control) levels. All statistical computations were performed at the 0.05 level of significance using the statistical package of Microsoft Excel 2010.

Results and Discussion. A portable glucometer was used to measure glucose levels from tissue homogenates of ornamental fish as a means of assessing responses of the host following exposure to air as a handling stress. Figure 1 shows the protocol that was developed using this approach.

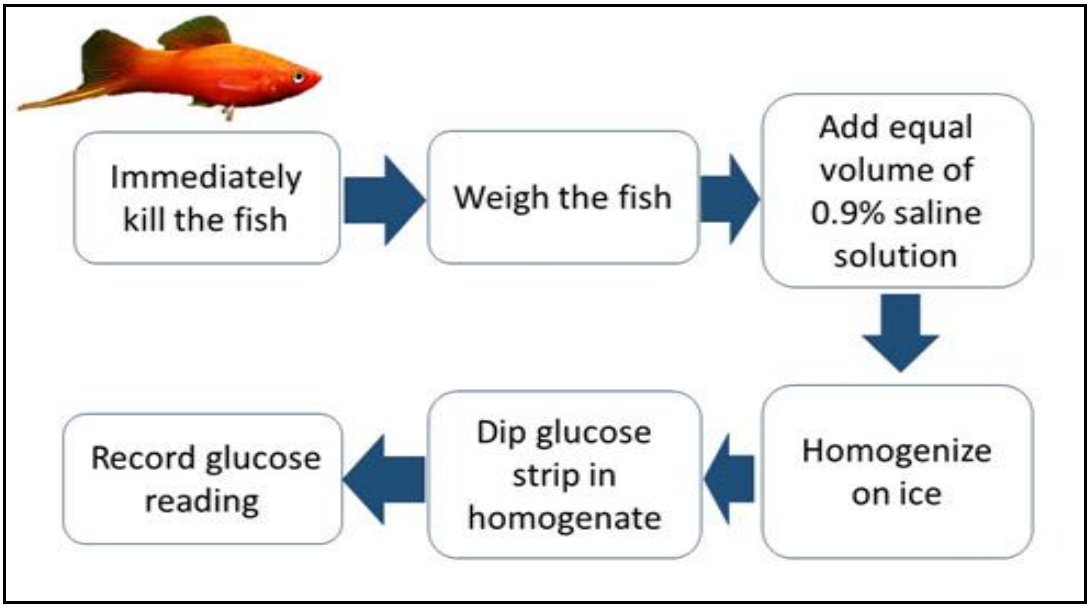

Figure 1. Schematic diagram of measuring tissue glucose in ornamental fish using a portable glucometer.

Briefly, individual fish from the control (pre-stress), immediately after air exposure and recovery (30 min post-air exposure), were extracted immediately from the container, killed and weighed. The fish were placed in individual 1.5 mL microfuge tubes and an equal volume of normal saline solution (0.9% NaCl w/v) was added. The fish were homogenized on ice using a plastic pestle, centrifuged at 4000 rpm for 30 sec and the supernatant was transferred to a new microfuge tube. A disposable glucose strip was attached to the glucometer, then the tip of the strip was dipped into the tissue homogenate. Glucose level was immediately read and recorded. After recording, the glucose strip was carefully removed and properly disposed. The surface of the glucometer was disinfected with 75% ethanol after every reading to prevent contamination. All tissue homogenates were placed on ice during the assays.

Tissue glucose was monitored in swordtail before air exposure, immediately after air exposure for 3 min and during recovery (30 min post-air exposure). Figure 2 shows the levels of glucose in the fish. Swordtail exhibited a significant rise in tissue glucose immediately after exposure to air for 3 min. During recovery, the tissue glucose decreased and the levels were returning to the pre-air exposure (control) levels.

In the present study, tissue glucose in swordtail increased following exposure to a handling stress. In this case, handling stress was in the form of air exposure for 3 min. The results obtained in this study were consistent with the observed increase in blood glucose following a stress episode (Iwama et al 1995; Morgan & Iwama 1997; Wells & Pankhurst 1999; Gomes 2007; Caipang et al 2014). The increased level of tissue glucose that were observed in fish immediately after exposure to a stressor is an indicator that the organism is likely undergoing a stress hyperglycemia, which is an evolutionarily preserved response that enables the host to survive during periods of severe stress (Barreto & Volpato 2006; Soeters & Soeters 2012; Marik & Bellomo 2013). However, tissue glucose did not remain at elevated levels during recovery, which were in contrast to earlier studies on fish (Gomes 2007; Caipang et al 2014). Gomes (2007) observed an elevated blood glucose in pirarucu (*Arapaima gigas*) even during recovery at 24 hours post-air exposure, while Caipang et al (2014) observed two peaks in juvenile Atlantic cod (*Gadus morhua*) during recovery following acute handling stress. The differences on the regulation of glucose levels in fish as a response to a stressor could be due to the species of fish, the type and duration of the stress episode and the sampling times that were employed during the conduct of the studies (Swift 1983; Barton & Schreck 1987).

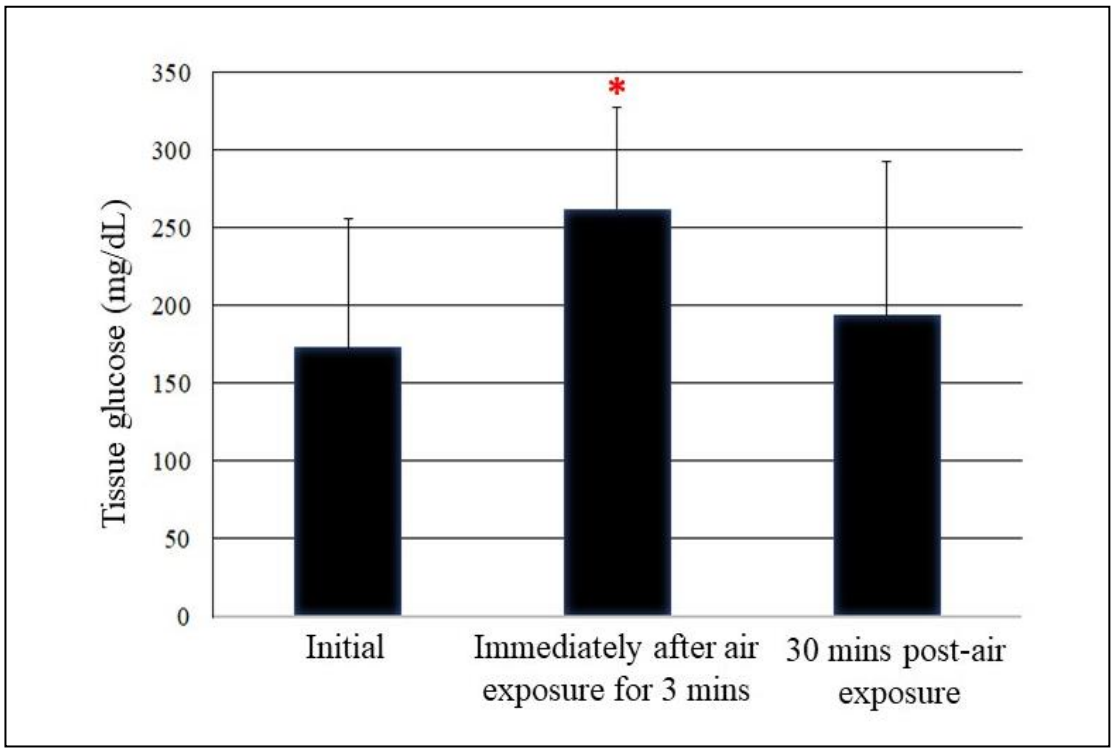

Figure 2. Tissue glucose in swordtail as monitored by a portable glucometer following exposure to air; the column bar with an asterisk indicates significant difference from the initial group at $p<0.05$ (N=7).

Recent improvements in the design of portable glucometers allow the measurements of glucose levels below 2 mmol L^{-1} (Wells & Pankhurst 1999). In our study, the minimum level of glucose that could be detected by the device was 1 mmol L^{-1}, indicating that this can detect even the relatively low glucose values typical of unstressed fish (Wood et al 1983; Madsen 1990). Because of this, the use of glucose as a stress indicator may be monitored far better than the range of tests that were conducted by Morgan & Iwama (1997). Portable glucometers have been utilized to measure glucose levels in fish during field samplings and in areas where there are limitations to access with laboratory-based assays in assessing the stress response (Iwama et al 1995; Wells & Pankhurst 1999). In these studies, the feasibility of using these handheld devices was tested in foodfish, namely, coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*) respectively. These were in contrast with the present study, where whole body (tissue) glucose was measured from ornamental fish. Because of its size, whole body was used to obtain samples in monitoring glucose levels from swordtail. Our results were in accordance with the expected pattern of increase, namely glucose levels significantly elevated following handling stress. In addition, this also indicates that for small fishes, the use of whole body as the biological material to measure various indices of stress is feasible both for laboratory assays and using portable devices.

Conclusions. Taken together, our results demonstrated that a portable glucometer has the potential to measure glucose levels in small fish using whole body (tissue) as the biological material. Monitoring tissue glucose in small ornamental fish using a glucometer can be used as an index of the secondary stress response in fish. This handheld device is easy to use and is readily accessible in resource-limited areas where regular field samplings for monitoring health and welfare in fish are conducted. Future studies should focus on establishing the correlation between cortisol levels and tissue glucose in response to stress. Moreover, there is also the need to validate the robustness of measuring tissue glucose using portable devices in comparison with the values obtained

using more sensitive laboratory-based assays. It is noteworthy to mention that these portable devices will only be suitable for use in the ornamental fish industry if reference values are well-established for particular species or strains of fish.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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