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Best practices for non-lethal blood sampling of fish *via* the caudal vasculature

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Abstract

Blood sampling through the caudal vasculature is a widely used technique in fish biology for investigating organismal health and physiology. In live fishes, it can provide a quick, easy and relatively non-invasive method for obtaining a blood sample (*cf.* cannulation and cardiac puncture). Here, a general set of recommendations are provided for optimizing the blood sampling protocol that reflects best practices in animal welfare and sample integrity. This includes selecting appropriate use of anaesthetics for blood sampling as well as restraint techniques for situations where sedation is not used. In addition, ideal sampling environments where the fish can freely ventilate and strategies for minimizing handling time are discussed. This study summarizes the techniques used for extracting blood from the caudal vasculature in live fishes, highlighting the phlebotomy itself, the timing of sampling events and acceptable blood sample volumes. This study further discuss considerations for selecting appropriate physiological metrics when sampling in the caudal region and the potential benefits that this technique provides with respect to long-term biological assessments. Although general guidelines for blood sampling are provided here, it should be recognized that contextual considerations (*e.g.*, taxonomic diversity, legal matters, environmental constraints) may influence the approach to blood sampling. Overall, it can be concluded that when done properly, blood sampling live fishes through the caudal vasculature is quick, efficient and minimally invasive, thus promoting conditions where live release of focal animals is possible.

KEYWORDS

animal welfare, aquaculture, caudal puncture, elasmobranch, field study, live release, teleost

1 | INTRODUCTION

The circulatory system is an important window through which the health and physiology of fishes can be examined. Blood samples from fish can provide information related to stress, disease, nutrition, reproduction and whole-organism responses to environmental challenges (*e.g.*, temperature, exercise, salinity, starvation, pH; *e.g.*, Lermen *et al.*, 2004; Wood *et al.*, 2010; Clark *et al.*, 2011b; Lawrence *et al.*, 2015). Several blood sampling techniques have been developed. Common techniques include cannulation (Axelsson & Fritsche, 1994; Caldwell *et al.*, 2006; Soivio *et al.*, 1975), gill and heart punctures (Di Marco *et al.*, 1999; Goldstein *et al.*, 1964; Kaleeswaran *et al.*, 2016; Railo *et al.*, 1985), tail ablation (Matsuyama *et al.*, 1990; Scholz *et al.*, 2004; Sellaththurai *et al.*, 2019) and caudal puncture (Caldwell *et al.*, 2006; Lawrence *et al.*, 2018). The latter method is one of the most widely used techniques in fish biology because it is quick and, if done properly, has minimal impacts on the welfare of the fish (Canada Department of Fisheries and Oceans, 2004; Houston, 1990). Despite its widespread use, only a handful of reviews have attempted to describe the methods, applications and benefits of caudal puncture (*e.g*., Canada Department of Fisheries and Oceans, 2004; Duman *et al*., 2019; Houston, 1990). Nonetheless, previous reviews have discussed more broadly about phlebotomy (*i.e*., the process of withdrawing blood through a needle) and less specifically about caudal puncture, and there continues to be misinformation on the merits and methods of sampling the blood of fishes (see Duman *et al*., 2019, and a critique by Cooke *et al*., 2019). Consequently, the aim of this review is to highlight the uses and benefits of caudal puncture and its applications in a diversity of cultured and wild fishes in laboratory and field settings. It will include ways in which researchers can implement "best practices" into blood sampling procedures which are defined as activities and measures that simultaneously have a net benefit on animal welfare while enabling the collection of physiologically valid and relevant samples (Table 1). In particularly, this will address the appropriate use of anaesthesia in blood sampling and the potential alternatives if sedation is not merited. Further, it will provide recommendations for sampling environments that should facilitate ease of biopsy while minimizing stress to the animal. Finally, it will discuss in detail the specific techniques associated with caudal blood sampling as well as highlight concerns related to sample timing and volumes.

2 | THE USE OF ANAESTHESIA IN CAUDAL PUNCTURE

Several common methods of inducing sedation in fishes may be used to facilitate fish handling and caudal puncture. This typically involves the application of pharmaceuticals through the surrounding water (*e.g*., MS-222, clove oil, benzocaine; reviewed in Neiffer & Stamper, 2009), electrical currents (reviewed in Reid *et al*., 2019) or altering the physical environment (*e.g*., CO_2, low temperatures; Erikson, 2008; Roth *et al*., 2009; Trushenski *et al*., 2013). As anaesthetics primarily serve to immobilize a fish, these agents can provide conditions to draw blood quickly from the animal without physical restraint and minimize stress-related impacts. In addition, larger fishes may require a light dose of anaesthesia (*i.e*., the movements of fish are minimized) to avoid the animal from inflicting self-harm (*i.e*., thrashing and flopping around) as well as to protect the researcher from the animal's movements (*e.g*., Reavill, 2006; Trushenski *et al*., 2013; Ueda *et al*., 2017). Typically, this involves using a lighter dose of the anaesthetic than would be used for more complicated surgical procedures and allows the animal to recover relatively quickly (Javahery *et al*., 2012; Trushenski *et al*., 2013; Ueda *et al*., 2017).

The use of anaesthetics has several disadvantages with respect to caudal puncture on live-released fish that should be considered. In many field and aquaculture settings, expediting sampling times is of critical importance to maintain the optimal welfare of the animal. Many of the anaesthesia methods discussed may extend captivity times as a result of slow induction durations and/or lengthy recovery periods (Hikasa *et al*., 1986; Mylonas *et al*., 2005; reviewed in Popovic *et al*., 2012, Neiffer & Stamper, 2009). Consequently, if the blood parameter of interest changes rapidly in a new environmental context (*i.e*., the anaesthesia bath), then using such agents could potentially result in erroneous data. Furthermore, it is possible that the agent itself can alter the blood physiological status of the fish, potentially confounding with treatment-level effects (Carter *et al*., 2011; Frick *et al*., 2009; Gholipour Kanani *et al*., 2011; Holloway *et al*., 2004; Iwama *et al*., 1989; Larter & Rees, 2017; Rothwell *et al*., 2005). Finally, long recovery durations associated with the use of many sedation agents (Trushenski *et al*., 2013) can be a significant issue for the live release of fish back into the wild. Any lingering effects of the sedation agent may contribute to alterations in natural behaviour (Losey & Hugie, 1994; Mettam *et al*., 2011; Prystay *et al*., 2017) or lead to post-release predation (reviewed in Raby *et al*., 2014). Lingering effects would be suboptimal for studies in which post-release behaviour is monitored (*i.e*., telemetry studies; see Brownscombe *et al*., 2019) or in cases where the loss of an individual from a species at risk would be unacceptable. In addition, experimental limitations such as the need to repeat sample individuals (see later) may often preclude the use of sedation before blood sampling.

An important legal consideration is that the immediate release of fish back into the wild after exposure to anaesthetics (*e.g*., MS-222, clove oil) is prohibited in some jurisdictions. For example, for use of MS-222 in fishes, the U.S. Food and Drug Administration recommends a 21-day holding period to ensure that the chemical is completely removed from the tissues (FDA, 2019). The goal of such legislation is to protect the general public from eating fishes that may be contaminated with one of these chemical agents, which could result in adverse human health effects (reviewed in Trushenski *et al*., 2013). Appropriately, many anaesthetics have strict legal requirements on their use and disposal (reviewed in Trushenski *et al*., 2013), meaning using anaesthetics in remote environments and field applications is difficult given the numerous legal and logistical challenges of chemical anaesthetics. Chemical anaesthetics in field settings are not recommended and, as an alternative, the use of low-voltage electricity is suggested [either as electric gloves (Abrams *et al*., 2018) or as a portable electrosedation system unit (Prystay *et al*., 2017)]. Low-voltage electricity has been proposed as an anaesthetic to aid in fish restraint because of relatively short recovery times after exposure (*i.e*., seconds; Vandergoot *et al*., 2011; Trushenski & Bowker, 2012; Ward *et al*., 2017; Abrams *et al*., 2018; Reid *et al*., 2019). Indeed, because of its relatively low and transient impacts, electricity is widely used as an anaesthetic in aquaculture (*e.g*., Chatakondi & Kelly, 2019; Nguyen *et al*., 2018; Rucinque *et al*., 2018; Trushenski *et al*., 2017). Nonetheless, it is possible, and often preferable, to sample fish from the caudal vasculature without the use of any anaesthesia (as described later). Blood sampling is a routine and rapid technique such that the use of topical analgesics before or after blood sampling is not needed. Beyond that, the efficacy of topical analgesics in fish is poorly understood, and topical analgesics would be washed away by water. Indeed, it is recommended that anaesthetics be used only when the behaviour of the

TABLE 1 Summary of considerations for maintaining best practices for non-lethal blood sampling of fish through the caudal vasculature

Point of interest	Considerations	Best practice recommendations
Anaesthesia	• Several drawbacks of its use: a Long induction/recovery times b Blood parameter could be affected by time/chemical c Behavioural impairments d Disposal of chemicals and legal restrictions	• Quick and simple nature of caudal blood sampling often does not require anaesthetics • Used only when: a Behaviour of the fish is a danger to itself/researchers b Experimental protocol requires it (*e.g.*, more extensive sampling) • If anaesthesia must be used: a Electrosedation b Light dose of a chemical anaesthetic (*e.g.*, MS-222, clove oil, metomidate)
Sampling environment	• Environment should facilitate blood sampling: a Minimize stress and handling of fish b Ease of access to caudal vasculature c Rapid sample collection d Safety of researchers and animals	• Use of a holding device where the movements of fish are constrained: a Padded V-trough b Cooler c Fish holding bags • Gills of fish should be submerged at all times, allowing free ventilation • Clean and well-oxygenated water in restraint device • Minimize air exposure durations (<10 s) • Fish restrained by hand in combination with the sampling constraint device (*e.g.*, V-trough, cooler) to: a Prevent fish self-harm and injury (*e.g.*, thrashing, rolling, moving around) b Expedite sampling times and improve ease of sampling c Protect researchers from harm
Needle entry and blood extraction	• Fish anatomy and size will dictate sampling devices and needle entry location • Entry location should avoid damaging vital organs and minimize stress	• Sampling typically done using either a heparinized syringe tipped with a needle (21 or 23 G) or a vacutainer • Typically, needles are inserted into the ventral midline of the animal, posterior to the anal fin, in the caudal peduncle region • Needle is inserted at a shallow, acute angle (~45°) through the musculature until it reaches the vertebral column • If blood is not captured immediately, rotate needle or draw the needle back slightly • After the needle is removed, if bleeding occurs, it should be halted by applying pressure to the wound or using a tissue adhesive such as Vetbond • Lateral midline needle insertions can be used if ventral sampling cannot be used
Sample timing	• Obtain the blood sample as quickly as possible • Rapid blood sampling is required to: a Preserve integrity and validity of blood metric of interest b Minimize stress on animal and ensure optimal welfare • Timing will be parameter specific	• Recommended maximal sampling duration is 3 min • Sampling area and conditions should be optimized: a Advance planning of sampling workflow and use of "dry runs" b Focal fish should be kept nearby (*e.g.*, tanks, net-pens) or sampled close to point of capture c Use of anaesthesia for difficult-to-handle fish d Sampling devices and tools should be ready e Experienced/trained personnel should conduct sampling
Blood volumes	• Blood volumes need to be considered for endpoint of interest and animal welfare • Taking too much blood can harm fish and can adversely affect physiological metrics of interest, including: a Haemodilution b Cardiovascular changes c Neuroendocrine responses • Must balance between animal's welfare and experimental endpoints of interest	• No accepted correct blood volume recommendations • 0.1%–10% of fish mass blood volumes have been suggested • Impacts of sample volume can be monitored by changes in blood haematocrit • Consult with veterinarians at host institutions on what appropriate blood volumes are given context and experimental endpoints

Note. Best practices are intended to maintain welfare of fish while also ensuring samples that are physiologically valid and relevant. It is acknowledged that study-specific and species-specific constraints will influence the extent to which the best practices outlined here can be applied. Small refinements may be needed.

fish or the experimental protocol necessitates it, given how easy and quick it is to sample blood from live fish. For context, no anaesthesia (or analgesia) is used for routine withdrawal of blood in humans.

3 | SAMPLING ENVIRONMENT AND ANIMAL CARE

Designing a blood sampling protocol for a particular study or species should involve optimizing the environment in which the fish is sampled. Water-breathing fishes take up oxygen across the gills, which do not function well in air. Consequently, exposing the gills to air can be stressful to the fish and can cause significant alterations in the physiological indices measured in the blood (*e.g.*, hypoxemia, metabolic acidosis, depletion of high-energy substrates; Ferguson & Tufts, 1992; Suski *et al*., 2007; Giomi *et al*., 2008; Cicia *et al*., 2012). As such, it is recommended that blood sampling occur while the gills of fish are submerged in well-oxygenated water (*e.g*., Figure 1) or irrigated through a supply of flowing water directed across the gills (through the mouth). For teleosts, a water-filled V-shaped trough or cooler was used, where the fish is held ventral-side up, permitting access to the caudal vasculature (see Appendix S1; Cooke *et al*., 2005; Cooke *et al*., 2008c). Clean water should be continually cycled through the trough to ensure that oxygen levels are sufficient for the study species. This can be achieved through a powered pump or by manually draining and refilling the system. Alternatively, the use of in-water restraining systems such as fish holding bags (Figure 1a; Raby *et al*., 2012; Donaldson *et al*., 2013; Gagne *et al*., 2017; Twardek *et al*., 2018) can also permit blood sampling to occur in an environment where the fish is freely allowed to ventilate (*i.e*., gills submerged throughout the procedure). If these options are not available and sampling must occur while the fish is out of water, sampling should be expedited to minimize the impacts of air exposure on fish welfare. If it cannot be avoided, a good science-based rule of thumb is that air exposure should be <10 s (Cook *et al*., 2015), acknowledging that the negative effects of air exposure vary widely with temperature and species.

Barring the use of anaesthetics, fish should be restrained during caudal puncture. Failure to properly restrain a fish during blood sampling could lead to undue stress on the animal, prolonged sampling durations and physical injuries (*e.g*., scrapes, bruises, injuries from falling), particularly in the case of large, powerful fishes or in situations where the fish is vigorous and unruly. Using devices such as padded

FIGURE 1 (a) Blood can also be collected by holding fish in "fish-holding bags" in the river/water (Photo credit: Graham Raby). (b) Under optimal conditions, a fish is held in a padded sampling trough while still having its gills submerged underwater. The animal is restrained during the procedure to prevent injury and undue stress. All preparations for the sampling event have been made in advance to facilitate ease and timeliness of the sampling event (Photo credit: Steven Cooke). (c) Use of a vacutainer to obtain a blood sample from the caudal vasculature of a sockeye salmon. The needle is inserted into the tissue immediately posterior to the posterior aspect of the anal fin. The vacutainer cup is held at a slight angle. Because the needle is bevelled, it may need to be rotated for blood to begin to flow into the vacutainer. Note that the fish is held supine and, while not visible, its head and gills are immersed in well-oxygenated water (Photo credit: Amy Teffer)

FIGURE 2 Water-filled padded fish sampling trough. The head of the fish is placed at the end with water inflow from a pump to ensure that oxygen levels remain high throughout the sampling event. A measuring tape has been integrated into the trough to simplify processing (Photo credit: Steven Cooke)

V-troughs (Figure 1b and Figure 2; Crossin *et al*., 2007; Clark *et al*., 2011a, 2011b; Raby *et al*., 2013) or other comparable containment devices (*e.g*., fish-restraint boxes; Swift, 1981) can provide an environment to cradle the fish during sampling and prevent self-inflicted harm. In conjunction with these devices, the fish should ideally be physically held in place by a researcher to ensure that the animal has minimal opportunity to move around while being sampled (*e.g*., Warne & Balment, 1995). Restraint typically involves holding the animal with enough force to confine it yet not so much to cause physical injury or substantial mucous loss (a protective coating that provides immune defence against pathogens; reviewed in Gomez *et al*., 2013). "Fish gloves" (*i.e*., gloves coated with tacky material to improve grip) are not recommended given their propensity to remove the mucous coat, which could lead to secondary infection (reviewed in Brownscombe *et al*., 2017). Control of the animal helps minimize the risk of unnecessary injury to the fish and the researchers and can help make caudal puncture quicker and easier. Often, the fish is held ventral-side up and, depending on the species, this may induce a tonic immobility reflex that serves to further immobilize the fish (reviewed in Wells *et al*., 2005). In the case of fish that are particularly strong and prone to "escape" during sampling, it can be useful to hold a wide net at the head-end of a trough. This has been particularly effective in work using live-sampled adult Pacific salmon. The use of suitable personal protective equipment, such as safety glasses, should be considered, as a sudden movement by the fish may dislodge the sampling needle and turn it into a projectile.

4 | NEEDLE ENTRYWAYS AND SPECIFIC EXTRACTION TECHNIQUES

A common practice is to insert a heparinized needle-tipped syringe or a needle and heparinized vacutainer into the midline of the ventral surface of the caudal peduncle posterior to the anal fin (see Figures 1, Appendix S1), although the anatomy of the fish (*e.g*., body size, scale thickness, distance to blood vessels) will largely drive the specific gauge (often 21 or 23 G) and length of the needle used and will likely take a degree of range-finding in new species and settings. Generally, the caudal vasculature lies immediately ventral to the centrum of the vertebral column and is contained within the haemal arch (Figures 3 and 4). Consequently, finding the vertebral column of the fish is often the primary internal landmark for locating the caudal vasculature. Depending on the species, several scales may need to be removed to allow for easy insertion of the needle and to prevent the needle from getting clogged. Upon piercing the epidermis, the needle should usually be kept at a shallow, acute angle (~45°; see Figures 1 and 3B for examples, Appendix S1) to the posterior end of the fish and gradually inserted through the caudal musculature until reaching the vertebrae (Figures 3 and 4). At this point, the vasculature is pierced, and blood can be drawn into the syringe or vacutainer. If blood does not immediately start flowing upon the initial piercing, then the needle can be drawn back slightly from the spinal column as the opening of the needle may not be sitting within the haemal canal (Appendix S1). Alternatively, because needles have an asymmetrical taper, rotating the tip of the needle while in place may help blood enter the needle tip. Generally, caudal puncture provides relatively easy access to the fish's haemal arch in most fish body styles (*e.g*., fusiform, compressiform and sagittiform; Brauner *et al*., 1993; Kieffer *et al*., 2001; Mandelman & Skomal, 2009; Jeffries *et al*., 2011; Lawrence *et al*., 2018). Although needle insertion and blood sampling can hypothetically occur along the entire length of the ventral surface of the caudal peduncle, sampling becomes easier when the needle is inserted closer to the anal fin. Besides ease of access, sampling in the caudal peduncle region ensures that the needle passes only through the caudal musculature and is far removed from vital organs and tissues that occur anterior to the anal fin (*cf*. Duman *et al*., 2019). Avoiding damage to these areas is particularly relevant in instances where live fish are being sampled (Cooke *et al*., 2016). It is also recommended to halt bleeding after withdrawing the needle, which can be achieved by briefly placing a finger or thumb over the puncture site with light pressure (*i.e*., well-aimed direct pressure, minimize rubbing to prevent mucous loss). An attempt was made to use Vetbond on its own or use Vetbond to glue a small piece of latex over the bleeding site, but it was found that pressure is more effective when needed. For most species, there is no bleeding after the needle is withdrawn.

Non-lethal blood sampling of live fishes can also occur through less orthodox entry points. Although the concept remains the same as in caudal puncture (*i.e*., accessing the haemal arch quickly), needles can be inserted along the lateral midline of the fish (Rapp *et al*., 2014). This technique has also been miniaturized such that small fish [*e.g*., zebrafish (*Danio rerio*; Hamilton, 1822)] can be sampled in a non-lethal fashion (Zang *et al*., 2013, 2015) and can allow for repeated sampling in some instances (Zang *et al*., 2013). Presumably, lateral blood sampling is used where either access through the ventral caudal peduncle is limited or the animal's anatomical features prevent sampling in that area altogether. For example, the tapered body and long anal fin in gourami (Osphronemidae) make accessing the ventral surface of the caudal peduncle difficult, and thus, lateral blood sampling is the preferred technique. It is also worth highlighting that the great anatomical and taxonomic diversity of fishes (Helfman *et al*., 2009) makes it challenging to standardize blood sampling techniques across all species. Indeed, the techniques outlined here are unlikely to be appropriate in all given contexts, especially in cases where the fish exhibits a "non-normal" anatomical body style [*e.g*., a leafy sea dragon (*Phycodurus eques*, Günther, 1865) or giant oceanic manta ray (*Mobula birostris*, Walbaum, 1792)] or is particularly large [*e.g*., a whale shark (*Rhincodon typus*, Müller and Henle, 1839)]. Consequently, before experimentation, the techniques and procedures used in blood sampling should be optimized for the particular context to maximize the sampling's effectiveness while simultaneously ensuring optimal animal welfare.

5 | CONSIDERATIONS FOR TIMING IN SAMPLING EVENTS

The timing of the blood sampling event should be a primary consideration during caudal puncture. Blood sampling from a live fish often requires that the animal be collected from its environment (in either cultured or wild settings) to be briefly exposed to air and handled during the sampling event. A 3 min maximum for the time between fish capture and the completion of blood sampling has been suggested (Lawrence *et al*., 2018), although it is important to remember that the nature of the blood parameter of interest will determine the appropriate maximal sampling durations used in the study. For example, circulating concentrations of catecholamines can change quite rapidly (*i.e*., seconds to minutes; Pottinger, 2008) in response to an acute handling stressor, whereas changes in glucocorticoids, blood lactate and glucose occur over longer durations (*i.e*., minutes to hours; Soivio & Oikari, 1976; Pickering & Pottinger, 1989; Lawrence *et al*., 2018; reviewed in Wendelaar Bonga, 1997). Minimizing capture, handling and sampling times not only ensures a high-quality blood sample but also maximizes the welfare of the animal by minimizing prolonged stressor exposure. Minimizing stress is of particular relevance for fish that are to be released back into the wild (Cooke *et al*., 2016) and in cases where repeated blood sampling occurs on the same individual (Cook *et al*., 2012; Frick *et al*., 2009; Jeffries *et al*., 2011).

Broadly, collection and handling times can be minimized by having well-designed sampling areas. For example, in many studies where riverside biopsy of migratory salmon was performed, captured fish were held in a net-pen in proximity to the sampling station. Individuals were then quickly netted and moved to a nearby (*e.g*., 5–10 m) water-filled trough where a team of researchers were ready (Figure 1c; Cooke *et al*., 2006; Dick *et al*., 2018). This process took < 2 min and had a minimal impact on the fish. Sampling durations can also be minimized by having a well-trained and experienced team of personnel. In addition, some form of sedation (*e.g*., CO_2, electricity, MS-222) can be used in appropriate contexts to help calm the animal, which would conceivably expedite sampling, thereby reducing handling stress. Together, planning and experience are key to ensuring caudal puncture is done efficiently to simultaneously maximize sample quality and animal welfare.

6 | HOW MUCH BLOOD CAN BE DRAWN?

Drawing too much blood, whether through single or repeated samples over a relatively short time span, could have negative physiological consequences to the fish, including haemodilution, cardiovascular changes or neuroendocrine responses (Duff & Olson, 1989; Fazio *et al*., 2015; Lane, 1979; Nishimura *et al*., 1979). There is no widely accepted rule for how much blood can be drawn from a live fish [*e.g*., 10% blood volume (BV) recommended by McGill University Animal Care, but 0.1% BV by the Canada Department of Fisheries and Oceans; Canada Department of Fisheries and Oceans 2004; McGill University, 2017]. Nonetheless, BVs do scale with body size. BV is typically 3%–4% of body mass in teleost fishes and 5%–8% of body mass in elasmobranchs (Olson, 1992). For example, a 1 kg rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) could be expected to have ~40 ml of blood, and thus, a researcher would want to collect < 4 ml of blood to stay below the 10% threshold. Indeed, there is considerable variability in drawn BVs from the order of a few microlitres in small fish (Cook *et al*., 2012; Jeffrey *et al*., 2014; Zang *et al*., 2013, 2015) to upwards of a few millilitres in larger individuals (Choromanski *et al*., 2017; Clark *et al*., 2011a, 2011b; Cooper & Morris, 1998; Robinson *et al*., 2013). Most teleost fishes have a resting haematocrit of ~20%–40% (Fánge, 1992; Fazio, 2019; Houston, 1997; Lawrence *et al*., 2018). The volume occupied by the red cells and leukocytes, as well as a general loss of fluid volume during plasma transfer, contributes to the lower-than-expected plasma yield when compared to the initial blood sample, and thus, a 1 ml blood sample should produce ~0.5–0.7 ml of plasma. For example, when blood from bluegill (*Lepomis macrochirus*, Rafinesque, 1810; ~100–160 mm and 100–120 g in size) is sampled, ~0.1–0.3 ml of whole blood yields ~0.05–0.15 ml of plasma (Abrams *et al*., 2018; Cook *et al*., 2012; Lawrence *et al*., 2019), which is sufficient to measure variables like cortisol, glucose, lactate and ions. Nevertheless, some individuals and species are simply too small to obtain a non-lethal blood sample, especially in the field and in non-euthanized fish. Ensuring to minimize sampling volume is a good idea when repeated sampling is required, which prevents issues associated with haemodilution (Duff & Olson, 1989; Fazio *et al*., 2015; Nishimura *et al*., 1979). Haemodilution can be monitored through observing changes in haematocrit and may be offset by re-injecting red cells suspended in saline back into the circulatory system of the fish, specifically when using catheters (*e.g*., Rodela *et al*., 2012; Rogers *et al*., 2003; Zimmer & Wood, 2014). The latter case is unlikely to occur outside a controlled laboratory setting. The balance rests between drawing a sufficient volume of blood to meet the analytical endpoints of the study and the potential impacts that blood removal may have on the welfare of the fish. Veterinarians at hosting institutions (*e.g*., Institutional Animal Care and Use Committee in the USA, Canadian Council on Animal

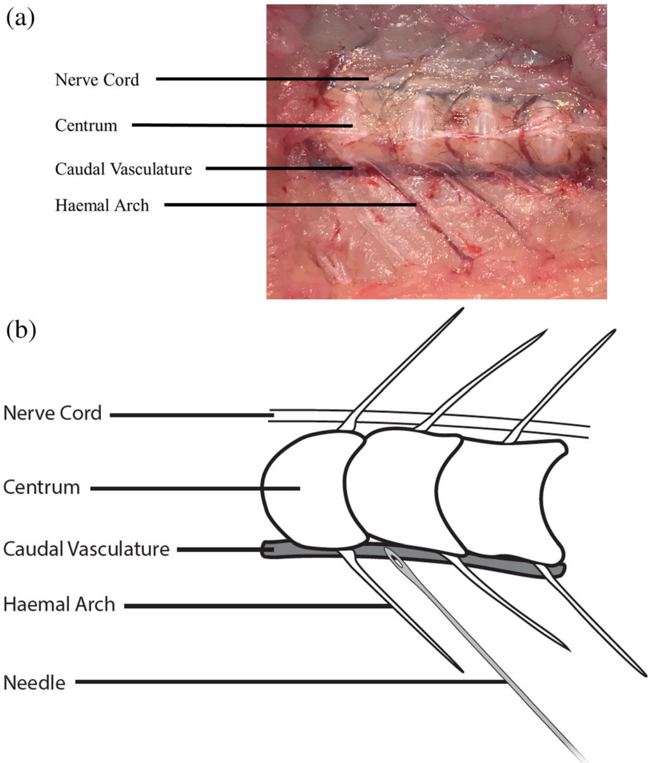

FIGURE 3 (a) Internal anatomy of the caudal vasculature and associated structures of a largemouth bass (*Micropterus salmoides*; Lacépède, 1802) and (b) a cartoon of the caudal vasculature with the needle placement for drawing blood. For point of reference, the image represents the sagittal plane of the caudal peduncle, with the dorsal surface of the animal being at the top of the image (Photo credit: Alice Abrams and Paul Parsons)

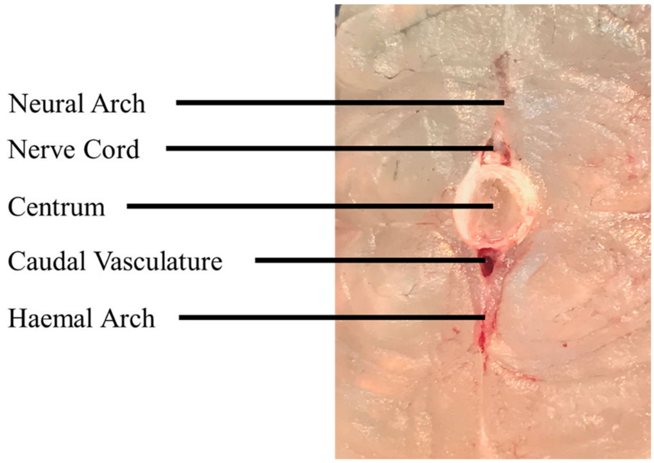

FIGURE 4 Transverse cross section of a largemouth bass's (*Micropterus salmoides*; Lacépède, 1802) caudal peduncle demonstrating the vasculature and associated structures. For point of reference, the dorsal surface of the animal is the top of the image (Photo credit: Alice Abrams)

Care in Canada, the Home Office in the UK) can also be consulted for their expertise regarding blood withdrawals from various taxa and may have guidelines for volumes that should be discussed according to the project design and research goals.

7 | ON ENDPOINTS OF INTEREST

During caudal puncture, as the needle pierces the haemal arch, it is likely that blood is obtained from both arterial and venous vasculature because the two vessels are in proximity (Figures 3 and 4). Given that caudal puncture is a "blind" procedure in this respect, one cannot be certain as to the venous–arterial mixture of the sample blood – often a caudal sample – contains both venous and arterial blood (Esbaugh *et al*., 2016; Mandelman & Skomal, 2009; O'Neill *et al*., 1998). As such, caution is required when this technique is selected for use in measuring parameters where it differs between the two subdivisions of the circulatory system. For this reason, caudal puncture is not ideal for measuring partial pressures of respiratory gases (*e.g*., O_2 and CO_2) because gas levels differ markedly between veins and arteries (Wood *et al*., 1979). Use of cannulation (Axelsson & Fritsche, 1994; Caldwell *et al*., 2006; Eliason *et al*., 2013; Soivio *et al*., 1975) is more appropriate for measuring respiratory gases. For other variables commonly measured with fish blood, the venous–arterial mixture is unimportant. For example, caudal puncture can be used to assess a wide variety of steroid hormones (*e.g*., cortisol, sex steroids; Rosenblum *et al*., 1987; Pickering *et al*., 1991; Bernier & Peter, 2001; Acerete *et al*., 2004; Barcellos *et al*., 2004; Lawrence *et al*., 2018), circulating proteins and triglycerides (Hasler *et al*., 2011) and ions (Ferreira-Martins *et al*., 2016; Marshall *et al*., 1999; Morris, 1980; Parry, 1961; Sui *et al*., 2016; Tunnah *et al*., 2016) that are presumably homogenous in their concentrations throughout the circulatory system.

8 | CONSIDERATIONS FOR POST-RELEASE IMPACTS AND MONITORING

In contrast to other blood sampling techniques, caudal puncture should be expected to have negligible effects on post-sampling survival. There are many examples of fish being blood sampled and surviving in the short- and long term. Indeed, a frontier in modern biology is examining individual variation in physiological status and health across hours (Cousineau *et al*., 2014; Deng *et al*., 2000; Jeffries *et al*., 2011; Vijayan & Moon, 1994), days (Caldwell *et al*., 2006; Djordjevic *et al*., 2012; Hanson & Cooke, 2009), weeks (Jeffries *et al*., 2011; Teffer *et al*., 2019) and even years (Cook *et al*., 2011). By combining non-lethal blood sampling with biotelemetry/biologging (see Cooke *et al*., 2008b), it is possible to assess how physiological and infection status relates to behaviour (Birnie-Gauvin *et al*., 2019; Cooke *et al*., 2008a; Hasler *et al*., 2011; Teffer *et al*., 2018) or survival (Birnie-Gauvin *et al*., 2019; Jeffries *et al*., 2011; Young *et al*., 2006a). Such research has been used to understand how fisheries interactions influence the fate and survival of wild fish (Thompson *et al*., 2008) and how physiology, infections and health status predict migration success for Pacific salmon (Bass *et al*., 2019; Hasler *et al*., 2011). Aquaculture studies have also used non-lethal blood sampling events in assessing how stressors (*e.g*., air exposure, handling, forced swimming) can affect the animal's physiology and health (*e.g*., Bayunova *et al*., 2002; Hamlin *et al*., 2008; Tort *et al*., 2001). In some instances, blood can also offer an interesting tissue through which disease state is assessed, with similar community compositions of infectious agents being observed between non-lethal blood samples, non-destructive gill samples and destructive multi-tissue sampling (*e.g*., Teffer & Miller, 2019). Together, non-lethal blood sampling can provide a wealth of physiological information that can help appreciate how the whole organism is affected by environmental challenges.

9 | CONCLUSIONS

Caudal puncture for sampling blood in live fishes is widely used technique by fisheries science professionals. With adequate practice and refinement of procedures, it is possible to quickly (*i.e*., in seconds to minutes) obtain a non-lethal blood sample from the caudal vasculature of a fish without the use of anaesthetics or euthanasia, particularly in large fishes. Best practices are outlined to guide researchers embarking on studies that use this phlebotomy technique (Table 1). Caudal puncture can also be used across a diversity of species and sampling locations (*e.g*., in the laboratory, aboard marine vessels, adjacent small freshwater streams, caged aquaculture facilities). Given the relatively minimal invasiveness of caudal puncture (*cf*. cannulation and cardiac puncture) combined with its relative ease of use and effectiveness, this technique provides an optimal procedure for use in field settings where live release is an ideal or necessary endpoint. As fish biologists use non-model species and settings at increasing rates, optimizing blood sampling is critical for sample quality and animal welfare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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