CALCULATION OF FASTING BLOOD SUGAR AND RANDOM BLOOD SUGAR OF *CLARIAS GAREIPINUS* IN THREE COMMERCIAL FISH FARM

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Abstract

We used methods of collecting micro samples of whole blood and plasma for the measurement of haematocrit and blood glucose. We made used of two handheld glucose metres designed for use by human diabetes patients which return valid results with catfish blood. Metre A uses glucose oxidase method, with 1µl sample volume while Metre B, C and D uses pyrroloquinoline quinine glucose dehydrogenase and they have a variable sample volume of 0.3µl, 0.6µl and 1.5µl respectively. We also presented two methods of anaesthesia using MS-222 tricane and Ice-cold facility water. Ice-cold water yielded more consistent glucose reading, since test with MS-222 tricane was able to prove to us that it increases blood glucose level in catfish. Although, the mean glucose concentration was similar in both anaesthesia method (p=0.3376, t-test), in catfish, we were unable to detect an effect of sex differences on blood glucose level and therefore, combined result from both sexes. Additionally, four sets of experiments were carried out which blood glucose levels were monitored to know how catfish will respond to glucose level over time. We fasted the fish to determine the changes in glucose level in absence of food. Method of postprandial glucose test was also involved. We also carried out random blood sugar test, and we performed intraperitoneal glucose tolerance test to know the time it required for the catfish to return glucose level to homeostasis.

Keywords: FASTING BLOOD SUGAR, HAEMATOCRIT, PLASMA

{**Citation:** Usman I. M., Solomon J. R. Calculation of fasting blood sugar and random blood sugar of *Clarias Gareipinus* in three commercial fish farm. American Journal of Research Communication, 2014, 2(12): 63-100} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Our understanding of human endocrine pancreas function has been greatly enhanced by comparative studies on catfish. Perhaps the most important work on pancreas function utilizing fish was performed by Macleod (1922), who used the large, isolated principal islets of angler fish and sculpin to definitively establish that the islets secrete insulin, with no contribution from acinar tissue. This pioneering study confirmed the work of Banting and Best (1922), who extracted insulin from the pancreas of dogs, and showed that injecting it into pancreatectomized dogs alleviated diabetes. For their discovery of insulin, Banting and Macleod shared the Nobel Prize in physiology or medicine in 1923. Since then, pancreas function has been studied in a wide variety of fish, and the literature contains a wealth of comparative data for fish blood glucose levels. Recently, catfish blood glucose studies have been added to this list (Elo *et al*., 2007; Moss *et al*., 2009). The catfish is a small, freshwater animal that has become a popular and informative developmental and genetic model. Catfish pancreas specification and morphogenesis have been well studied (Kinkel and Prince, 2009), making this organism attractive for further development into a physiological model of pancreas function. The catfish islet, like that of other fish, is organized similarly to the human islet and consists of α, β-, sigma-, and ε-cells, with pancreatic polypeptide antibody-reactive cells also reported (Li *et al*., 2009). Additionally, several catfish models have been established for studying b-cell or islet regeneration (Moss *et al*., 2009; Curado *et al.*, 2007). These studies demonstrate the ability of the catfish islet to regenerate following isletectomy or b-cell-specific ablation. Studies such as these have the potential to significantly enhance diabetesrelated research. However, we currently lack tools for evaluating cat islet function.

An important parameter for evaluating islet function is blood glucose level, which is measured, for example, to determine whether the islet is secreting a sufficient amount of insulin. Existing methods used for other animals need to be scaleddown or redesigned. Fish

biologists have traditionally used the glucose oxidase assay, which is also used for the clinical assay of human blood. However, these studies have utilized larger fish for which it is relatively easy to sample blood, and to collect adequate sample volumes.

As an alternative to either of these potential sampling methods, we tested two hand-held meters used by diabetic humans for measuring blood glucose. The meters have several advantages over traditional laboratory methods, including speed and portability: glucose can be measured within seconds of blood collection, on site. More important, the hand-held meters require only a microliter or less of blood, giving this method a distinct advantage over traditional laboratory methods. Here we present the details of tests of both meters. We found that both Meter A, which uses the glucose oxidase method, and Meter B, which uses pyrroloquinoline quinine (PQQ) glucose dehydrogenase, were consistent with results from a laboratory glucose oxidase assay. We also present methods for applying anesthesia, for testing fasted and postprandial glucose levels, and for performing an intraperitoneal glucose tolerance test (IP-GTT). These methods will enable catfish researchers to study pancreatic function, and will also open the way to studies of metabolic disorders and diseases that perturb glucose homeostasis. As glucose homeostasis involves multiple organs and cell types, including the liver, skeletal muscle, and adipose tissue, these new methodologies will have very broad application.

Fasting Blood Sugar

This test requires a 12 hour fast. You should wait to eat and/or take a hypoglycemic agent (insulin or oral medication) until after test has been drawn. Eating and digesting foods called carbohydrates form glucose (blood sugar). Glucose is needed by body to provide energy to carry out normal activities. Insulin is needed by the body to allow glucose to go into the cells and be used as energy, without insulin, the level of glucose will rise. Diabetes is a disease that

occurs when either the pancreas is not able to produce insulin or the pancreas makes insulin, but it does not work as it should. Fasting blood sugar is a part of diabetic evaluation and management. An FBS greater than 126mg/dl on more than one occasion usually indicates diabetes.

Post Prandial Test

This test is carried out 2 hour after eating. This test is used to see if someone with diabetes is taking the right amount of insulin.

Random Blood Sugar (RBS)

This measures the blood glucose regardless of when food was taking last. Random testing is useful because glucose level do not vary widely throughout the day. Blood glucose level that vary widely may mean a problem. This test is also called a casual blood glucose test.

Significance of the Study

The knowledge gained from this research has made *C. gariepinus* a popular and informative developmental and genetic model. The catfish pancreas specification and morphogenesis have been well studied, making this organism attractive for further development into a physiological model of pancreas function. Within this, we could be able to determine whether the islet of *C. gariepinus* is secreting sufficient amount of insulin or not. The two (2) metres, tested, metre A and metre B, use different chemistries for measuring glucose in whole blood of C. *gariepinus*. It shows that catfish, blood glucose is dynamically regulated, and that glucose metabolism is consistent with reports on other omnivorous fish. Thus, the practical significance of these data is that only one metre should be used for experimental tests so that there is consistency for comparing across measurements.

Literature review

Description of the genus and species

The catfish genus can be defined as displaying an eel shape, having an elongated cylindrical body with dorsal and anal fins being extremely long (nearly reaching or reaching the caudal fin) both fins containing only soft fin rays (Figure 1). The outer pectoral ray is in the form of a spine and the pelvic fin normally has six soft trays. The head is flattened, highly ossified, the skull bones (above and on the sides) forming a casque and the body is covered with a smooth scaleless skin. The skin is generally darkly pigmented on the dorsal and lateral parts of the body. The colour is uniform marbled and changes from greyish olive to blackish according to the substrate. On exposure to light skin the color generally becomes lighter. They have four pairs of unbranched barbels, one nasal, one maxillar (longest and most mobile) on the vomer and two mandibulars (inner and outer) on the jaw. Tooth plates are present on the jaws as well as on the vomer. The major function of the barbels is prey detection.A supra-branchial or accessory respiratory organ, composed of a paired pear-shaped air-chamber containing two arborescent structures is generally present. These arborescent or cauliflower-like structures located on the secondhand forth branchial arcs, are supported by cartilage and covered by highly vascularised tissue which can absorb oxygen from atmospheric air (Moussa, 1956). The airchamber communicates with the pharynx and with the gillchamber. The accessory air breathing organ allows the fish to survive for many hours out of the water or for many weeks in muddy marshes.

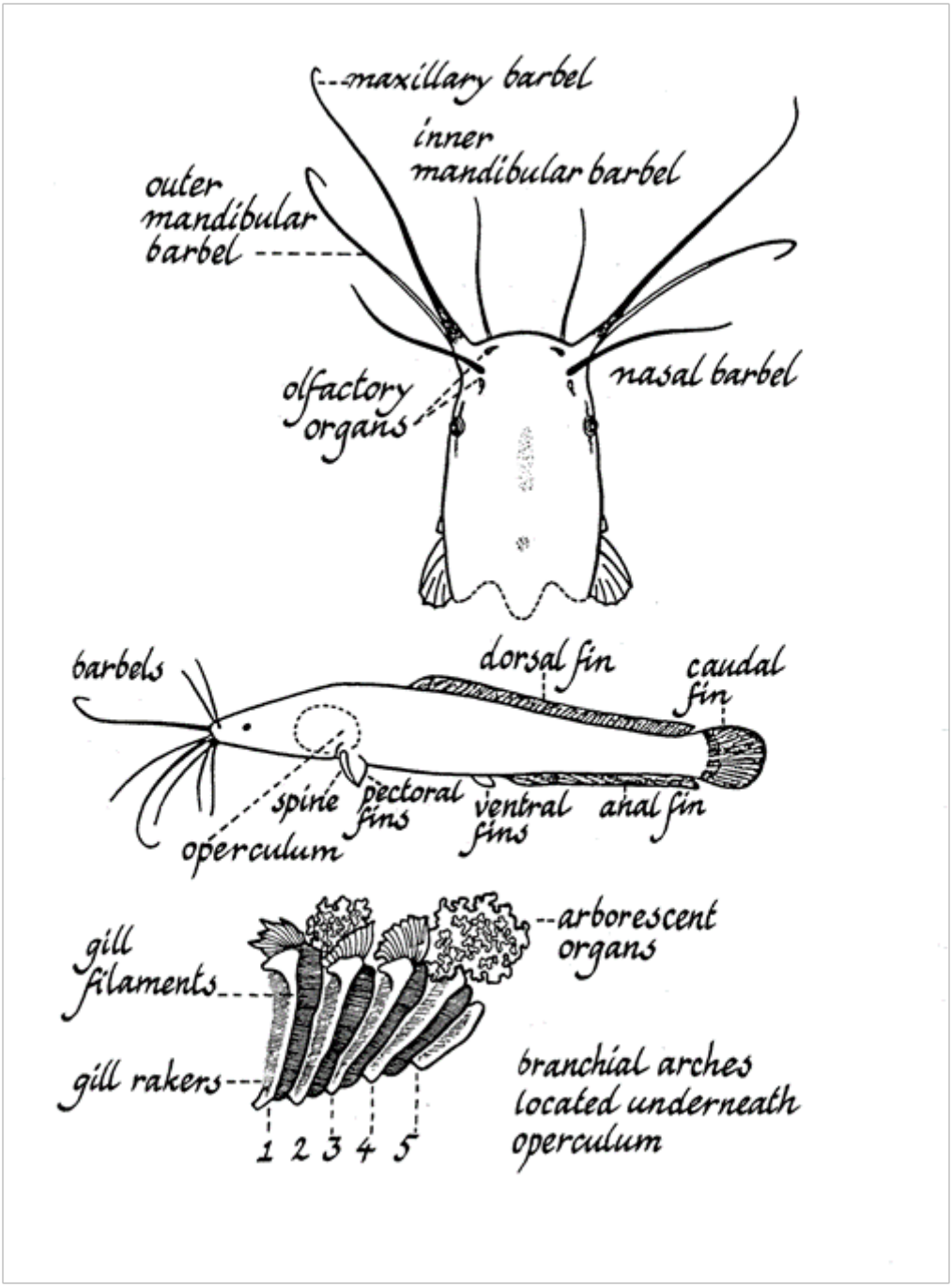

Figure 1: Morphological characteristics of *C. gariepinus*

The male and females of *C. gariepinus* can be easily recognized as the male has a distinct sexual papilla, located just behind the anus. This sexual papilla is absent in females (Figure 2).

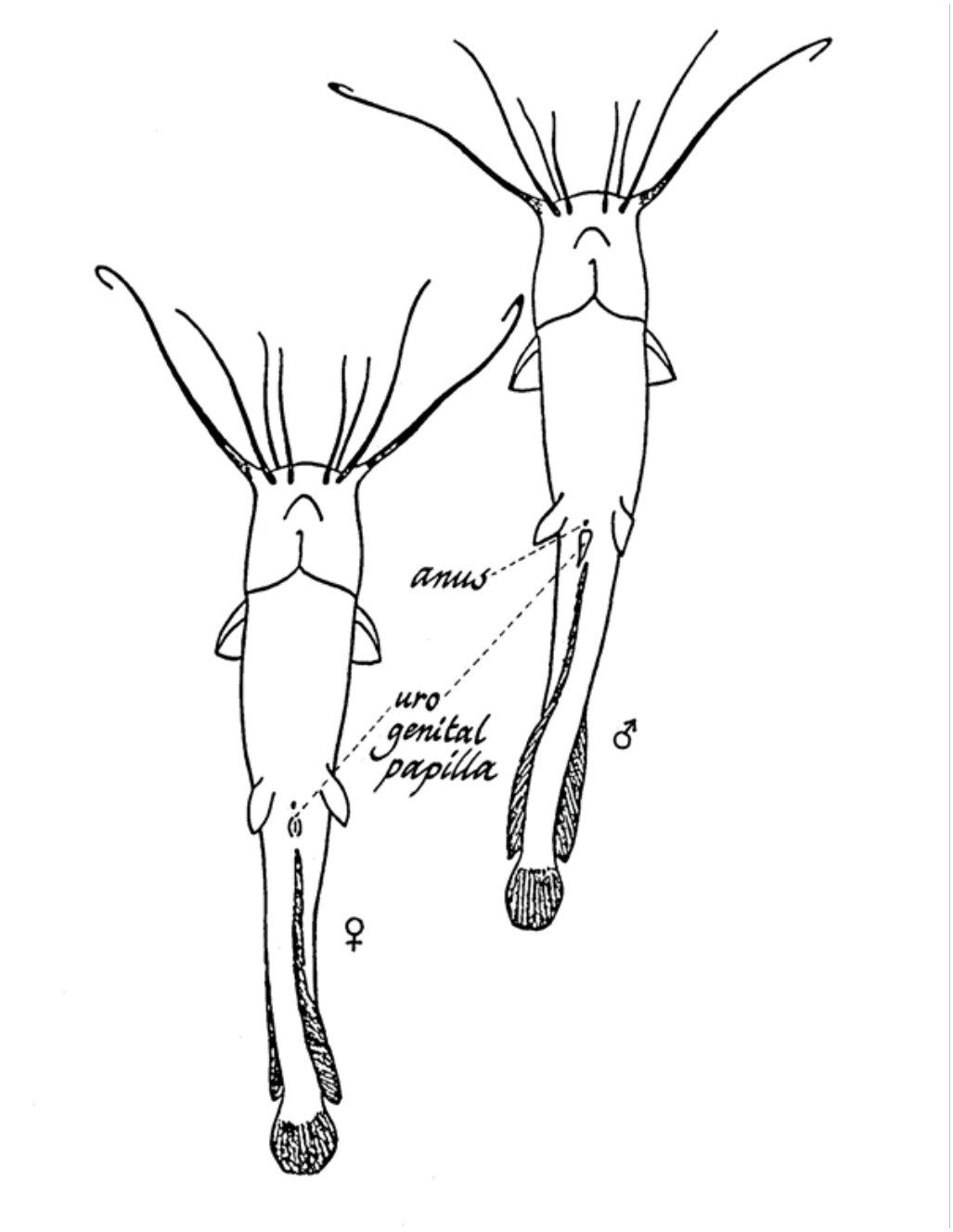

Figure 2: Sexual characteristics of *C. gariepinus*.

Habitat

Clarias spp. inhabit calm waters from lakes, streams, rivers, swamps to floodplains, some of which are subject to seasonal drying. The most common habitats frequented are floodplain

swamps and pools in which the catfish can survive during the dry seasons due to the presence of the accessory air breathing organs (Bruton, 1979; Clay, 1979).

Natural food and feeding

Although numerous studies on the food composition of *C. gariepinus* have been carried out, a consistent pattern has not emerged and they are generally classified as omnivores or predators. Micha (1973) examined catfishes from the river Ubangui (Central African Republic) and found that *C. lazera* (= *C. gariepinus*) fed mainly on aquatic insects, fish and debris of higher plants. They also feed on terrestrial insects, mollusc and fruits. Similarly, Bruton (1979b) found that catfish in Lake Sibaya (South Africa) fed mainly on fish or crustacea, and that terrestrial and aquatic insects were an important part of the diet of juvenile and adult fish which inhabit shallow areas. However, molluscs, diatoms, arachnids, plant debris were the minor food items consumed in this lake. Munro (1967) studied the feeding habits of *C. gariepinus* in Lake McIIwaine (Zimbabwe) and found that feed composition changes as fish became larger. Diptera, particularly chironomid pupae, predominate in the diet of the smallest group but become progressively less important with increasing size. Zooplankton became more important with increasing size and predominates in the diet of the largest fish. Most of the minor food groups also showed a progressive increase or decrease in importance in relation to increasing size (Figure 3). The greater importance of zooplankton in the diet of large fish was believed to be due to the increased gape and number of gill rakers of the larger fish (Jubb, 1961; Groenewald, 1964); presumably resulting in a more efficient filter feeding.

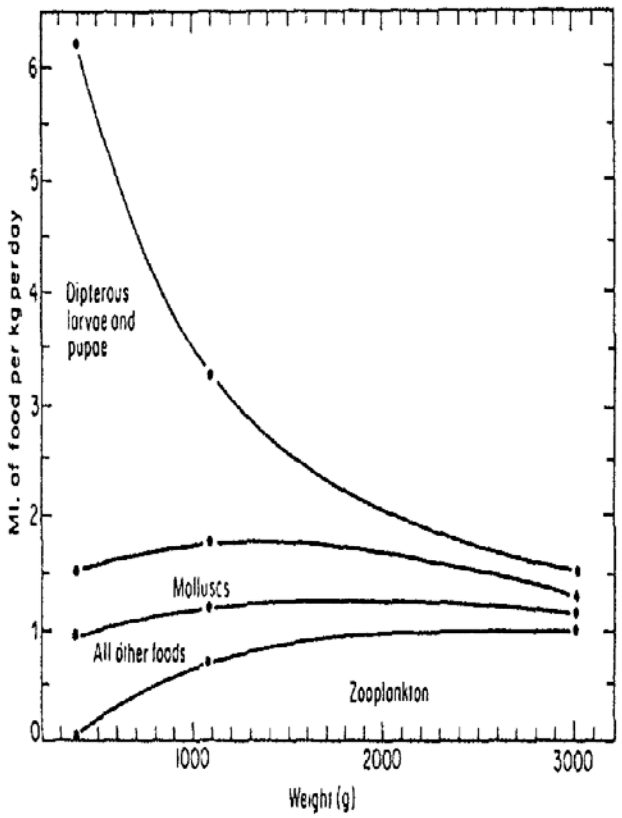

Figure 3: Apparent changes in the composition of the mean daily ration of *C. gariepinus* in relation to increasing size. Source: Munro, 1967

Spataru *et al*. (1987) studied the feeding habits of *C. gariepinus* in Lake Kinneret (Israel) and found that preyed fish were the most abundant food component (81%) and constituted the highest biomass. In conclusion, we can consider *C. gariepinus* as a slow moving omnivorous predatory fish which feeds on a variety of foods items from minute zooplankton to fish half of its own length or 10% of its own body weight. In order to feed on this wide variety of organisms in different situations *C. gariepinus* is equipped with a wide array of anatomical adaptations for feeding under low visibility (Bruton, 1979b) including;

A wide mouth capable of considerable vertical displacement for engulfing large prey or large volumes of water during filter feeding.

A broad band of recurved teeth on the jaws and pharyngeal teeth preventing prey from escaping.

An abundant network of sensory organs on the body, head, lips and circumoral barbels. These barbels are extensively used for prey detection and fixation. Hecht and Applebaum (1988) found that *C. gariepinus* with barbels were 22.6% more efficient at catching prey than those without. This could indicate that tactile behaviour is important in the prey catching processes.

A wide, rounded caudal fin, typical for fish which ambush their prey.

Long gill rakers on the five branchial arches.

A short and dilatable oesophagu which opens into a distinct muscular stomach (mechanical digestion) and a simple thin walled intestine.

Slow, methodical searching is the normal predatory tactic of *C. gariepinus*, with catfish grasping their prey by suction; a negative pressure (suction) being created by a sudden increase of the bucco-pharyngeal chamber.

An important aspect of predation by *C. gariepinus* is their ability to switch feeding from one type of prey to another. In Lake Sibaya (South Africa), catfish ignore (or cannot catch) fish prey during daylight and feed mainly on invertebrates, which are abundant and relatively easy to catch. By contrast, at night, when fish prey become more vulnerable, they switch their feeding habits to fish prey

(Bruton, 1979). In general, fish prey provides far more energy per unit weight than other prey items. However, switching feeding habits relies on the existence of at least two alternate abundant preys.

Natural reproduction

C. gariepinus shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes of *C. gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a raise in

water level due to rainfall (de Graaf *et al*., 1995). An example of maturation and spawning of *C. gariepinus* in Lake Victoria (Kenya) is presented in Figure 4; reproduction starting in March just after the start of the first heavy rains as is indicated by the decrease in the Gonado Somatic Index1 (G.S.I.). Natural reproduction is completed in July and the G.S.I. remains low till November, thereafter the oocytes start maturing gradually and become ripe again in March.

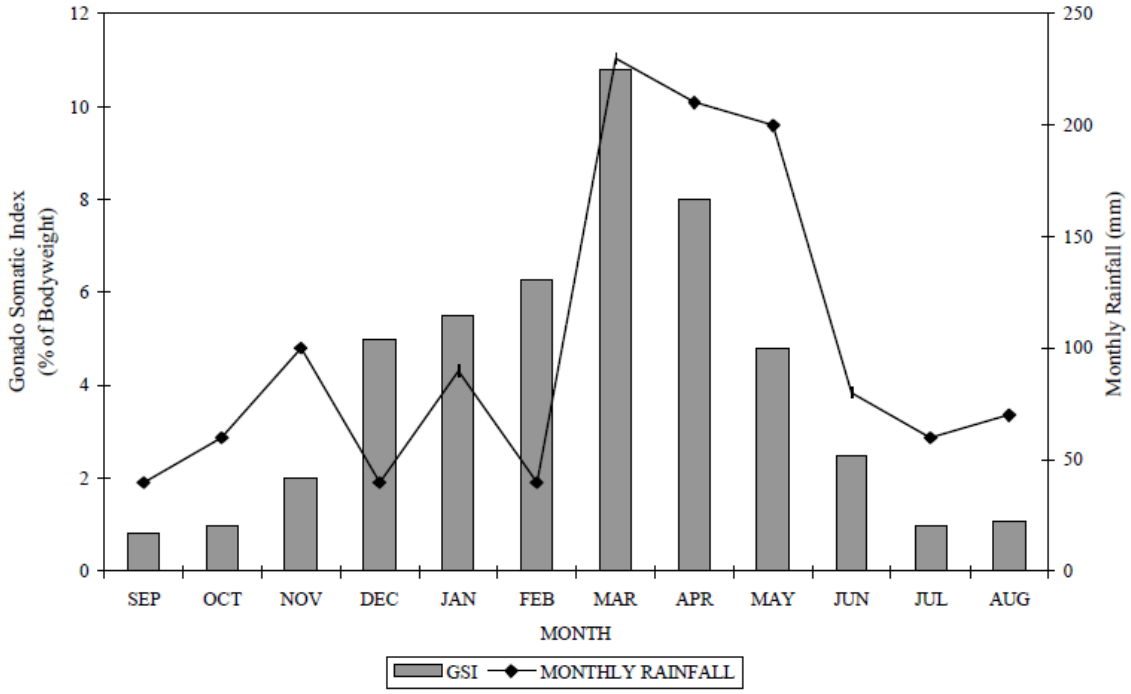

Figure 4: The Gonado Somatic Index (% of body weight) of *C. mossambicus* (= *C. gariepinus*) and the monthly registered rainfall (mm), Nyanza Gulf, Lake Victoria, Kenya.. After; Owiti and Dadzie, 1989.

Spawning usually takes place at night in the shallow inundated areas of the rivers lakes and streams. Courtship is preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow waters between isolated pairs of males and females. The mating posture, a form of amplexus (the male lies in a Ushape curved around the head of the

female) is held for several seconds (see Figure 5). A batch of milt and eggs is released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. The pair usually rest after mating (from seconds up to several minutes) and then resume mating.

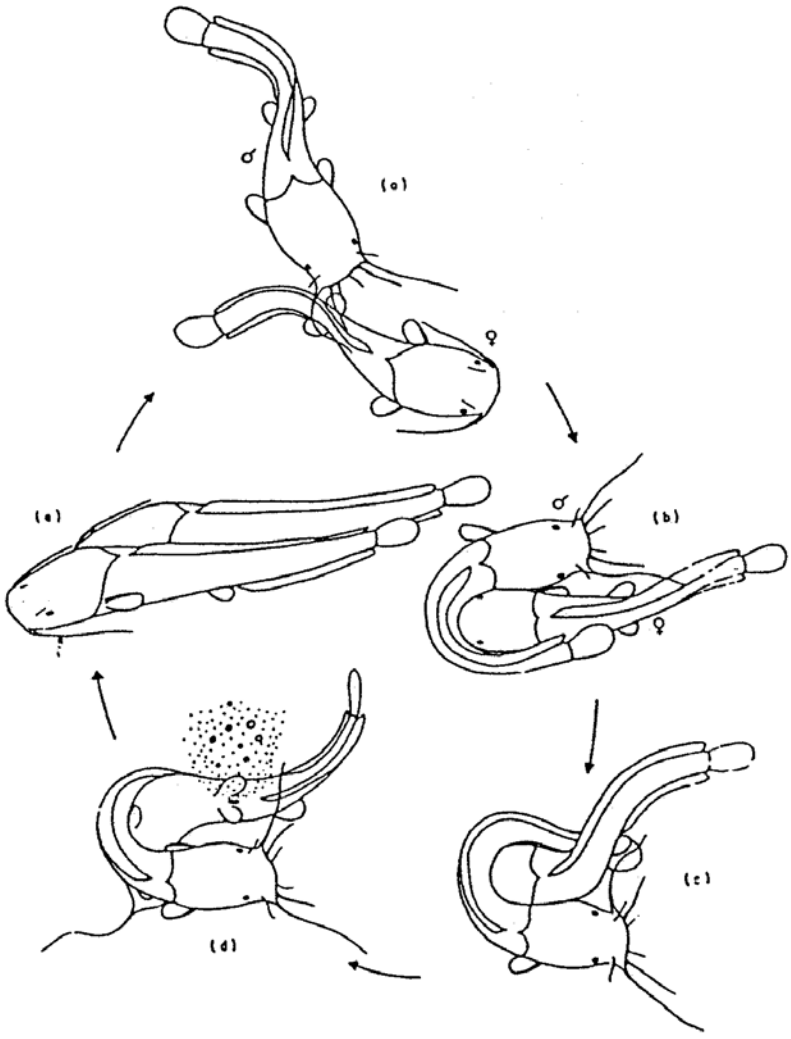

Figure 5: The courtship ritual of *C. gariepinus*.. Source: Bruton, 1979.

There is no parental care for ensuring the survival of the catfish offspring except by the careful choice of a suitable site. Development of eggs and larvae is rapid and the larvae are capable of swimming within 48-72 hours after fertilization at 23-28^{O}C.

Materials and Method

Experimental Catfish

Hybrid Catfish (*Clarias gariepinus*) were purchased from three (3) commercial fish farms (i.e. Gwagwalada, Kwali and Kuje). This cat fish which has been artificially crossbreed were fed to satisfaction with floating commercial pellet feed. Fasting experiment, glucose tolerance testing, glucose oxidase and casual blood glucose test (i.e. RBS) were carried out.

Length and Weight Measurement

Length in centimetre was measured from the anterior-most point of the mouth, to the posterior-most region of the caudal peduncle, using metre rule (cm). Weight in grams was also measured by putting the fish into a small plastic bucket of facility water on a scale and subtracting the non-fish weight. The length of the fishes were between, 50.0 $\pm$ 55.0cm, 38.0 $\pm$ 44cm, and 20.0 $\pm$ 30cm while their weight are taking to be 800.0 $\pm$ 900g, 650.0 $\pm$ 700g and 200.0 $\pm$ 300g respectively.

Anesthesia

MS-222 (tricaine; Sigma) was used at 0.02% in facility water, at 28.5^0C (Westerfield, 1995). Hypothermia used ice-cold facility water (Iwama and Ackerman, 1994). The cold water was held in a beaker in an ice bucket to maintain temperature. To anesthetize fish for measuring blood glucose, fish were transferred to a container containing either MS-222 or ice-cold water and then monitored for signs that they had reached stage III, plane 2 of anesthesia, namely, loss of equilibrium, loss of operculum movements, and loss of reactivity (Iwama and Ackerman, 1994; Brown, 1993). For both anesthetics, stage III was typically reached within 60s. Fish were sacrificed and blood glucose was measured immediately.

To anesthetize fish for IP injection, fish were placed into 17^OC facility water, and the container was not on ice. Ice made from facility water was gradually added to the water, to decrease water temperature to 12^OC over several minutes. This procedure gradually brought

the fish to stage II of anesthesia, in which muscle tone is decreased enough to allow handling of the fish, and opercular movements are decreased but still present (Iwama and Ackerman, 1994; Brown, 1993). Recovery from stage II anesthesia was typically within several seconds of returning the fish to 28.5^{O}C facility water.

Whole blood collection

To obtain whole blood, fish were anesthetized then decapitated by cutting cleanly through the pectoral girdle with scissors. The cut was immediately anterior to the articulation of the pectoral fin with the girdle, and severed the heart. Whole blood was analyzed immediately by applying a test strip directly to the cardiac blood. For repeated measurement of a sample, whole blood was collected by holding a heparinized 100 µL microcapillary tube (Sarstedt) adjacent to the severed heart, or a heparinized 40mm microhematocrit tube (StatSpin). With this method, the quantity of blood collected depends on the size of the fish. We found that approximately 10 µL was typical. The tube was briefly spun to collect the sample, and glucose was measured immediately. With improper blood handling, hemolysis may occur, which can potentially affect glucose measurement (Brown, 1993). Potential causes of hemolysis are shearing with a needle and storage at incorrect temperature. Our blood-handling procedures avoided those practices, and we used collection tubes designed specifically for blood collection.

Hematocrit

Whole blood was collected using 40mm heparinized microhematocrit tubes (StatSpin). Samples were spun for 120s at 13,700g in a CritSpin hematocrit centrifuge (StatSpin), and hematocrit value was read with a digital reader (StatSpin).

Glucose meters

The following glucose meters and test strip sample volumes were used-Meter A: OneTouch Ultra, 1 µL sample (LifeScan); Meter B: FreeStyle Lite, 0.3 µL sample (Abbott); Meter C:

Accu-Chek Aviva, 0.6 μL sample (Roche Diagnostics); Meter D: Accu-Chek Compact Plus, 1.5 μL sample (Roche Diagnostics). For each meter, the appropriate control solution was used each time a new vial of test strips was opened, following manufacturer's instructions.

Meter precision

Before blood collection, fish were fasted for 24 h. To generate a range of values, a subset of fish were additionally fed their normal meal 30 min before testing, or injected with 1mg/g glucose solution. Whole blood was collected from each individual, and measured 2–6 times with either Meter A, which uses glucose oxidase, or Meter B, which uses PQQ glucose dehydrogenase. A new test strip was used for each measurement. The resulting values were sorted into range categories (Table 1), and coefficient of variation (CV) was calculated. For some species of vertebrates, including several species of fish (Charvin and Young, 1970), there are sex differences with respect to blood glucose levels. For catfish, we were unable to detect an effect of sex on blood glucose level (unpublished data), and therefore combined results from both sexes. A study of several other freshwater catfish species also found no sex differences with respect to glucose level (Van Vuren and Hattingh, 1978).

Glucose oxidase assay

To perform a glucose oxidase assay, we collected approximately 80–90μL of whole blood and then separated plasma as follows. Whole blood was collected using a tube assembly consisting of a 40mm heparinized microhematocrit tube (StatSpin) inserted through the split cap of a StatSpin SS1E blood collection tube, and held by a 100μL microcentrifuge tube (Sarstedt). Both the Sarstedt tube and the StatSpin SS1E are designed for whole blood collection and consist of a capillary tube inserted into a larger microcentrifuge-style tube.

TABLE 1: Meter Precision Data for Catfish Whole Blood

	Meter A			Meter B		
Glucose range	Low	Mid	High	Low	Mild	High
Mean glucose concentration, mg/dL	46	88	307	33	71	220
Pooled standard deviation	3.42	5.17	23.36	2.62	3.11	15.15
Coefficient of variation (%)	7.41	5.85	7.61	7.92	4.39	6.88
Sample size	16	12	9	12	8	3
Total measurements	33	28	31	28	20	15

However, we found that substituting the narrower microhematocrit tube for the Sarstedt microcapillary tube was more efficient for collecting microsamples than using either the Sarstedt or StatSpin collection tubes. Further, the short height of the StatSpin SS1E microcentrifuge tube prevented efficient collection of the sample from the microhematocrit tube during centrifugation. We found that transferring the split cap with microhematocrit tube to the taller Sarstedt microcentrifuge tube resulted in an assembly that allowed complete collection of the blood sample into the centrifuge tube by spinning down briefly using a mini centrifuge.

Whole blood was collected from 16 large fish, using one tube assembly per fish; then, the blood was pooled for a sample size of 80–90 mL, and gently mixed. Blood was collected within 10 min. Glucose was measured twice with Meter A and twice with Meter B; then, plasma (approximately 30 mL) was immediately separated from the remaining sample and used for glucose measurement with a YSI 2300 STAT Plus Analyzer (Yellow Springs Instruments, Inc.). To maximize the volume of plasma that could be separated, we made a scaled-down version of the StatSampler Micro Blood Collector (StatSpin, SS2U), an

assembly that includes a microtube containing a barrier gel that separates blood cells from plasma. We made a smaller version by transferring approximately one-third of the gel to a 0.5mL PCR tube, and spinning for 20s at 13,000g. The whole-blood sample was centrifuged for 14s at 10,000g to separate plasma. The plasma sample was measured twice with the YSI Analyzer. For this procedure, there was an unavoidable time delay of approximately 20 min between blood collection and measurement. Therefore, tubes were kept on a cold rack (4^{o}C) throughout the procedure to minimize glycolytic activity. To test for an effect of the time delay on the glucose value, we collected whole blood as described, and measured the sample at 10 min postcollection using Meter A and Meter B. After an additional 20 min, the sample was measured again and the values were compared. The sample was measured twice with both meters at both time points and the entire experiment was performed four times. We found that the time delay had a negligible affect on the average value for Meter B (glucose increased by 2.5mg/dL, on average, after 20 min). However, for Meter A there was a significant decrease (11.63mg/dL, on average, after 20 min), thus indicating a technical constraint in our ability to directly compare the performance of Meter A with the YSI Analyzer. We concluded that, given the delay between blood collection and measurement, a correction factor of 11.63mg/dL should be applied for Meter A.

Fasting

Tanks were taken offline during the fasting period and food was withheld. We observed that stress can raise glucose levels in catfish, as reported for other fish (Van Vuren and Hattingh, 1978; Groff and Zinkl, 1999). To minimize stress, we avoided overcrowding by housing fish at 12–15 fish per 100 L tank, and maintaining catfish. Fish were fasted for a maximum of 4 days, and blood glucose was measured in a subset of fish every 24 h, using Meter B.

Postprandial glucose

Fish were fasted for 4 days, then fed their normal dry meal, and allowed to feed until no food remained, which took approximately 3 min. Blood glucose was measured using Meter B.

Glucose tolerance test

D-glucose (Sigma) was dissolved in Cortland salt solution, pH 7.45, at 0.5mg/μL (formulation shown in Table 2). Glucose solution or vehicle was injected IP at 1mg/g fish weight. For IP injection, a surgical table was constructed consisting of a 60mm Petri dish holding a soft sponge, and set into a pipet tip box lid. The surgical table was set into a larger, deeper container (2.4 L Rubbermaid container). All containers were flooded with cold facility water. The sponge was cut in half, and a shallow trough was cut into the flat face. The trough was used for holding the fish securely during injection. The sponge was saturated with cold facility water. Saturation of the sponge was important for maintaining anesthesia during injection as well as for providing water to the gills for respiration. Each fish was weighed, anesthetized in the largest container of the surgical table setup, and transferred to the saturated sponge as soon as stage II anesthesia was reached, and body movements had slowed enough for handling. The surgical table (sponge, Petri dish, and box lid) with fish was immediately transferred to an adjacent dissecting microscope stage (Leica M165 FC) for injection. Injection was performed under 7.3x magnification using a 35-gauge beveled steel needle and a 10 mL NanoFil syringe (World Precision Instruments) attached to an UltraMicro- Pump III driven by a Micro4 Controller (World Precision Instruments). The needle was inserted into the midline of the ventral posterior abdomen, between the pelvic fins. The injection site was closer to the insertion of the fins on the pelvic girdle than to the anus. The needle was directed cranially, to reduce the possibility of damage to internal organs (Reavill, 2006). Immediately following injection, the fish was returned to 28.5^{O}C facility water for recovery. Blood glucose was measured using Meter A.

Table 2: Cortland Salt Solution

NaCl	7.25 g/L	124.1mM
KCl	0.38 g/L	5.1mM
Na_2HPO_4	0.41 g/L	2.9mM
$MgSO_4.7H_2O$	0.24 g/L	1.9mM
$CaCl_2.2H_2O$	0.16 g/L	1.4mM
$NaHCO_3$	1.00 g/L	11.9mM
Polyvinylpyrrolidone	40.00 g/L	4%
Heparin	10,000 USP Units/L	

Statistical analysis

Analysis was performed using GraphPad Prizm, v5.02. All t-tests were two-tailed, and were unpaired. Averages are reported as mean and standard deviation.

Results

Anesthesia

We compared two methods of anesthesia, MS-222 (tricaine) and ice-cold facility water (hypothermia). MS-222 is a known ion channel blocker (Frazier and Narahashi, 1975), and thus may interfere with b-cell function. Further, MS-222 has been reported to increase blood glucose in catfish (Brown, 1993; Charvin and Young, 1970). To test its effect on catfish, fish were fed their normal meal, fasted for 2 h, anesthetized, and measured for glucose level using Meter B. As shown in Figure 10, hypothermia yielded more consistent glucose readings than MS-222 treatment. Although the mean glucose concentration was similar for both anesthesia methods ($p=0.3376$, t-test), the CV was much higher for MS-222-treated fish (MS-222, CV=54%; hypothermia, CV=29%). We concluded that MS-222 may be interfering with normal ion channel function in the β-cells, and therefore chose hypothermia for subsequent analyses.

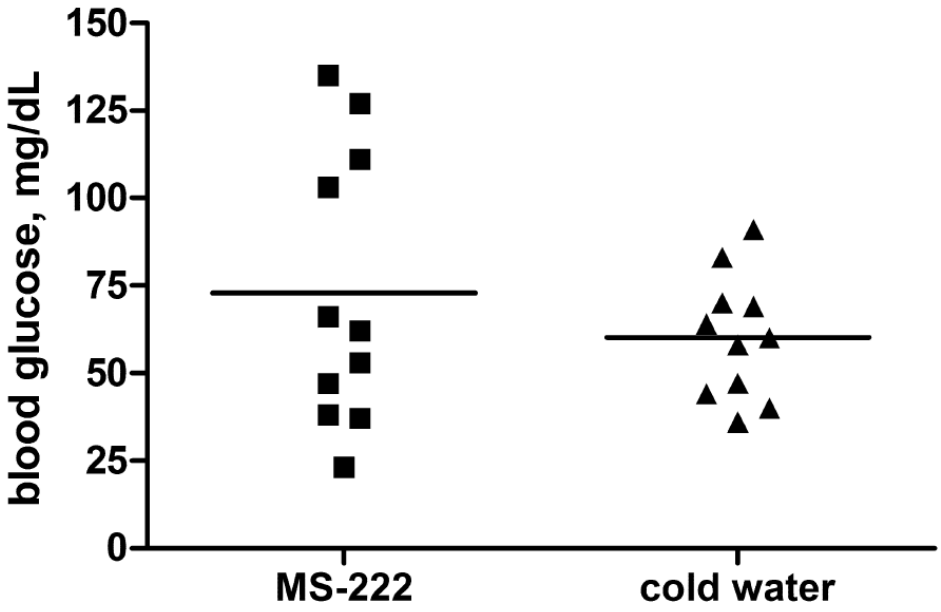

FIG. 6. Anesthetic comparison. Treatment with MS-222 causes increased variability in blood glucose relative to treatment with cold water. Fish were fasted for 2 h before treatment. Glucose was measured with Meter B. MS-222: mean and standard deviation, 72.91±39.21mg/dL; coefficient of variation (CV)=54%; n=11. Hypothermia: mean and standard deviation, 60.18±17.58mg/dL; CV=29%; n=11.

Blood glucose meter testing

To measure catfish blood glucose, we tested two handheld devices designed for measuring glucose in whole blood of human diabetics. First, we tested whether catfish blood is within the recommended hematocrit range for both meters. We measured the hematocrit of individual fish and found a mean of 31.50% ± 5.71% (n=12). This value was within the recommended range of both meters (Meter A, 30%–55%; Meter B, 15-65%; ranges according to manufacturer's instructions). Additionally, the catfish hematocrit was consistent with values reported for a variety of freshwater fish (Hattingh, 1972). For some fish species, hematocrit has been found to vary by sex and/or size (Li *et al*., 2009) For catfish, we found no difference in hematocrit for males versus females (males, 30.90% ± 5.52%, n = 5; females, 29.67% ± 4.37%, n = 6; p = 0.6880, t-test). To test for a correlation between hematocrit and

size, we measured length (centimetres) and weight (grams) of each fish. We performed linear regression to test the strength of the relationship between hematocrit and length, and calculated r^2 = 0.05212(n = 9). For the relationship between hematocrit and weight, r^2 = 0.002928 (n = 11). We concluded that hematocrit cannot be predicted by either length or weight, and therefore fish were not separated by size for glucose testing.

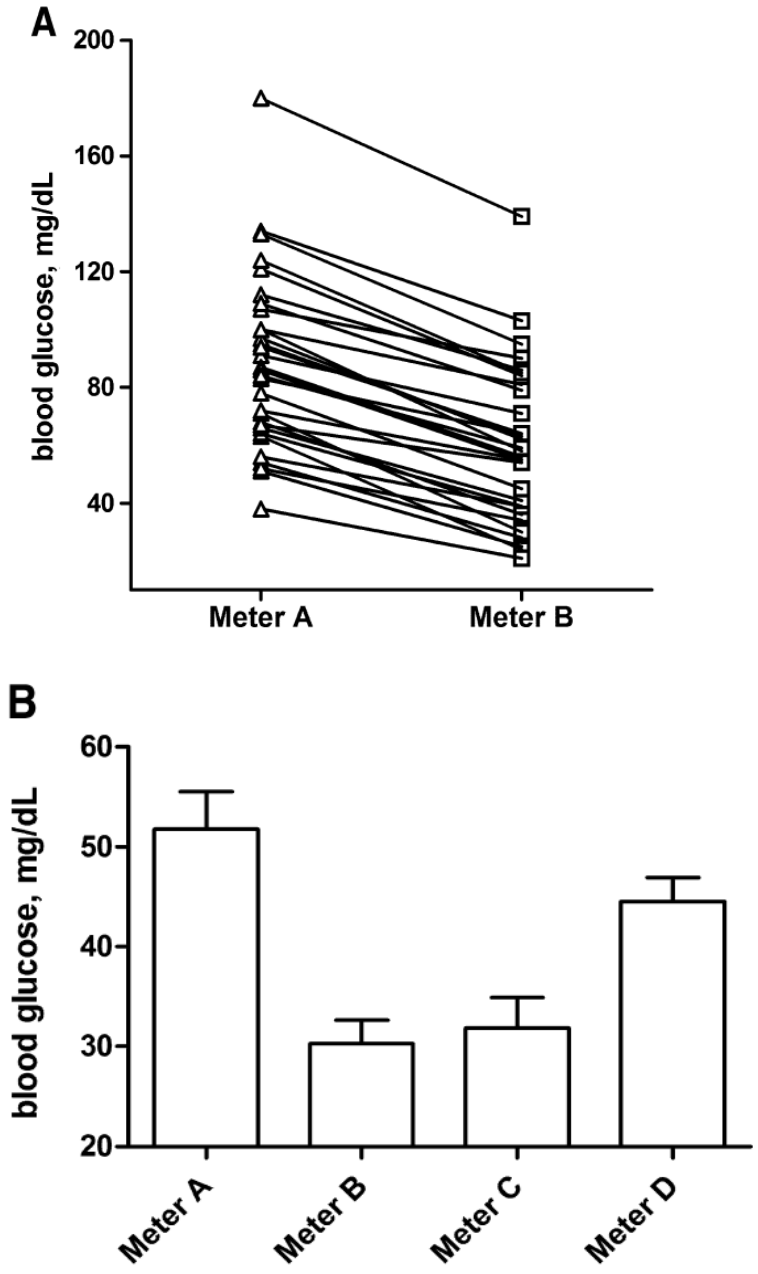

FIG. 7. Glucose meter comparisons.

(A) Two different glucose meters give different results. A blood sample was collected from individual fish, and each sample (n=31) was measured immediately with both meters. A broad range of glucose values was compared by measuring fish that had been recently fed (beginning at 15 min postprandial) as well as fish that had been fasted for up to 8 days. (B) Blood glucose measured with pyrroloquinoline quinone glucose dehydrogenase (Meters B–D) using different sample sizes, compared with glucose oxidase (Meter A). Test strip sample volumes: Meter A, 1.0 μL; Meter B, 0.3 μL; Meter C, 0.6 μL; Meter D, 1.5 μL. Samples (n=10) from individual fish were measured once with each meter. Order of meter use was randomized. Tukey's multiple comparison posttest revealed that Meter B versus Meter C was not statistically significant ($p>0.05$). All other comparisons were significant at $p<0.001$, except Meter D versus Meter A was significant at $p<0.05$. Data are mean and standard deviation. Next, we tested the precision and accuracy of both meters with catfish blood, as the meters use different chemistries to measure glucose. Meter A uses glucose oxidase and Meter B uses PQQ glucose dehydrogenase. The principles of immobilized enzyme technology in glucose testing have been reviewed (Hones *et al.*, 2008). To measure precision, whole blood was collected from individuals using heparinized capillary tubes, and measured repeatedly. According to the manufacturers, both test strips are compatible with heparinized blood. Meter precision was tested over three ranges for both meters: Meter A low, 28—59mg/dL; mid, 60—129; high, 130—550; Meter B low, 21—44mg/dL; mid, 45—105; high, 106—280. For both test strip chemistries, the CVs were similar to those reported in test strip package inserts for human blood (Table 1).

In a further comparison of the two meters, blood from 31 individuals was measured once with each meter, and the values were compared. Meter B returned values that were 28 mg/dL lower, on average, than Meter A ($p<0.0001$, paired t-test, Fig. 11A). To confirm that order of meter use did not affect blood glucose values in this comparison, five of the samples were

measured first with Meter B followed by Meter A, and five of the samples were measured first with Meter A followed by Meter B. Order of meter use did not significantly affect the value (Meter B first vs. second, p = 0.4873; Meter A first vs. second, p=0.2831, paired t-tests). These tests suggested that at least one of the meters may not be accurate. To further test the accuracy of thermometers, we asked whether the lower values given by Meter B were a consequence of the different test strip chemistries, or a consequence of the difference in sample volume (0.3 μL for Meter B vs. 1.0 μL for Meter A). We measured blood glucose using two additional meters that employ the same enzyme as Meter B, PQQ glucose dehydrogenase, but that use larger sample volumes. Meter C requires a 0.6 μL blood sample, and Meter D requires 1.5 μL. The catfish hematocrit falls into the range for both Meter C (20%–70%) and Meter D (25%–65%). We collected a blood sample from individual fish, and measured each sample once with each meter. We found that for test strips employing PQQ glucose dehydrogenase, sample volume may have an effect on the glucose value, as smaller sample volume is associated with lower glucose measurement (Fig. 11B, repeated measures ANOVA, $p<0.0001$). However, the average blood glucose value from Meter D, which uses the largest sample volume of the four meters, was slightly lower than the average blood glucose value from Meter A, which uses glucose oxidase chemistry (Meter D, 42.06$\pm$12.15mg/dL vs. Meter A, 45.15$\pm$16.27mg/dL; $p<0.05$; Tukey's multiple comparison test). This suggested that differences in test strip technology, beyond possible effects of the sample volume, may be contributing to the discrepancy between the meters. For example, although the test strips for Meter C and D both use the same enzyme, coenzyme, and mediator, they employ different indicators. (Hones *et al.*, 2008). Next, we directly tested the accuracy of Meter A and B by comparing meter results with those from a clinical laboratory glucose oxidase assay. We measured a pooled sample using both meters and a YSI 2300 Analyzer and found that Meter A measured 106.63$\pm$8.49 mg/dL; Meter B measured 114$\pm$9.90

mg/dL; and the YSI Analyzer measured 121±0mg/dL. The performance standard set by the International Organization for Standardization (ISO 15197) requires that meters measure within ±20% of the laboratory measurement for 95% of the samples (Bell, 2008). Measurements with Meter A were within 11.9% and measurements with Meter B were within 5.8%. For Meter A, we applied a correction factor as detailed in the Materials and Methods section.

Monitoring blood glucose homeostasis

To demonstrate the utility of blood glucose measurement in catfish, we performed four sets of experiments in which we monitored changes in blood glucose levels over time. We fasted the fish over the course of 4 days to determine the changes in glucose level in the absence of food, and followed the fasting with a postprandial glucose test, we carried out random blood sugar test, and we performed an IP-GTT to determine the time required for the catfish to return glucose levels to homeostasis. With fasting, glucose levels rose slightly after 2 days, and dropped significantly by 3 days (Fig. 12). We concluded that fasting for 3 days is sufficient to bring blood glucose to a baseline level. After 4 days of fasting, the mean was not reduced further compared to 3 days; however, the CV was reduced after 4 days of fasting, compared to shorter fasts (Fig. 12A). Refeeding the fish following a 4-day fast revealed that postprandial glucose peaks by 30 min (Fig. 12B). Next, we developed a GTT that utilized IP injection. For performing a GTT, glucose could simply be added to the tank water, to be taken up orally and through the gills. Indeed, such a test has been performed on catfish with success (Gleeson *et al*., 2007). However, there may be experimental questions in which it is desirable to give each fish the same amount of glucose, relative to weight. Therefore, we developed a method for IP injection into catfish. We followed the general recommendations found in Perry and Reid (Perry and Ried, 1994). with respect to vehicle, injection volume, and controls. For vehicle we used Cortland salt solution, a physiological saline formulated for

freshwater fish (Wolf, 1963; Perry *et al.*, 1984). We used an injection volume of 2 µL/g body weight, and controls included vehicle-only injections and no injection controls. Before testing, fish were fasted for 3 days and then challenged with high glucose, and clearance time was determined. We found that blood glucose peaks at 30 min postinjection and recovers to normal levels by 6 h (Fig. 13). Additionally, glucose levels following injection with vehicle alone were not significantly elevated relative to non injected controls. This demonstrates that any stress caused by the injection itself has a minimal effect on glucose level.

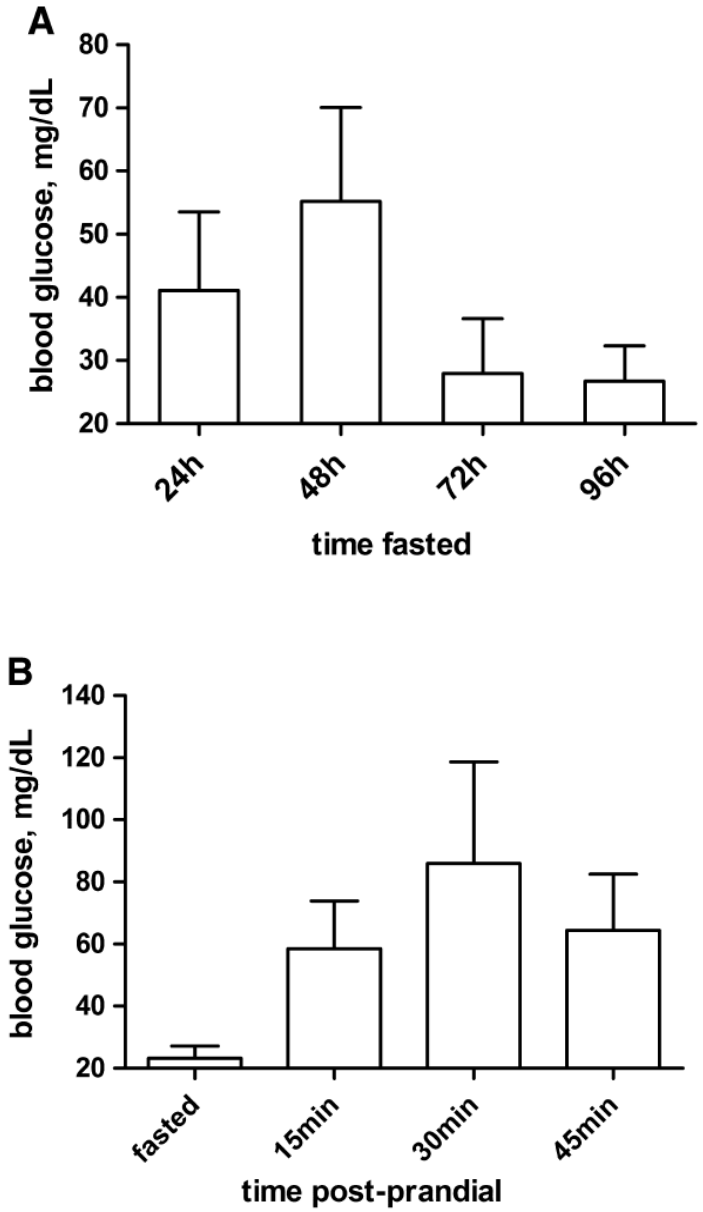

FIG. 8. Blood glucose response to fasting and feeding.

(A) Fasting blood glucose at 24 h: CV=30%, n=35; 48 h: CV=27%, n=11; 72 h: CV=31%, n=25; 96 h: CV=21%, n=15. Kruskal–Wallis test with Dunn's posttest showed the following: 24 h versus 48 h, $p>0.05$; 24 h versus 72 h, $p<0.01$; 72 h versus 96 h, $p>0.05$. Data are mean and standard deviation. (B) Postprandial blood glucose. Fish were fasted for 4 days, and then fed. Sample size is a minimum of 10 fish per time point. All fed groups are significantly different from fasted, $p<0.01$; glucose at 30 min versus 15 min, $p<0.05$; glucose at 45 min cannot be statistically discriminated from glucose at 15 or 30 min, $p>0.05$. ANOVA with Tukey's posttest. Data are mean and standard deviation. Glucose was measured with Meter B for both experiments.

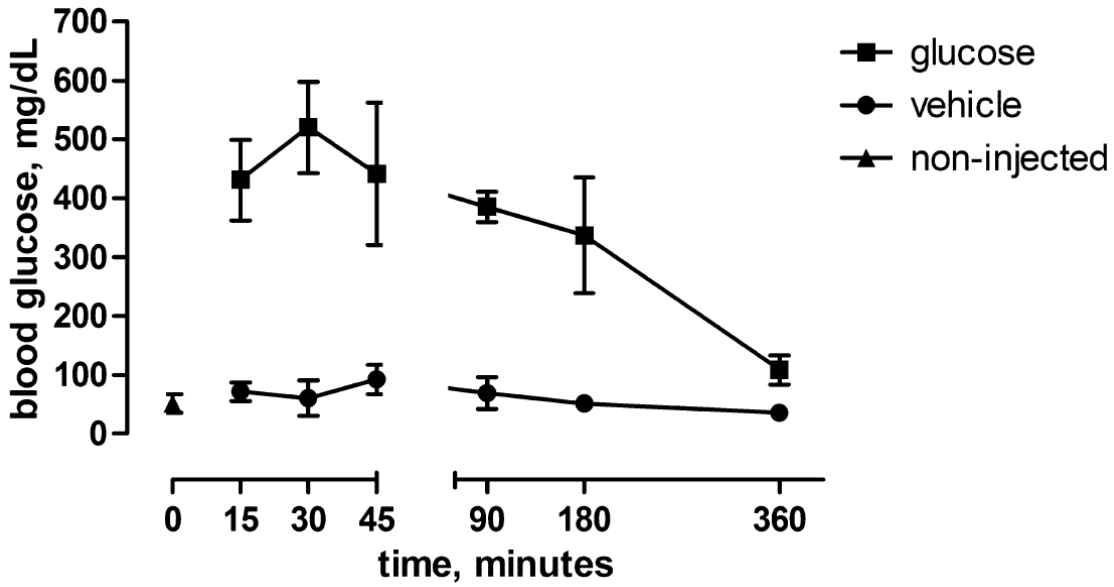

FIG. 9. Glucose tolerance test.

Fish were injected with 1mg/g glucose solution or vehicle. Glucose was measured with Meter A. Samples sizes: glucose-injected, n=4–6; vehicle-injected, n=3–7; noninjected, n=10. ANOVA with Bonferroni's posttest showed no significant difference between vehicle-injected and noninjected controls for any time point. All glucose-injected were significantly different from vehicle-injected at $p<0.001$, except that for 360 min postinjection there was no significant difference ($p>0.05$). Data are mean and standard deviation.

Discussion

Studies on carbohydrate utilization in fish show that warm water, omnivorous fishes utilize carbohydrates to a much greater extent than do coldwater, carnivorous catfish (Stone, 2003; Wilson, 1994). As catfish are warm water omnivores (Spence *et al*., 2008) carbohydrates are likely an important component of their diet. Indeed, recent work on adult catfish has demonstrated that the amount of carbohydrate in the diet is positively correlated with growth rate (Robison *et al.*, 2008). In light of these studies, it is not surprising that catfish express 18 glucose transporters, (Tiseng *et al*., 2009) as well as a number of genes, including hexokinases, that are important for glucose metabolism (Robison *et al*., 2008; Gonzalez *et al*., 2009). Additionally, deficiency in the glucose transporter s/c2a1a (solute carrier family 2 [facilitated glucose transporter], member 1a, formerly glut1) has been shown to cause a suite of neural defects in embryonic catfish (Jensen *et al*., 2006). We anticipate that studies of glucose metabolism utilizing the catfish model will become increasingly important. Here, we have presented methods for the analysis of blood glucose homeostasis in adult catfish and have found that important considerations for measuring blood glucose include choice of anesthetic, blood collection method, and glucose assay method.

Anesthetics and blood collection

We found that anesthetics should be tested for obvious effects on glucose level. In our tests on catfish, treatment with the commonly used MS-222 rapidly affected blood glucose levels, as reported for other freshwater fish (Charvin and Young, 1970). MS-222 treatment produced highly variable glucose levels compared to anesthetizing with cold water. Although it is known that MS-222 is a nerve ion channel blocker (Frazier *et al*., 1975), it is not known whether MS-222 also directly affects b-cell ion channels and therefore insulin secretion. Anesthetics in general alter glucose levels (Doyne and Egan, 2003), and some have been shown to alter insulin secretion by acting directly on β -cell channels. To cite two examples,

tetracaine alters Ca^{2+} uptake or efflux from β-cells, depending on dosage (Brison *et al*., 1971; Norlund and Sehlin, 1985), and isoflurane decreases insulin release from b-cells by opening ATP-sensitive potassium channels (Tanaka *et al*., 2009). Thus, for glucose metabolism studies, appropriate anesthetic is critical. For sampling blood, we found that decapitation was a reliable and easy method. A drawback is that it precludes repeatedly measuring individuals for time course studies. A potential alternative is cardiac puncture on live fish, which would allow repeated measurements from the same individual. In theory, it should be possible to withdraw 30%–50% of total blood volume without adverse affects, based on work in larger catfish (Groff and Zinkl, 1999). In practice, the small size of the fish heart makes this procedure quite difficult. Although the location of the beating heart can easily be determined externally, inserting the needle into the heart chamber accurately requires precise anatomical knowledge, and significant surgical skill. One group has performed catfish cardiac puncture for blood sampling, and has utilized pulled glass capillary pipettes to remove a blood volume of approximately 50nL (Moss *et al*., 2009). With currently available meters, the smallest blood sample that can be measured for glucose is 300 nL. Therefore, the small amount of blood that can be retrieved requires that the sample be diluted, or combined with other samples, to bring it to assay volume. The relative advantages and disadvantages of cardiac puncture should be carefully evaluated when designing experiments. To collect blood for downstream assays requiring whole blood or plasma, we used two different collection tubes, a 100µL heparinized microcapillary tube, and a 40mm heparinized microhematocrit tube. For both tubes, blood is drawn up via capillary action. We ultimately preferred the microhematocrit tube over the microcapillary tube, as its smaller diameter and thinner walls permitted collection of a larger sample volume. To minimize the possibility of collecting fluids other than blood, we found it was important to hold the collection tube in lose proximity to, but not touching, the exposed heart chamber.

Conclusion

Glucose homeostasis

Glucose metabolism studies commonly employ measurement of blood glucose following fasting, feeding, or a glucose challenge. Here, we have presented methods for performing these tests on adult catfish, and have used the tests to demonstrate that catfish blood glucose is dynamically regulated. We monitored blood glucose level over the course of 4 days of fasting, and found that blood glucose rose after 2 days of fasting, and then decreased to a baseline level by 3 days. A similar pattern for fasting glucose, in which glucose was elevated on day 2, was reported for the goldfish species *Carassius auratus* (Charvin and Young, 1970; Groff and Zinkl, 1999), *C. auratus* is a warm water omnivorous fish, and is in the family Cyprinidae. It is possible that short-term fasting causes stress, which may in turn increase plasma cortisol. This could explain the increased glucose level observed on day 2 of the fast. Cortisol has been shown to increase liver gluconeogenesis in fish (Navarro and Gutierrez, 1995), but the effect of fasting on cortisol level has not been studied in catfish. During long-term fasting, maintenance of blood glucose at a baseline level has been observed in a variety of fish, and this maintenance has been attributed to gluconeogenesis (Saurez and Mommsen, 1987). We found that injection of glucose produced peak blood glucose levels within 30 min following injection, and that blood glucose levels returned to the control level within 6 h. This 30-min peak was consistent with our postprandial experiment, which showed a peak in glucose 30 min after feeding. Additionally, this time frame for clearance is consistent with studies in another freshwater omnivorous fish. Oral administration of glucose raised blood glucose in C. carpio to a maximum within 1 h, and glucose returned to baseline by 5 h (Fruruichi and Yone, 1981). Similarly, intravenous injection of glucose into the marine omnivore Opsanus tau (oyster toadfish) resulted in peak blood glucose by 30 min, followed by clearance by around 6 h (Tashima and Cahill, 1968). A recent article has reported IP-GTT

in catfish and found peak glucose at 45 min rather than 30 min, with clearance time not determined (Gonzelez and Ortega, 2009). The difference in peak time between their study and the current one might be attributed to a difference in the two fish populations, and/or the different test strip chemistry employed (phenanthroline quinone glucose dehydrogenase used in the previous study, whereas we used glucose oxidase). There may also have been a difference in the level of stress induced by the injection procedure, as the control values were higher than we found in the current study. We have shown that catfish blood glucose is dynamically regulated, and that glucose metabolism is consistent with reports on other omnivorous fish. Our studies show that catfish, like other omnivores, metabolize glucose faster than carnivorous fish (Stone, 2003). The relatively fast glucose metabolism of catfish should facilitate laboratory studies of pancreas and liver function. Other tissues involved in glucose homeostasis include skeletal muscle and adipose, and numerous factors affect circulating glucose level, including insulin sensitivity of peripheral tissues and plasma glucocorticoid levels. Thus, the methods presented here will be useful for studying the function of multiple tissues with respect to glucose metabolism in catfish. We anticipate that additional methods and techniques for studying glucose metabolism will be developed in the near future. An important tool that is currently lacking for pancreatic islet studies is an assay for determining circulating insulin level. The ability to assay insulin would be a powerful complement to glucose measurement, and would enhance our ability to study carbohydrate metabolism in the catfish model. As pointed out by Moon and Foster, ‘‘Carbohydrates are key to the metabolism of all vertebrates, including fish species” (Moon and Foster, 1995). Here we have demonstrated that the catfish is an important new tool for carbohydrate utilization studies in a tractable, genetic vertebrate model.

Recommendations

Glucose meter testing

A large number of studies have compared various glucose meters using human blood and have shown that there can be significant differences in performance, for example (Kimberly *et al*., 2006; Weinzimer *et al*., 2005). Such studies have shown that, although each meter may meet the accuracy requirements of the International Organization for Standardization, they do not necessarily agree with each other precisely. Therefore, human patients are advised to use one meter for managing blood glucose rather than comparing across meters. Additionally, studies have comparedmeters for use on nonhumans, including dogs, cats, and birds, and have reached similar conclusions with respect to meter use. (Wess and Reush, 2000; Lieske *et al*., 2002). Here, we tested the suitability of two human glucose meters for use with catfish blood. We conclude that although both meters are accurate with respect to a laboratory glucose assay, for consistency within and between experiments, only one meter should be used. The two meters tested, Meter A and Meter B, use different chemistries for measuring glucose in whole blood. In an initial test of both meters, we determined that the average catfish hematocrit fell into the range of both meters, and that neither body weight, length, nor sex influenced hematocrit value. For reading the hematocrit value, we tried three commonly used commercially available readers: a card-style reader, in which the microhematocrit tube sits over a sliding scale and the packed cell volume is read manually; a disc-style reader, in which the scale is placed directly over the tube rotor for reading manually; and a digital reader, in which the tube is read electronically. We found that the manual readers were not practical, because they require a minimum 9 μL blood volume, which we could not typically draw. By contrast, the digital reader could measure hematocrit using as little as approximately 5 μL blood volume. Hematocrit measurement is useful for the study of a

variety of diseases, including anemias, infections by parasites, and some metabolic disorders (Shafizadeh *et al.*, 2002).

After determining that both glucose meters were suitable with respect to catfish hematocrit, we went on to test precision and accuracy. Both meters were slightly less precise than reported for human blood. However, as the CV was <8% across a range of glucose values for either meter, we concluded that both meters give reliable measures.

In our comparison of all four meters, we found that we could discriminate statistically between them, with the exception that Meter B could not be discriminated from Meter C. Although we tested for differences attributable to sample volume, this parameter is simply a proxy for general differences in test strip technology. The strips differ in a number of ways that could potentially affect glucose measurement, including the specific mediator and indicators employed, and number and orientation of electrodes (Hones *et al*., 2008). In further testing, we directly compared the performance of Meter A and B with a clinical laboratory test. We found that both meters fall within the 20% error cutoff deemed acceptable for human patients. Ultimately, we concluded that although all four brands of test strips and meters give somewhat different values, they perform similarly from a clinical perspective. The practical significance of these data is that only one meter should be used for experimental tests so that there is consistency for comparing across measurements.

References

1. Anderson, R.M., Bosch, J.A., Goll, M.G., Hesselson, D., Dong, P.D. and Shin D. (2009). Loss of Dnmt1 catalytic activity reveals multiple roles for DNA methylation during pancreas development and regeneration. *Dev Biol;*334:213–223.

2. Banting. F. G. and Best C. H. (1922). The internal secretion of the pancreas. *J. Lab. Clin. Med.*, 7:251–266.
3. Bell, D.E., Sasse, T.A. (2008). Evaluation of the Performance of the TRUEread Blood Glucose Monitoring System Using the ISO 15197:2003 Accuracy and User Evaluation Requirements., TR331 Rev., 1st Edition. Ft. Lauderdale, FL: Home Diagnostics, Inc., pp. 1–5.
4. Brand, M., Granato, M., Nusslein-Volhard, C. (2002). Keeping and raising zebrafish. In: Zebrafish: A Practical Approach. Nusslein-Volhard C, Dahm R (eds.). Oxford: Oxford University Press, pp. 7–37.
5. Brisson, G.R., Camu, F., Malaisse-Lagae, F., Malaisse, W.J. Effect of a local anesthetic upon calcium uptake and insulin secretion by isolated islets of Langerhans. *Life Sci*, 1971;10:445–448.
6. Brown, L.A. (1993). Anesthesia and restraint. In: Fish Medicine. Stoskopf MK (ed.). Philadelphia: W.B. Saunders Company, pp. 79–90.
7. Bruton, M.N. (1979). The breeding biology and early development of *Clarias gariepinus* (Pisces, clariidae) in lake Sibaya, South Africa, with a review of breeding species of the subgenus *Clarias* (Clarias). *Trans. Zool. Soc. London*, 35:1-45.
8. Chavin, W. and Young, J.E. (1970). Factors in the determination of normal serum glucose levels of goldfish, *Carassius auratus* L. *Comp. Biochem Physiol*; 33:629–653.
9. Clay, D. (1979). Population biology, growth and feeding of the African catfish, *Clarias gariepinus*, with special reference to juveniles and their importance in fish culture. *Arch. Hydrobiol*., 87 (4): 453-482.
10. Curado, S, Anderson, R. M., Jungblut, B., Mumm, J., Schroeter, E., Stainier, D. Y (2007). Conditional targeted cell ablation in zebrafish: a new tool for regeneration studies. *Dev Dyn;* 236:1025–1035.
11. Doyle, M.E. and Egan, J.M. (2003). Pharmacological agents that directly modulate insulin secretion. *Pharmacol Rev*.; 55:105–131.
12. Elo, B, Villano, C.M., Govorko, D. (2007) White, L.A. Larval zebrafish as a model for glucose metabolism: expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds. *J Mol Endocrinol;* 38:433–440.

13. Frazier, D.T. and Narahashi, T. T. (1975). (MS-222): effects on ionic conductances of squid axon membranes. Eur J Pharmacol;33:313–317.

14. Furuichi, M. and Yone, Y. (1981). Change of blood sugar and plasma insulin levels of fishes in glucose tolerance test. *Bull Jpn Soc Sci Fish*;47:761–764.

15. Gleeson, M., Connaughton, V., Arneson L.S. (2007). Induction of hyperglycaemia in zebrafish (Danio rerio) leads to morphological changes in the retina. *Acta Diabetol*; 44:157–163.

16. Gonzalez-Alvarez, R., Ortega-Cuellar, D., Hernandez-Mendoza, A., Moreno-Arriola, E., Villasenor-Mendoza, K., Galvez-Mariscal, A. (2009). The hexokinase gene family in the zebrafish: structure, expression, functional and phylogenetic analysis. *Comp Biochem Physiol B Biochem Mol Biol*;152:189–195.

17. Groenewald, A.A.v.J. (1964). Observations on the food habits of *Clarias gariepinus* Burchell, the South African freshwater Barbel (Pisces: Clariidae) in Transvaal. *Hydrobiologia*, 23:267-273.

18. Groff, J.M. and Zinkl, J.G (1999). Hematology and clinical chemistry of cyprinid fish. Common carp and goldfish. *Vet Clin North Am Exot Anim Pract*; 2:741–776.

19. Hattingh, J. (1972). Observations on the blood physiology of five South African freshwater fish. *J Fish Biol*; 4:555–563.

20. Hattingh, J. (1973). Some blood parameters of the yellowfish (*Barbus holubi*) and the barbel (*Clarias gariepinus*). *Zool Afr*.,;8:35–39.

21. Hecht, T. and Appelbaum, S. (1988). Observations on intraspecific aggression and coeval sibling cannibalism by larval and juvenile *Clarias gariepinus* (Clariidae: Pisces) under controlled conditions. *J. Zool., Lond.*, 214: 21-44.

22. Hogendoorn, H., and Vismans, M.M. (1980). Controlled propagation of the African catfish, *Clarias lazera* (C&V). II. Artificial reproduction. *Aquaculture*, 21: 39-53.

23. Hones, J., Muller, P. and Surridge, N. (2008). The technology behind glucose meters: test strips. *Diabetes Technol Ther*; 10:S1–S10.

24. Iwama, G.K. and Ackerman, P.A. (1994). Anaesthetics. In: Analytical Techniques. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier,, pp. 1–15.

25. Jensen, P.J., Gitlin, J.D., Carayannopoulos, M.O. (2006). GLUT1 deficiency links nutrient availability and apoptosis during embryonic development. *J Biol Chem*; 281:13382–13387.

26. Jones, C.M. and Grutter, A.S. (2005). Parasitic isopods (Gnathia sp.) reduce haematocrit in captive blackeye thicklip (Labridae) on the Great Barrier Reef. *J Fish Biol*; 66:860–864.

27. Jubb, R.A. (1961). An illustrated guide to the fresh water fishes of the Zambezi River, lake Kariba, Pungwe, Sabi, Lundi and Limpopo Rivers. Bulawayo: Stuart Manning.

28. Kilpatrick, E.S., Rumley, A.G. and Rumley, C.N. (1995). The effect of haemolysis on blood glucose meter measurement. *Diabet Med*;12:341–343.

29. Kimberly, M.M., Vesper, H.W., Caudill, S.P., Ethridge, S.F., Archibold, E., Porter, K.H., Myers, G.L. (2006). Variability among five over-the-counter blood glucose monitors. *Clin Chim Acta*; 364:292–297.

30. Kinkel, M. D. and Prince, V.E. (2009). On the diabetic menu: zebrafish as a model for pancreas development and function. Bioessays; 31:139–152.

31. Koerber, A. S. and Kalishman, J. (2009). Preparing for a semiannual IACUC inspection of a satellite zebrafish (Danio rerio) facility. *J Am Assoc Lab Anim Sci*; 48:65–75.

32. Li, Z., Wen, C., Peng, J., Korzh, V., Gong, Z. (2009). Generation of living color transgenic zebrafish to trace somatostatin-expressing cells and endocrine pancreas organization. *Differentiation*; 77:128–134.

33. 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.

34. Macleod, J.J.R (1922). The source of insulin. *J. Metab. Res*.; 2:149–172.

35. Micha, J.C. (1976). Synthèse des essais de reproduction, d'alevinage et de production chez un silure Africain: *Clarias lazera* Val. Symp. FAO/CPCA on Aquaculture in Africa. Accra, Ghana. *CIFA Techn. Pap*. 4 (1): 450-473.

36. Moon, T.W. and Foster, G.D. (1995). Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. In: Biochemistry and Molecular Biology of Fishes, Vol.4: Metabolic Biochemistry. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier, pp. 65–100.

37. Moro, E., Gnugge, L., Braghetta, P., Bortolussi, M., Argenton, F. (2009). Analysis of beta cell proliferation dynamics in zebrafish. *Dev Biol;* 332:299–308.

38. Moss, J. B., Koustubhan, P., Greenman, M., Parsons, M. J., Walter, I. and Moss LG (2009). Regeneration of the pancreas in adult zebrafish. *Diabetes;* 58:1844–1851.

39. Moussa, T.A. (1956). Morphology of the accessory airbreathing organs of the teleost *Clarias lazera* (C&V). *J. Morph.*, 98:125-160.

40. Moussa, T.A. (1956). Morphology of the accessory airbreathing organs of the teleost *Clarias lazera* (C&V). *J. Morph.*, 98:125-160.

41. Munro, J. L. (1967). The food of a community of East African freshwater fishes. *J. Zool., Lond*. 151:389-415.

42. Navarro, I. and Gutierrez, J. (1995) Fasting and starvation. In: Biochemistry and Molecular Biology of Fishes, Vol. 4: Metabolic Biochemistry. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier, pp. 393–434.

43. Norlund, L. and Sehlin, J. (1985). Effect of tetracaine and glibenclamide on $45Ca^{2+}$ handling by isolated pancreatic islets. *Br. J. Pharmacol*; 85:127–132.

44. Owiti, D.O. and Dadzie S. (1989). Maturity, fecundity and the effect of reduced rainfall on the spawning rhythm of a siluroid catfish, *Clarias mossambicus* (Peters). *Aquaculture and fisheries management*, 20: 355-368.

45. Perry, S.F. and Reid, D.G. (1994). Injection techniques. In: Biochemistry and Molecular Biology of Fishes, Vol. 3: Analytical Techniques. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier, pp. 85–92.

46. Perry, S.F., Davie, P.S., Daxboeck, C., Ellis, A.G., Smith, D.G. Perfusion methods for the study of gill physiology. In: Fish Physiology Volume X: Gills, Part B: Ion and Water Transfer. Hoar WS, Randall DJ (eds.). Orlando, FL: Academic Press, Inc., 1984, pp. 325–388.

47. Pisharath. H., Rhee, J.M., Swanson, M.A., Leach, S.D., Parsons, M.J. (2007) Targeted ablation of beta cells in the embryonic zebrafish pancreas using *E. coli* nitroreductase. *Mech Dev*; 124:218–229.

48. Reavill, D.R. (2006). Common diagnostic and clinical techniques for fish. *Vet Clin North Am Exot Anim Pract;* 9:223–235.

49. Richter, C.J.J. (1979). The african catfish, *Clarias lazera* (C&V), a new possibility for fish culture in tropical regions. Misc. Pap. Landbouwhogeschool, Wageningen, No. 13: 51-71.

50. Robison, B.D., Drew, R.E., Murdoch, G.K., Powell, M., Rodnick, K. J, Settles M. (2008). Sexual dimorphism in hepatic gene expression and the response to dietary

carbohydrate manipulation in the zebrafish (Danio rerio). *Comparative Biochemistry and Physiology*, Part D;3:141–154.

51. Shafizadeh, E., Paw, B. H., Foott, H., Liao, E.C., Barut, B.A., Cope, J.J., Zon, L.I., Lin, S. (2002). Characterization of zebrafish merlot/Chablis as non-mammalian vertebrate models for severe congenital anemia due to protein 4.1 deficiency. *Development*; 129:4359–4370.

52. Spataru, P., Viveen, W.J.A.R. and Gophen, M. (1987). Food composition of *Clarias gariepinus* (= *C. lazera*), (Cypriniformes, Claridae) in Lake Kinneret (Israel), *Hydrobiologica*, 144: 77-82.

53. Spence, R., Gerlach, G., Lawrence, C., Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biol Rev Camb Philos Soc*; 83:13–34.

54. Stone, D. A. J. (2003). Dietary carbohydrate utilization by fish. *Rev Fish Sci*; 11:337–369.

55. Suarez, R. and Mommsen, T.P. (1987). Gluconeogenesis in teleost fishes. *Can J Zool*; 65:1869–1882.

56. Tanaka, K., Kawano, T., Tomino, T., Kawano, H., Okada, T., Oshita, S. (2009). Mechanisms of impaired glucose tolerance and insulin secretion during isoflurane anesthesia. *Anesthesiology*, 111:1044–1051.

57. Tashima, L. and Cahill, G.F. Jr. (1968). Effects of insulin in the toadfish, Opsanus tau. *Gen Comp Endocrinol*; 11:262–271.

58. Tiso, N., Moro, E., Argenton, F. (2009). Zebrafish pancreas development. *Mol Cell Endocrinol;* 312:24–30.

59. Tseng, Y.C., Chen, R.D., Lee, J.R., Liu, S.T., Lee, S.J., Hwang, P.P. (2009). Specific expression and regulation of glucose transporters in zebrafish ionocytes. *Am J Physiol Regul Integr Comp Physiol*; 297:R275–R290.

60. Van Vuren, J.H.J., Hattingh, J. A. (1978). Seasonal study of the haematology of wild freshwater fish. *J. Fish Biol;* 13:305–313.

61. Weinzimer, S.A., Beck, R.W., Chase, H.P., Fox, L.A., Buckingham, B.A., Tamborlane, W.V. (2005). Accuracy of newer-generation home blood glucose meters in a diabetes research in children network (DirecNet) inpatient exercise study. *Diabetes Technol Ther*; 7:675–680.

62. Wess, G. and Reusch, C. (2000). Assessment of five portable blood glucose meters for use in cats. *Am J Vet Res*; 61:1587–1592.

63. Wess, G. and Reusch, C. (2000). Evaluation of five portable blood glucose meters for use in dogs. *J Am Vet Med Assoc*; 216:203–209.

64. Westerfield, M. (1995). The Zebrafish Book. Eugene, University of Oregon Press.

65. Wilson, R. P. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*; 124:67–80.

66. Wolf, K. (1963). Physiological salines for fresh-water teleosts. *The Progressive Fish-Culturist*; 25:135–140.