A simple and rapid method for blood collection from walking catfish, *Clarias batrachus* (Linneaus, 1758)

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Abstract

Blood is collected from experimental animals for a wide range of scientific purposes including; hematology, clinical biochemistry parameters, immunology, studies in bacteriology, parasitology and investigations in reproductive performance and health. The number of methods employed to collect blood from fish include; the puncture of caudal vein, dorsal aorta or cardiac vessels and the severance of the caudal vein. Unfortunately, all these procedures are practically found to be slow and stressful to *Clarias batrachus*, including the popular caudal vein approach, likely due to the small size of caudal veins relative to the size of the species. In line with the universal ethical recommendations for taking blood from small research animals, we propose an alternative one-operator approach for *C. batrachus* that is simple, rapid and without the need to sacrifice the fish as with other methods. This procedure targets the dorsal aorta (a relatively larger blood vessel) in a sedated fish, punctured by inserting a needle directly from the anterior part of the anal fin about 2-5 mm behind the genital papilla, to draw the desired amount of blood. The technique is a one-operator procedure not requiring the help of an assistant or any special equipment to restrain the fish. The operation of the protocol is unique since it permits the continuous collection of blood from the same experimental fish over a varied time course and reduces the need for a large number of replicate animals. The advantages of the proposed protocol are also highlighted and discussed in detail.

Keywords: Haematology, Catfish, Aquaculture, Blood sampling, *Clarias batrachus*

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Introduction

Clarias batrachus is one of the most notable catfishes in Asia (Argungu *et al.,* 2012) and its popularity has increased significantly as an important commercial aquaculture species (Zonneveld *et al.,* 1988; Knud-Hansen *et al.,* 1990; Kumari and Sahoo, 2006; Sahoo *et al.,* 2007) and for aquarium trade in recent times among the fish farming communities in Asia (Ng and Kottelat, 2008). This is better appreciated, considering that consumers prefer it more, than *Clarias gariepinus* in some parts of Asia because of its excellent taste and rough muscle mass. This is in spite of the better growth performance achieved with the latter species when both are farmed (Rahman *et al*., 1995). *C. batrachus* typically attains a standard length of 22.5-30 cm, although individuals twice this size has been reported (Masterson, 2007). Lately, research efforts on *C. batrachus* are focused on its reproductive performance, especially with regards to selection of matured candidates for induced breeding. To carry out such studies effectively, the need arises for researchers to collect blood from the experimental animals, for further processing (Kori-Slakpere, 1985).

Collection of blood from experimental fish is for a wide range of scientific purposes including hematology, clinical biochemistry parameters, studies in bacteriology, parasitology and investigations in reproductive performance and health (DFO, 2004). A number of methods are available for blood collection from fish, including the puncture of caudal vein, dorsal aorta or cardiac vessels, decapitation and the severance of caudal vein (MUAWC, 2008). However, the choice of sampling technique utilized by a researcher depends on a number of factors such as the health status and size of fish, the quantity of blood required to be collected and the fate of the animal (terminal or non-lethal) in a study (Svobodora and Vykusova, 1991). Overall, the chosen sampling approach should be the least stressful, to avoid interference with the outcome of the study. This consideration makes it necessary for the chosen methodology to be refined, in order to also comply with International ethical and procedural requirements, such as the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (MUAWC, 2008).

The most popular technique, targeting the caudal vein to draw blood (MUAWC, 2008) is practically slow and stressful, particularly when applied to *C. batrachus.* This is likely due to the small size of caudal veins in the species. Furthermore, the other conventional methods are very cumbersome and less convenient to sample blood in the species (via non-lethal means). This is particularly a challenge in experiments which require continuous blood collection over a varied time course and need the animal to be kept alive.

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It is an ethical obligation for researchers to ensure that laboratory animals in their care during experimentation are not subjected to unnecessary stress (DFO, 2004). Consistent with this therefore, the objective of the present exposition was to develop a one-operator approach that is simple and rapid for sampling blood from *C. batrachus*, without the need to sacrifice the fish. The advantages of the proposed protocol are also highlighted and discussed in detail.

Materials and methods

The specimens we used to develop this protocol were *C. batrachus* brood fish, with an average body weight and total length of 186 g and 28 cm, respectively. The live fish were caught from the wild by fishermen in Negeri Sembilan, Malaysia where they were purchased and transported to University Putra Malaysia Aquaculture hatchery unit, Puchong-Selangor, Malaysia. The fish samples were kept in fiber glass tanks (2 tones capacity) for about two months, under observation during acclimation in our research facility. The average water quality conditions in the tanks during this period were maintained at 26.5ºC, 5.7 ppm and 7.6 for temperature, dissolved oxygen and pH, respectively, and were confirmed optimal to maintain sound health of the species.

Using an appropriate net, we randomly removed individual fish from the holding tank and immersed it into anesthetic solution (containing 2.5 mL of clove oil solutions diluted in 5 L of water), for 4-6 min. Before the blood collection procedure, we subjected the fish samples to morphometric measurements, to determine their body weight, as well as total and standard lengths. Complete sedation was confirmed when a fish no longer responded to external stimuli, yet slight gill movements were still observable.

Blood collection was accomplished as follows; the completely sedated fish was removed with the aid of a net from the anesthetic solution and placed on a clean towel on its side. Using the towel, the fish was carefully lifted to a comfortable handling position with one hand, in such a way that the fish was upside down and the head away from, but tail nearer to the individual carrying out the procedure. With the other hand, a 23G needle was inserted into the fish at a point anterior to the anal fin, and about 2-5 mm behind the genital papilla. The insertion of the needle into the fish was at a perpendicular angle to the ventral surface (at approximately 90º), until some blood entered into the syringe or when the needle made contact with the vertebral column (hard impenetrable surface). Once the needle touched the vertebral column, it was withdrawn slightly, approximately 1 mm, so that the blood vessel overlying the vertebral column could then be sampled easily and rapidly. This was done by gently pulling on the plunger by maintaining consistent pressure until the desired quantity of blood is drawn into the syringe. The collected blood

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sample was slowly but quickly transferred into collection tubes (containing EDTA or heparin or treated as may be desired per individual experimental set up). The sampled fish was then transferred to a recovery tank, containing clean, aerated water (also prepared before the commencement of the procedure), where it recovered fully and was swimming freely within 45-60 minutes.

The tube containing sampled blood was gently mixed with the anti-coagulant (as the case may be) by carefully turning it upside down to mix the contents. The tubes were then kept in a sampling box containing ice for further analysis.

Results

Using the presently proposed protocol, we successfully collected 0.5 mL of blood from each of the 24 fish (3 replicate fish per treatment, of 8 treatments) during each of 6 hourly time course sampling periods, within 24 hours (a total of 4 collections per fish, each yielding a total of 2.5 mL of blood). The fish sampled ranged in size between 90 g and 160 g and were made up of equal numbers of male and female individuals. Among these sampled fish, over 75% (made up of some 20 individuals) recovered fully and survived the blood collection. Similarly, we were also able to use this same proposed procedure to collect 1 mL of blood from each of 40 individual fish (males and females) of *Channa striata* species, during haematological and biochemical studies. All the fish in this experimental trial (ranging in size between 75 g and 310 g) made 100% recovery and survived, thereafter to later be subjected to further experimentation.

Discussion

According to MUAWC (2008), the most popular technique for blood sampling from fish is the caudal vein method. Attempts were made by other workers using this popular method, to sample blood from over ten individuals of *C. batrachus* in our laboratory, but were met with little success. These attempts failed in spite of help offered by experienced hands in blood collection from other species of catfish, using the caudal vein technique. This challenge may explain why some researchers previously working on the same species, *C. batrachus*, resorted to completely severing off the caudal peduncle to obtain the desired quantity of blood from the species (Kulshrestha and Mandal, 1982; Maheswaran *et al.*, 2008; Kumar *et al.*, 2010). Consistent with this speculation, some authors reported sectioning the caudal peduncle (Srivastav and Srivastav, 1988), whereas others had to stun the animals (Raja and Sapkal, 2011) to collect enough blood samples due to the difficulty and stressful nature of the operation when conventionally targeting the caudal vein in the species. The procedure used in these trials indicate that, not only is the popular method slow and stressful to *C.*

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batrachus, it is also cumbersome. That those earlier workers neither mentioned the survival rates after their procedure nor used other conventional methods, is additional evidence to suspect that those methods were considered less convenient. The difficulty of blood collection from *C. batrachus* could therefore, be attributed to the small size of caudal veins and peculiarities in the physiology of its cardio-vascular system relative to the moderate size of the species.

The cardio-vascular system of the walking catfish, *C. batrachus* is that of a primitive, air-breathing fish (Graham, 1997), a similarity of this characteristic which it shares with snakehead fish, *Channa striata* (Aliyu-Paiko *et al*., 2010). These air-breathing species are much unlike their typical water-breathing counterparts in the circulation systems. The system in water-breathing fish is reported to be a single "in-series" arrangement, in which blood from the veins enters the heart via *sinus venosus* with pressure below that in the atmosphere. The venous blood is then forced to the gills via the ventral aorta by the heart. Upon oxygenation in the gills, the blood does not return to the heart like in mammals, but arterial blood is forced into the dorsal aorta that runs just beneath the vertebral column and to further be dispersed into the different tissues and organs via arteries, arterioles and the capillaries (Olsen, 2011). The heart pumps out the blood to the systemic circulation through the respiratory organs, with clear separation between respiratory and systemic circulations (Ishimatsu *et al*., 1979). A secondary circulation system has also been identified in fish; an arterial-capillary-venous system derived from the primary circulation which supplies the fins, tail and skin with blood. Capillaries are drained into a large collecting vein that flows towards the head. In salmonids, a "caudal heart" helps to force blood from the secondary capillaries system in the tail into the caudal vein, driven by skeletal muscle contractions. The venous blood is then returned to the heart, by the posterior cardinal vein, which is also located beneath the vertebral column. All the venous blood collects into one vessel (*Sinus venosus*) before entering the heart. Therefore, skeletal muscle contractions, vasodilators and capillary attraction play important roles in the transportation of venous blood (Farrell *et al*., 2001; Sandblom and Axelsson, 2007).

In "air-breathing" fish however, this clear separation is not in place, where mixing of oxygenated and deoxygenated blood occurs in some species. Among these, some species have evolved breathing organs between the gills and systemic circulation so that mixing does not occur, whereas in many others like *Channa* (and probably *C. batrachus*) the arrangement is such that oxygenated blood from the air-breathing organs is sent to the venous system and returned to the heart, before it is pumped to the systemic circulation (Satchell, 1976). Overall, these

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modifications due to the air-breathing nature of the fish have affected the arrangement of the central cardiovascular system of the species to show several unique deviations from that of the typical teleost design (Ishimatsu, 2012). The most notable among these deviations is the presence of anterior and posterior ventral aortae (Ishimatsu and Itazawa, 1983) as opposed to the single dorsal aorta in water-breathing fish**.** Blood oxygen pressure is the highest in the anterior cardinal vein, which receives oxygenated blood from the suprabranchial organ, and is lowered at the common cardinal vein and *sinus venosus* when mixed with venous blood from posterior cardinal and hepatic veins, respectively (Ishimatsu *et al*., 1979). According to Satchell (1976), the advantage of this drainage of oxygenated blood into the venous system is such that the lower pressure at the peripheral end of the respiratory capillaries, caused by their exposure to the *vis a fonte* of the central veins rather than the arterial pressure of the dorsal aorta lowers the average pressure along their lengths. Therefore, the combination of 1) presence of two aortae, 2) the lack of anatomical division in the venous system and 3) the absence of sino-artrial valves, which according to Johansen (1971) play an important role in ventricular filling in other teleosts, all contribute to lowering the pressure of blood in caudal veins of "air-breathing" fish such as *C. batrachus*, making blood collection at the caudal vein difficult in the species. Added to this, vasodilators and skeletal muscle contraction play important roles on the pressure during transportation of venous blood (Sandblom and Axelsson, 2007; Farrell *et al*., 2001) and proper anesthesia (which is part of the blood collection procedure) severely affects muscle contraction. This may be additional evidence to explain the lack of success in collecting blood from the species using the popular protocol.

Considering that *C. batrachus* is a threatened species (Hossain *et al*., 2006; Ahmad *et al.*, 2012) and is reported to be vanishing in some parts of Asia (Binoy, 2010), further unnecessary waste of individuals cannot be afforded, especially through experimental designs which are developed with the sole purpose of maximizing blood collection. This formed one of the important reasons for the development of the present protocol, to make blood collection easier and more rapid. Our procedure was not only rapid and of minimal stress to the species, but is also non-lethal and does not require the help of an assistant to restrain the fish or any sophisticated equipment such a "V" board fish holder commonly used during most of the existing protocols.

Considering the positive attributes mentioned above, the procedure described in our proposed protocol comply consistently with the ethical requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose

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(MUAWC, 2008). The important advantage which it has over other conventional approaches being a one-operator procedure makes it suitable for broader applications, where it is likely to prove useful in collecting blood from different species. Lastly, the only limitation to the proposed protocol for now is that it was tested on only two species (*Clarias batrachus* and *Channa striata*). It is expected that operators may wish to test the procedure on a wide range of species, especially the primitive, air-breathing species, to investigate its effectiveness and applicability to the species targeted.

Blood collection from *Clarias batrachus* by an individual operator could be made simple and rapid when carried out based on the procedure described in this protocol. This is a fundamental achievement, considering that the species is a promising aquaculture candidate and one of the economically important indigenous freshwater fishes in Southeast Asia. The fact that the species is reported to be approaching extinction due to its scarcity in the wild and competition with *Clarias gariepinus*, makes the development of this protocol timely. Therefore, any research practice or cultural operation that duly contributes to further depletion of the species must be avoided, in favor of conservation friendly efforts. This blood sampling methodology promises to support conservation efforts of this economically important fish by enhancing blood sampling from the species for various investigations.

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