









REVIEW PAPER

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

Michael J. Lawrence^{1,2}  | Graham D. Raby³  | Amy K. Teffer^{4,5}  |
Ken M. Jeffries²  | Andy J. Danylchuk⁵  | Erika J. Eliason⁶  |
Caleb T. Hasler⁷  | Timothy D. Clark⁸  | Steven J. Cooke¹

¹Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Sciences, Carleton University, Ottawa, Ontario, Canada

²Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

³Great Lakes Institute for Environmental Science, University of Windsor, Windsor, Ontario, Canada

⁴Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

⁵Department of Environmental Conservation, University of Massachusetts Amherst, Amherst, Massachusetts

⁶Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California

⁷Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada

⁸School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia

Correspondence

Michael J. Lawrence, Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.
Email: m_lawrence27@live.ca

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; Sellathurai *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 particular, 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., CO₂, 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; Rucinke *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
<i>Anaesthesia</i>	<ul style="list-style-type: none"> Several drawbacks of its use: <ol style="list-style-type: none"> Long induction/recovery times Blood parameter could be affected by time/chemical Behavioural impairments Disposal of chemicals and legal restrictions 	<ul style="list-style-type: none"> Quick and simple nature of caudal blood sampling often does not require anaesthetics Used only when: <ol style="list-style-type: none"> Behaviour of the fish is a danger to itself/researchers Experimental protocol requires it (e.g., more extensive sampling) If anaesthesia must be used: <ol style="list-style-type: none"> Electrosedation Light dose of a chemical anaesthetic (e.g., MS-222, clove oil, metomidate)
<i>Sampling environment</i>	<ul style="list-style-type: none"> Environment should facilitate blood sampling: <ol style="list-style-type: none"> Minimize stress and handling of fish Ease of access to caudal vasculature Rapid sample collection Safety of researchers and animals 	<ul style="list-style-type: none"> Use of a holding device where the movements of fish are constrained: <ol style="list-style-type: none"> Padded V-trough Cooler 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: <ol style="list-style-type: none"> Prevent fish self-harm and injury (e.g., thrashing, rolling, moving around) Expedite sampling times and improve ease of sampling Protect researchers from harm
<i>Needle entry and blood extraction</i>	<ul style="list-style-type: none"> Fish anatomy and size will dictate sampling devices and needle entry location Entry location should avoid damaging vital organs and minimize stress 	<ul style="list-style-type: none"> 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
<i>Sample timing</i>	<ul style="list-style-type: none"> Obtain the blood sample as quickly as possible Rapid blood sampling is required to: <ol style="list-style-type: none"> Preserve integrity and validity of blood metric of interest Minimize stress on animal and ensure optimal welfare Timing will be parameter specific 	<ul style="list-style-type: none"> Recommended maximal sampling duration is 3 min Sampling area and conditions should be optimized: <ol style="list-style-type: none"> Advance planning of sampling workflow and use of "dry runs" Focal fish should be kept nearby (e.g., tanks, net-pens) or sampled close to point of capture Use of anaesthesia for difficult-to-handle fish Sampling devices and tools should be ready Experienced/trained personnel should conduct sampling
<i>Blood volumes</i>	<ul style="list-style-type: none"> 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: <ol style="list-style-type: none"> Haemodilution Cardiovascular changes Neuroendocrine responses Must balance between animal's welfare and experimental endpoints of interest 	<ul style="list-style-type: none"> 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 ($\sim 45^\circ$; 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., CO₂, 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% (Fänge, 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-ethanized 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

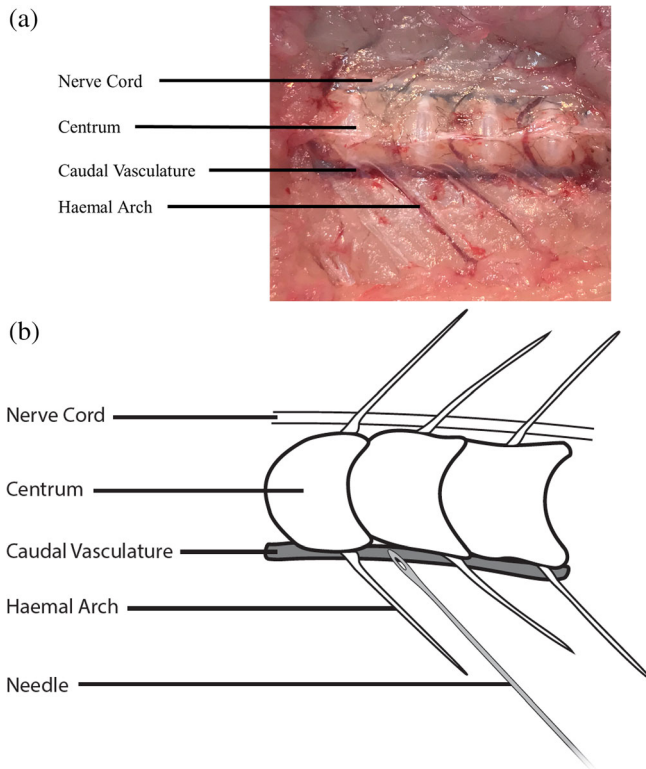


FIGURE 3 (a) Internal anatomy of the caudal vasculature and associated structures of a largemouth bass (*Micropterus salmoides*; Lacépède, 1802) and (b) a cartoon of the caudal vasculature with the needle placement for drawing blood. For point of reference, the image represents the sagittal plane of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

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.*, O₂ and CO₂) 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

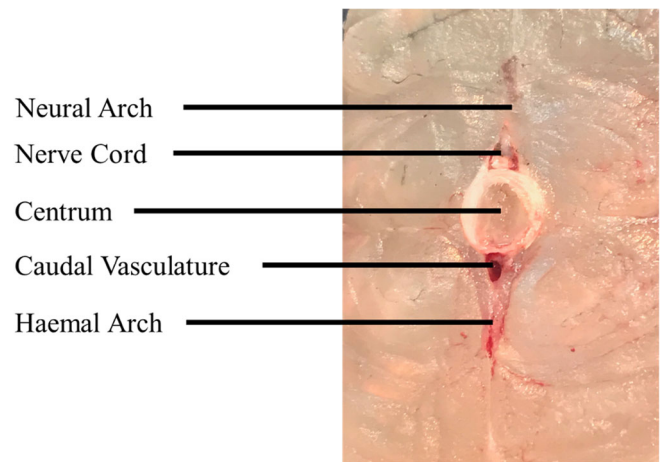


FIGURE 4 Transverse cross section of a largemouth bass's (*Micropterus salmoides*; Lacépède, 1802) caudal peduncle demonstrating the vasculature and associated structures. For point of reference, the dorsal surface of the animal is the top of the image (Photo credit: Alice Abrams)

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.

ORCID

Michael J. Lawrence  <https://orcid.org/0000-0002-4801-1580>

Graham D. Raby  <https://orcid.org/0000-0002-0549-4017>

Amy K. Teffer  <https://orcid.org/0000-0001-5322-2617>

Ken M. Jeffries  <https://orcid.org/0000-0002-7466-1915>

Andy J. Danylchuk  <https://orcid.org/0000-0002-8363-0782>

Erika J. Eliason  <https://orcid.org/0000-0002-0120-7498>

Caleb T. Hasler  <https://orcid.org/0000-0003-3242-7415>

Timothy D. Clark  <https://orcid.org/0000-0001-8738-3347>

REFERENCES

- Abrams, A. E., Rous, A. M., Brooks, J. L., Lawrence, M. J., Midwood, J. D., Doka, S. E., & Cooke, S. J. (2018). Comparing immobilization, recovery, and stress indicators associated with electric fish handling gloves and a portable Electrosedation system. *Transactions of the American Fisheries Society*, 147(2), 390–399.
- Acerete, L., Balasch, J. C., Espinosa, E., Josa, A., & Tort, L. (2004). Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. *Aquaculture*, 237(1–4), 167–178.
- Axelsson, M., & Fritsche, R. (1994). Cannulation techniques. *Analytical Techniques*, 3, 17–36.
- Barcellos, L. J. G., Kreutz, L. C., de Souza, C., Rodrigues, L. B., Fioreze, I., Quevedo, R. M., ... de Almeida Lacerda, L. (2004). Hematological changes in jundiá (*Rhamdia quelen* Quoy and *Gaimard Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture*, 237(1–4), 229–236.
- Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2019). Fisheries capture and infectious agents are associated with travel rate and survival of Chinook salmon during spawning migration. *Fisheries Research*, 209, 156–166.
- Bayunova, L., Barannikova, I., & Semenkov, T. (2002). Sturgeon stress reactions in aquaculture. *Journal of Applied Ichthyology*, 18(4–6), 397–404.
- Bernier, N. J., & Peter, R. E. (2001). Appetite-suppressing effects of urotensin I and corticotropin-releasing hormone in goldfish (*Carassius auratus*). *Neuroendocrinology*, 73(4), 248–260.
- Birnie-Gauvin, K. H. F., Kristensen, M. L., Walton-Rabideau, S., Cooke, S. J., Willmore, W. G., Koed, A., & Aarestrup, K. (2019). Cortisol predicts migration timing and success in both Atlantic salmon and sea trout kelts. *Scientific Reports*, 9, 2422.
- Brauner, C. J., Val, A. L., & Randall, D. J. (1993). The effect of graded methaemoglobin levels on the swimming performance of Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Experimental Biology*, 185(1), 121–135.
- Brownscombe, J. W., Danylchuk, A. J., Chapman, J. M., Gutowsky, L. F., & Cooke, S. J. (2017). Best practices for catch-and-release recreational fisheries—angling tools and tactics. *Fisheries Research*, 186, 693–705.
- Brownscombe, J. W., Lédée, E. J., Raby, G. D., Struthers, D. P., Gutowsky, L. F., Nguyen, V. M., ... Vandergoot, C. S. (2019). Conducting and interpreting fish telemetry studies: Considerations for researchers and resource managers. *Reviews in Fish Biology and Fisheries*, 29(2), 369–400.
- Caldwell, S., Rummer, J. L., & Brauner, C. J. (2006). Blood sampling techniques and storage duration: Effects on the presence and magnitude of the red blood cell β -adrenergic response in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 144(2), 188–195.
- Canada Department of Fisheries and Oceans Canada. (2004). Canada Department of Fisheries and Oceans animal-user training template: 4.0 blood sampling of finfish. Available from https://www.ccc.ca/Documents/Education/DFO/4_Blood_Sampling_of_Finfish.pdf.
- Carter, K. M., Woodley, C. M., & Brown, R. S. (2011). A review of tricaine methanesulfonate for anesthesia of fish. *Reviews in Fish Biology and Fisheries*, 21(1), 51–59.
- Chatakondi, N. G., & Kelly, A. M. (2019). Evaluation of a portable electro-sedation system for anesthetizing mature channel catfish. *North American Journal of Aquaculture*, 81, 269–274.
- Choromanski, J. M., Bulman, F. E., Handsel, T. H., George, R. H., Batt, J. H., Harvey-Clark, C., & Gallant, J. J. (2017). Collection, transport and handling of the Greenland shark, *Somniosus microcephalus*. In M. Smith, D. Warmolts, D. Thoney, R. Hueter, M. Murray, & J. Ezcurra (Eds.), *Elasmobranch husbandry manual II: Recent advances in the care of sharks, rays and their relatives* (pp. 33–42). Ohio Biological Survey: Columbus, OH.
- Cicia, A. M., Schlenker, L. S., Sulikowski, J. A., & Mandelman, J. W. (2012). Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 162(2), 130–138.
- Clark, T. D., Donaldson, M. R., Drenner, S. M., Hinch, S. G., Patterson, D. A., Hills, J., ... Farrell, A. P. (2011a). The efficacy of field techniques for obtaining and storing blood samples from fishes. *Journal of Fish Biology*, 79(5), 1322–1333.
- Clark, T. D., Jeffries, K. M., Hinch, S. G., & Farrell, A. P. (2011b). Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *Journal of Experimental Biology*, 214(18), 3074–3081.

- Cook, K. V., Lennox, R. J., Hinch, S. G., & Cooke, S. J. (2015). Fish out of water: How much air is too much? *Fisheries*, 40(9), 452–461.
- Cook, K. V., O'Connor, C. M., Gilmour, K. M., & Cooke, S. J. (2011). The glucocorticoid stress response is repeatable between years in a wild teleost fish. *Journal of Comparative Physiology A*, 197(12), 1189–1196.
- Cook, K. V., O'Connor, C. M., McConnachie, S. H., Gilmour, K. M., & Cooke, S. J. (2012). Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 161(3), 337–343.
- Cooke, S. J., Crossin, G. T., Patterson, D. A., English, K. K., Hinch, S. G., Young, J. L., ... Farrell, A. P. (2005). Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon: Development and validation of a technique. *Journal of Fish Biology*, 67(5), 1342–1358.
- Cooke, S. J., Hinch, S. G., Crossin, G. T., Patterson, D. A., English, K. K., Healey, M. C., ... Farrell, A. P. (2008a). Physiological correlates of coastal arrival and river entry timing in late summer Fraser River sockeye salmon (*Oncorhynchus nerka*). *Behavioural Ecology*, 19, 747–758.
- Cooke, S. J., Hinch, S. G., Crossin, G. T., Patterson, D. A., English, K. K., Shrimpton, J. M., ... Farrell, A. P. (2006). Physiology of individual late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. *Canadian Journal of Fisheries and Aquatic Sciences*, 63(7), 1469–1480.
- Cooke, S. J., Hinch, S. G., Patterson, A. P. F. D. A., Miller-Saunders, K., Welch, D. W., Donaldson, M. R., ... Van Der Kraak, G. (2008b). Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behaviour, genomics and experimental biology: An interdisciplinary case study on adult Fraser River sockeye salmon. *Fisheries*, 33, 321–338.
- Cooke, S. J., Lawrence, M. J., Raby, G. D., Teffer, A. K., Jeffries, K. M., Danylchuk, A. J., & Clark, T. D. (2019). Comment: Practices for drawing blood samples from teleost fish. *North American Journal of Aquaculture*, 81(4), 424–426.
- Cooke, S. J., Suski, C. D., Danylchuk, S. E., Danylchuk, A. J., Donaldson, M. R., Pullen, C., ... Shultz, A. D. (2008c). Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology*, 73(6), 1351–1375.
- Cooke, S. J., Wilson, A. D., Elvidge, C. K., Lennox, R. J., Jepsen, N., Colotelo, A. H., & Brown, R. S. (2016). Ten practical realities for institutional animal care and use committees when evaluating protocols dealing with fish in the field. *Reviews in Fish Biology and Fisheries*, 26(1), 123–133.
- Cooper, A. R., & Morris, S. (1998). The blood respiratory, haematological, acid-base and ionic status of the port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anaesthesia and surgery: A comparison with sampling by direct caudal puncture. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 119(4), 895–903.
- Cousineau, A., Midwood, J. D., Stamplecoskie, K., King, G., Suski, C. D., & Cooke, S. J. (2014). Diel patterns of baseline glucocorticoids and stress responsiveness in a teleost fish (bluegill, *Lepomis macrochirus*). *Canadian Journal of Zoology*, 92(5), 417–421.
- Crossin, G. T., Hinch, S. G., Cooke, S. J., Welch, D. W., Batten, S. D., Patterson, D. A., ... Farrell, A. P. (2007). Behaviour and physiology of sockeye salmon homing through coastal waters to a natal river. *Marine Biology*, 152(4), 905–918.
- Deng, D. F., Refstie, S., Hemre, G. I., Crocker, C. E., Chen, H. Y., Cech, J. J., & Hung, S. S. O. (2000). A new technique of feeding, repeated sampling of blood and continuous collection of urine in white sturgeon. *Fish Physiology and Biochemistry*, 22(3), 191–197.
- Di Marco, P., McKenzie, D. J., Mandich, A., Bronzi, P., Cataldi, E., & Cataudella, S. (1999). Influence of sampling conditions on blood chemistry values of Adriatic sturgeon *Acipenser naccarii* (Bonaparte, 1836). *Journal of Applied Ichthyology*, 15(4–5), 73–77.
- Dick, M., Eliason, E. J., Patterson, D. A., Robinson, K. A., Hinch, S. G., & Cooke, S. J. (2018). Short-term physiological response profiles of tagged migrating adult sockeye salmon: A comparison of gastric insertion and external tagging methods. *Transactions of the American Fisheries Society*, 147(2), 300–315.
- Djordjevic, B., Kristensen, T., Øverli, Ø., Rosseland, B. O., & Kiessling, A. (2012). Effect of nutritional status and sampling intensity on recovery after dorsal aorta cannulation in free-swimming Atlantic salmon (*Salmo salar* L.). *Fish Physiology and Biochemistry*, 38(1), 259–272.
- Donaldson, M. R., Raby, G. D., Nguyen, V. N., Hinch, S. G., Patterson, D. A., Farrell, A. P., ... McConnachie, S. H. (2013). Evaluation of a simple technique for recovering fish from capture stress: Integrating physiology, biotelemetry, and social science to solve a conservation problem. *Canadian Journal of Fisheries and Aquatic Sciences*, 70(1), 90–100.
- Duff, D. W., & Olson, K. R. (1989). Response of rainbow trout to constant-pressure and constant-volume hemorrhage. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 257(6), R1307–R1314.
- Duman, M., Saticioglu, I. B., Suzer, B., & Altun, S. (2019). Practices for drawing blood samples from teleost fish. *North American Journal of Aquaculture*, 81(2), 119–125.
- Eliason, E. J., Clark, T. D., Hinch, S. G., & Farrell, A. P. (2013). Cardiorespiratory performance and blood chemistry during swimming and recovery in three populations of elite swimmers: Adult sockeye salmon. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 166(2), 385–397.
- Erikson, U. (2008). Live chilling and carbon dioxide sedation at slaughter of farmed Atlantic salmon: A description of a number of commercial case studies. *Journal of Applied Aquaculture*, 20(1), 38–61.
- Esbaugh, A. J., Ern, R., Nordi, W. M., & Johnson, A. S. (2016). Respiratory plasticity is insufficient to alleviate blood acid–base disturbances after acclimation to ocean acidification in the estuarine red drum, *Sciaenops ocellatus*. *Journal of Comparative Physiology B*, 186(1), 97–109.
- Fänge, R. (1992). 1 fish blood cells. In W. S. Hoar, D. J. Randall, & A. P. Farrell (Eds.), *Fish physiology* (pp. 1–54). New York, NY: Academic Press Inc.
- Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, 500, 237–242.
- Fazio, F., Piccione, G., Arfuso, F., & Faggio, C. (2015). Peripheral blood and head kidney haematopoietic tissue response to experimental blood loss in mullet (*Mugil cephalus*). *Marine Biology Research*, 11(2), 197–202.
- FDA (U.S. Food and Drug Administration). 2019. Database of approved animal drug products. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. VMRCVM Drug Information Laboratory. Available from <https://animaldrugatfda.fda.gov/adafda/views/#/home/previewsearch/200-226>.
- Ferguson, R. A., & Tufts, B. L. (1992). Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): Implications for "catch and release" fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, 49(6), 1157–1162.
- Ferreira-Martins, D., Coimbra, J., Antunes, C., & Wilson, J. M. (2016). Effects of salinity on upstream-migrating, spawning sea lamprey, *Petromyzon marinus*. *Conservation Physiology*, 4(1), cov064.
- Frick, L. H., Reina, R. D., & Walker, T. I. (2009). The physiological response of port Jackson sharks and Australian swellsharks to sedation, gill-net capture, and repeated sampling in captivity. *North American Journal of Fisheries Management*, 29(1), 127–139.
- Gagne, T. O., Ovitz, K. L., Griffin, L. P., Brownscombe, J. W., Cooke, S. J., & Danylchuk, A. J. (2017). Evaluating the consequences of catch-and-

- release recreational angling on golden dorado (*Salminus brasiliensis*) in Salta, Argentina. *Fisheries Research*, 186, 625–633.
- Gholipour Kanani, H., Mirzargar, S. S., Soltani, M., Ahmadi, M., Abrishamifar, A., Bahonar, A., & Yousefi, P. (2011). Anesthetic effect of tricaine methanesulfonate, clove oil and electroanesthesia on lysozyme activity of *Oncorhynchus mykiss*. *Iranian Journal of Fisheries Research*, 10(3), 3939–3402.
- Giomì, F., Raicevich, S., Giovanardi, O., Pranovi, F., Di Muro, P., & Beltramini, M. (2008). Catch me in winter! Seasonal variation in air temperature severely enhances physiological stress and mortality of species subjected to sorting operations and discarded during annual fishing activities. *Hydrobiologia*, 606, 195–202.
- Goldstein, L., Forster, R. P., & Fanelli, G. M., Jr. (1964). Gill blood flow and ammonia excretion in the marine teleost, *Myoxocephalus scorpius*. *Comparative Biochemistry and Physiology*, 12(4), 489–499.
- Gomez, D., Sunyer, J. O., & Salinas, I. (2013). The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens. *Fish & Shellfish Immunology*, 35(6), 1729–1739.
- Hamlin, H. J., Moore, B. C., Edwards, T. M., Larkin, I. L., Boggs, A., High, W. J., ... Guillette, L. J., Jr. (2008). Nitrate-induced elevations in circulating sex steroid concentrations in female Siberian sturgeon (*Acipenser baeri*) in commercial aquaculture. *Aquaculture*, 281(1–4), 118–125.
- Hanson, K. C., & Cooke, S. J. (2009). Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development. *Journal of Comparative Physiology B*, 179, 253–266.
- Hasler, C. T., Donaldson, M. R., Sunder, R. P., Guimond, E., Patterson, D. A., Mossop, B., ... Cooke, S. J. (2011). Osmoregulatory, metabolic, and nutritional condition of summer-run male Chinook salmon in relation to their fate and migratory behavior in a regulated river. *Endangered Species Research*, 14(1), 79–89.
- Helfman, G., Collette, B. B., Facey, D. E., & Bowen, B. W. (2009). *The diversity of fishes: Biology, evolution, and ecology*. Hoboken, NJ: John Wiley & Sons.
- Hikasa, Y., Takase, K., Ogasawara, T., & Ogasawara, S. (1986). Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. *Japanese Journal of Veterinary Science*, 48, 341–351.
- Holloway, A. C., Keene, J. L., Noakes, D. G., & Moccia, R. D. (2004). Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquaculture Research*, 35(11), 1025–1030.
- Houston, A. H. (1990). Blood and circulation. In C. B. Schreck & P. H. Moyses (Eds.), *Methods for fish biology* (pp. 273–322). Bethesda, MD: American Fisheries Society.
- Houston, A. H. (1997). Are the classical hematological variables acceptable indicators of fish health? *Transactions of the American Fisheries Society*, 126(6), 879–894.
- Iwama, G. K., McGeer, J. C., & Pawluk, M. P. (1989). The effects of five fish anaesthetics on acid–base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. *Canadian Journal of Zoology*, 67(8), 2065–2073.
- Javahery, S., Nekoubin, H., & Moradlu, A. H. (2012). Effect of anaesthesia with clove oil in fish. *Fish Physiology and Biochemistry*, 38(6), 1545–1552.
- Jeffrey, J. D., Gollock, M. J., & Gilmour, K. M. (2014). Social stress modulates the cortisol response to an acute stressor in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, 196, 8–16.
- Jeffries, K. M., Hinch, S. G., Donaldson, M. R., Gale, M. K., Burt, J. M., Thompson, L. A., ... Miller, K. M. (2011). Temporal changes in blood variables during final maturation and senescence in male sockeye salmon *Oncorhynchus nerka*: Reduced osmoregulatory ability can predict mortality. *Journal of Fish Biology*, 79(2), 449–465.
- Kaleeswaran, B., Ilavenil, S., Karthik, D., & Ravikumar, S. (2016). A new method of approaching the heart for rapid bleeding in fish. *South Indian Journal of Biological Sciences*, 2(3), 337–340.
- Kieffer, J. D., Wakefield, A. M., & Litvak, M. K. (2001). Juvenile sturgeon exhibit reduced physiological responses to exercise. *Journal of Experimental Biology*, 204(24), 4281–4289.
- Lane, H. C. (1979). Some haematological responses of normal and splenectomized rainbow trout *Salmo gairdneri* to a 12% blood loss. *Journal of Fish Biology*, 14(2), 159–164.
- Larter, K. F., & Rees, B. B. (2017). Influence of euthanasia method on blood and gill variables in normoxic and hypoxic gulf killifish *Fundulus grandis*. *Journal of Fish Biology*, 90(6), 2323–2343.
- Lawrence, M. J., Jain-Schlaepfer, S., Zolderdo, A. J., Algera, D. A., Gilmour, K. M., Gallagher, A. J., & Cooke, S. J. (2018). Are 3 minutes good enough for obtaining baseline physiological samples from teleost fish? *Canadian Journal of Zoology*, 96(7), 774–786.
- Lawrence, M. J., Wright, P. A., & Wood, C. M. (2015). Physiological and molecular responses of the goldfish (*Carassius auratus*) kidney to metabolic acidosis, and potential mechanisms of renal ammonia transport. *Journal of Experimental Biology*, 218(13), 2124–2135.
- Lawrence, M. J., Zolderdo, A. J., Godin, J. G. J., Mandelman, J. W., Gilmour, K. M., & Cooke, S. J. (2019). Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: A manipulative experimental field study. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 331(4), 253–261.
- Lermen, C. L., Lappe, R., Crestani, M., Vieira, V. P., Gioda, C. R., Schetinger, M. R. C., ... Morsch, V. M. (2004). Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. *Aquaculture*, 239(1–4), 497–507.
- Losey, G. S., & Hugie, D. M. (1994). Prior anesthesia impairs a chemically mediated fright response in a gobiid fish. *Journal of Chemical Ecology*, 20(8), 1877–1883.
- Mandelman, J. W., & Skomal, G. B. (2009). Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. *Journal of Comparative Physiology B*, 179(3), 267–277.
- Marshall, W. S., Emberley, T. R., Singer, T. D., Bryson, S. E., & McCormick, S. D. (1999). Time course of salinity adaptation in a strongly euryhaline estuarine teleost, *Fundulus heteroclitus*: A multivariable approach. *Journal of Experimental Biology*, 202(11), 1535–1544.
- Matsuyama, M., Adachi, S., Nagahama, Y., Maruyama, K., & Matsura, S. (1990). Diurnal rhythm of serum steroid hormone levels in the Japanese whiting, *Sillago japonica*, a daily-spawning teleost. *Fish Physiology and Biochemistry*, 8(4), 329–338.
- McGill University. (2017). SOP 403.01 – guidelines for blood collection volumes and frequency. Available from https://www.mcgill.ca/research/files/research/403-guidelines_blood_collection_volumes_and_frequency_-_sept_2017_0.pdf.
- Mettam, J. J., Oulton, L. J., McCrohan, C. R., & Sneddon, L. U. (2011). The efficacy of three types of analgesic drugs in reducing pain in the rainbow trout, *Oncorhynchus mykiss*. *Applied Animal Behaviour Science*, 133(3–4), 265–274.
- Morris, R. (1980). Blood composition and osmoregulation in ammocoete larvae. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(11), 1665–1679.
- Mylonas, C. C., Cardinaletti, G., Sigelaki, I., & Polzonetti-Magni, A. (2005). Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture*, 246(1–4), 467–481.
- Neiffer, D. L., & Stamper, M. A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. *ILAR Journal*, 50(4), 343–360.
- Nguyen, P. L., Haman, K., Carstensen, L., McKlveen, T., Cooney, P., & Gibson, S. (2018). Evaluation of three electrical outputs in an electro-

- immobilization system to reduce induction time during routine handling of fish at a hatchery. *North American Journal of Aquaculture*, 80(3), 239–248.
- Nishimura, H., Lunde, L. G., & Zucker, A. (1979). Renin response to hemorrhage and hypotension in the aglomerular toadfish *Opsanus tau*. *American Journal of Physiology-Heart and Circulatory Physiology*, 237(2), H105–H111.
- Olson, K. R. (1992). 3 blood and extracellular fluid volume regulation: Role of the renin-angiotensin system, Kallikrein-Kinin system, and atrial natriuretic peptides. In W. S. Hoar, D. J. Randall, & A. P. Farrell (Eds.), *Fish physiology* (pp. 135–254). New York, NY: Academic Press Inc.
- O'Neill, M. D., Wesp, H. M., Mensinger, A. F., & Hanlon, R. T. (1998). Initial baseline blood chemistry of the oyster toadfish, *Opsanus tau*. *The Biological Bulletin*, 195(2), 228–229.
- Parry, G. (1961). Osmotic and ionic changes in blood and muscle of migrating salmonids. *Journal of Experimental Biology*, 38(2), 411–427.
- Pickering, A. D., & Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry*, 7(1–6), 253–258.
- Pickering, A. D., Pottinger, T. G., Sumpter, J. P., Carragher, J. F., & Le Bail, P. Y. (1991). Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology*, 83(1), 86–93.
- Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., & Sauerborn Klobucar, R. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology*, 28(4), 553–564.
- Pottinger, T. G. (2008). The stress response in fish—mechanisms, effects and measurement. In E. J. Branson (Ed.), *Fish welfare* (pp. 32–48). Hoboken, NJ: Blackwell Publishing Ltd.
- Prystay, T. S., Elvidge, C. K., Twardek, W. M., Logan, J. M., Reid, C. H., Clarke, S. H., ... Cooke, S. J. (2017). Comparison of the behavioral consequences and recovery patterns of largemouth bass exposed to MS-222 or electrosedation. *Transactions of the American Fisheries Society*, 146(3), 556–566.
- Raby, G. D., Cooke, S. J., Cook, K. V., McConnachie, S. H., Donaldson, M. R., Hinch, S. G., ... Farrell, A. P. (2013). Resilience of pink salmon and chum salmon to simulated fisheries capture stress incurred upon arrival at spawning grounds. *Transactions of the American Fisheries Society*, 142(2), 524–539.
- Raby, G. D., Donaldson, M. R., Hinch, S. G., Patterson, D. A., Lotto, A. G., Robichaud, D., ... Cooke, S. J. (2012). Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. *Journal of Applied Ecology*, 49(1), 90–98.
- Raby, G. D., Packer, J. R., Danylchuk, A. J., & Cooke, S. J. (2014). The understudied and underappreciated role of predation in the mortality of fish released from fishing gears. *Fish and Fisheries*, 15(3), 489–505.
- Railo, E., Nikinmaa, M., & Soivio, A. (1985). Effects of sampling on blood parameters in the rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology*, 26(6), 725–732.
- Rapp, T., Hallermann, J., Cooke, S. J., Hetz, S. K., Wuertz, S., & Arlinghaus, R. (2014). Consequences of air exposure on the physiology and behavior of caught-and-released common carp in the laboratory and under natural conditions. *North American Journal of Fisheries Management*, 34(2), 232–246.
- Reavill, D. R. (2006). Common diagnostic and clinical techniques for fish. *Veterinary Clinics: Exotic Animal Practice*, 9(2), 223–235.
- Reid, C. H., Vandergoot, C. S., Midwood, J. D., Stevens, E. D., Bowker, J., & Cooke, S. J. (2019). On the electroimmobilization of fishes for research and practice: Opportunities, challenges, and research needs. *Fisheries*, 147, 390–399.
- Robinson, K. A., Hinch, S. G., Gale, M. K., Clark, T. D., Wilson, S. M., Donaldson, M. R., ... Patterson, D. A. (2013). Effects of post-capture ventilation assistance and elevated water temperature on sockeye salmon in a simulated capture-and-release experiment. *Conservation Physiology*, 1(1), cot015.
- Rodela, T. M., McDonald, M. D., Walsh, P. J., & Gilmour, K. M. (2012). Interactions between cortisol and rhesus glycoprotein expression in ureogenic toadfish, *Opsanus beta*. *Journal of Experimental Biology*, 215(2), 314–323.
- Rogers, J. T., Richards, J. G., & Wood, C. M. (2003). Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, 64(2), 215–234.
- Rosenblum, P. M., Pudney, J., & Callard, I. P. (1987). Gonadal morphology, enzyme histochemistry and plasma steroid levels during the annual reproductive cycle of male and female brown bullhead catfish, *Ictalurus nebulosus* Lesueur. *Journal of Fish Biology*, 31(3), 325–341.
- Roth, B., Imsland, A. K., & Foss, A. (2009). Live chilling of turbot and subsequent effect on behaviour, muscle stiffness, muscle quality, blood gases and chemistry. *Animal Welfare*, 18, 33–41.
- Rothwell, S. E., Black, S. E., Jerrett, A. R., & Forster, M. E. (2005). Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (*Oncorhynchus tshawytscha*) and snapper (*Pagrus auratus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 140(3), 289–298.
- Rucinke, D. S., Watanabe, A. L., & Molento, C. F. M. (2018). Electrical stunning in pacu (*Piaractus mesopotamicus*) using direct current waveform. *Aquaculture*, 497, 42–48.
- Scholz, S., Kordes, C., Hamann, J., & Gutzeit, H. O. (2004). Induction of vitellogenin in vivo and in vitro in the model teleost medaka (*Oryzias latipes*): Comparison of gene expression and protein levels. *Marine Environmental Research*, 57(3), 235–244.
- Sellathurai, S., Priyathilaka, T. T., & Lee, J. (2019). Molecular cloning, characterization, and expression level analysis of a marine teleost homolog of catalase from big belly seahorse (*Hippocampus abdominalis*). *Fish & Shellfish Immunology*, 89, 647–659.
- Soivio, A., Nynolm, K., & Westman, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *Journal of Experimental Biology*, 63(1), 207–217.
- Soivio, A., & Oikari, A. (1976). Haematological effects of stress on a teleost, *Esox lucius* L. *Journal of Fish Biology*, 8(5), 397–411.
- Sui, Y., Huang, X., Kong, H., Lu, W., & Wang, Y. (2016). Physiological responses to salinity increase in blood parrotfish (*Cichlasoma synspilum*♀ × *Cichlasoma citrinellum*♂). *Springerplus*, 5(1), 1246.
- Suski, C. D., Cooke, S. J., Danylchuk, A. J., O'Connor, C. M., Gravel, M. A., Redpath, T., ... Koppelman, J. B. (2007). Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 148(3), 664–673.
- Swift, D. J. (1981). A holding box system for physiological experiments on rainbow trout (*Salmo gairdneri* Richardson) requiring rapid blood sampling. *Journal of Fish Biology*, 18(3), 309–319.
- Teffer, A. K., Bass, A. L., Miller, K. M., Patterson, D. A., Juanes, F., & Hinch, S. G. (2018). Infections, fisheries capture, temperature, and host responses: Multistressor influences on survival and behaviour of adult Chinook salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(11), 2069–2083.
- Teffer, A. K., Hinch, S., Miller, K., Jeffries, K., Patterson, D., Cooke, S., ... Juanes, F. (2019). Cumulative effects of thermal and fisheries stressors reveal sex-specific effects on infection development and early mortality of adult coho salmon (*Oncorhynchus kisutch*). *Physiological and Biochemical Zoology*, 92(5), 505–529.
- Teffer, A. K., & Miller, K. M. (2019). A comparison of non-lethal and destructive methods for broad-based infectious agent screening of Chinook Salmon using high-throughput qPCR. *Journal of Aquatic Animal Health*, 31(3), 274–289.
- Thompson, L. A., Cooke, S. J., Donaldson, M. R., Hanson, K. C., Gingerich, A., Klefoth, T., & Arlinghaus, R. (2008). Physiology, behavior,

- and survival of angled and air-exposed largemouth bass. *North American Journal of Fisheries Management*, 28(4), 1059–1068.
- Tort, L., Montero, D., Robaina, L., Fernández-Palacios, H., & Izquierdo, M. S. (2001). Consistency of stress response to repeated handling in the gilthead sea bream *Sparus aurata* Linnaeus, 1758. *Aquaculture Research*, 32(7), 593–598.
- Trushenski, J. T., & Bowker, J. D. (2012). Effect of voltage and exposure time on fish response to electrosedation. *Journal of Fish and Wildlife Management*, 3(2), 276–287.
- Trushenski, J. T., Bowker, J. D., Cooke, S. J., Erdahl, D., Bell, T., MacMillan, J. R., ... Sharon, S. (2013). Issues regarding the use of sedatives in fisheries and the need for immediate-release options. *Transactions of the American Fisheries Society*, 142(1), 156–170.
- Trushenski, J. T., Bowzer, J. C., Bergman, A. M., & Bowker, J. D. (2017). Developing rested harvest strategies for rainbow trout. *North American Journal of Aquaculture*, 79(1), 36–52.
- Tunnah, L., MacKellar, S. R., Barnett, D. A., MacCormack, T. J., Stehfest, K. M., Morash, A. J., ... Currie, S. (2016). Physiological responses to hypersalinity correspond to nursery ground usage in two inshore shark species (*Mustelus antarcticus* and *Galeorhinus galeus*). *Journal of Experimental Biology*, 219(13), 2028–2038.
- Twardek, W. M., Gagne, T. O., Elmer, L. K., Cooke, S. J., Beere, M. C., & Danylchuk, A. J. (2018). Consequences of catch-and-release angling on the physiology, behaviour and survival of wild steelhead *Oncorhynchus mykiss* in the Bulkley River, British Columbia. *Fisheries Research*, 206, 235–246.
- Ueda, K., Yanagisawa, M., Murakumo, K., Matsumoto, Y., Sato, K., & Uchida, S. (2017). Physical examination, blood sampling, and sedation of large elasmobranchs. In M. Smith, D. Warmolts, D. Thoney, R. Hueter, M. Murray, & J. Ezcurra (Eds.), *Elasmobranch husbandry manual II: Recent advances in the care of sharks, rays and their relatives* (pp. 255–262). Ohio Biological Survey: Columbus, OH.
- Vandergoot, C. S., Murchie, K. J., Cooke, S. J., Dettmers, J. M., Bergstedt, R. A., & Fielder, D. G. (2011). Evaluation of two forms of electroanesthesia and carbon dioxide for short-term anesthesia in walleye. *North American Journal of Fisheries Management*, 31(5), 914–922.
- Vijayan, M. M., & Moon, T. W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Canadian Journal of Zoology*, 72(3), 379–386.
- Ward, T. D., Brownscombe, J. W., Gutowsky, L. F., Ballagh, R., Sakich, N., McLean, D., ... Cooke, S. J. (2017). Electric fish handling gloves provide effective immobilization and do not impede reflex recovery of adult largemouth Bass. *North American Journal of Fisheries Management*, 37(3), 652–659.
- Warne, J. M., & Balment, R. J. (1995). Effect of acute manipulation of blood volume and osmolality on plasma (AVT) in seawater flounder. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 269(5), R1107–R1112.
- Wells, R. M., McNeil, H., & MacDonald, J. A. (2005). Fish hypnosis: Induction of an atonic immobility reflex. *Marine and Freshwater Behaviour and Physiology*, 38(1), 71–78.
- Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, 77(3), 591–625.
- WOOD, C. M., McMahan, B. R., & McDonald, D. G. (1979). Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*: A comparison with other teleosts. *Journal of Experimental Biology*, 78(1), 167–179.
- Wood, C. M., Walsh, P. J., Kajimura, M., McClelland, G. B., & Chew, S. F. (2010). The influence of feeding and fasting on plasma metabolites in the dogfish shark (*Squalus acanthias*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 155(4), 435–444.
- Young, J. L., Hinch, S. G., Cooke, S. J., Crossin, G. T., Patterson, D. A., Farrell, A. P., ... English, K. K. (2006). Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, 63(5), 1067–1077.
- Zang, L., Shimada, Y., Nishimura, Y., Tanaka, T., & Nishimura, N. (2013). A novel, reliable method for repeated blood collection from aquarium fish. *Zebrafish*, 10(3), 425–432.
- Zang, L., Shimada, Y., Nishimura, Y., Tanaka, T., & Nishimura, N. (2015). Repeated blood collection for blood tests in adult zebrafish. *Journal of Visualized Experiments*, 102, 53272.
- Zimmer, A. M., & Wood, C. M. (2014). Exposure to acute severe hypoxia leads to increased urea loss and disruptions in acid-base and ionoregulatory balance in dogfish sharks (*Squalus acanthias*). *Physiological and Biochemical Zoology*, 87(5), 623–639.

SUPPORTING INFORMATION

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

How to cite this article: Lawrence MJ, Raby GD, Teffer AK, et al. Best practices for non-lethal blood sampling of fish via the caudal vasculature. *J Fish Biol.* 2020;97:4–15. <https://doi.org/10.1111/jfb.14339>