Received: 4 December 2023 | Accepted: 19 December 2023

DOI: 10.1111/jfb.15650

JOURNAL OF FISH BIOLOGY fsbi

COMMENT

Opportunities and challenges with transitioning to non-lethal sampling of wild fish for microbiome research

Lisa A. Kelly[1] | Christopher K. Yost[2,3] | Steven J. Cooke[1]

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

[2]Department of Biology, University of Regina, Regina, Saskatchewan, Canada

[3]Institute for Microbial Systems and Society, University of Regina, Regina, Saskatchewan, Canada

Correspondence
Lisa A. Kelly, Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, ON K1S 5B6, Canada.
Email: lisakelly.lak@gmail.com and lisakelly3@cmail.carleton.ca

Funding information
Natural Sciences and Engineering Research Council of Canada

Abstract

The microbial communities of fish are considered an integral part of maintaining the overall health and fitness of their host. Research has shown that resident microbes reside on various mucosal surfaces, such as the gills, skin, and gastrointestinal tract, and play a key role in various host functions, including digestion, immunity, and disease resistance. A second, more transient group of microbes reside in the digesta, or feces, and are primarily influenced by environmental factors such as the host diet. The vast majority of fish microbiome research currently uses lethal sampling to analyse any one of these mucosal and/or digesta microbial communities. The present paper discusses the various opportunities that non-lethal microbiome sampling offers, as well as some inherent challenges, with the ultimate goal of creating a sound argument for future researchers to transition to non-lethal sampling of wild fish in microbiome research. Doing so will reduce animal welfare and population impacts on fish while creating novel opportunities to link host microbial communities to an individual's behavior and survival across space and time (e.g., life-stages, seasons). Current lethal sampling efforts constrain our ability to understand the mechanistic ecological consequences of variation in microbiome communities in the wild. Transitioning to non-lethal sampling will open new frontiers in ecological and microbial research.

KEYWORDS
amplicon sequencing, fish, microbial communities, mucosal surfaces, non-lethal microbiome sampling

1 | INTRODUCTION

The bacterial microbiota of fish are a complex and dynamic community that inhabits the mucosal surfaces of fish, such as the skin, gills, and gastrointestinal tract (Legrand et al., 2020). These communities interact with the host to provide various beneficial services, including metabolic processes, immune functions, and disease resistance, that are important to fish health and fitness (Llewellyn et al., 2014). The composition and diversity of this collective microbiota are heavily influenced by both exogenous (i.e., environment, diet) and endogenous factors (i.e., host genetics and physiology; Spor et al., 2011). As such, the microbial community is highly dynamic and varies among individuals and within an individual across time and contexts (e.g., life-cycle stages; Boutin et al., 2014; Llewellyn et al., 2016).

Currently, the majority of microbiome studies conducted on fish involve lethal sampling and removal of whole digestive tracts, with a potential secondary sampling of the gill or skin microbiota (Gajardo et al., 2016; Uren Webster et al., 2018). Sampling will either use the whole digestive tract (Gajardo et al., 2016) or particular sections of the tract, such as the hindgut (Lyons et al., 2017) or digesta (Eichmiller et al., 2016). Comparative studies between the different gut compartments (i.e., proximal, mid, and distal intestine), as well as the digesta, have revealed that the microbial composition and diversity varied significantly, both between compartments within the intestinal tract and

between the intestinal mucosal layer and digesta (Gajardo et al., 2016; Nyholm et al., 2022). Resident (autochthonous) bacteria are found more commonly on the mucosal layer, in close association with the host epithelial cells, and are typically less diverse communities than the transient (allochthonous) community comprising the digesta, which are more heavily influenced by environmental factors (Gajardo et al., 2016; Legrand et al., 2020). Therefore, the research questions being asked (i.e., host-associated factors vs. effects of diet, for example) will necessitate which part of the gastrointestinal tract is required for sampling and whether lethal sampling is required.

Lethal sampling is much more commonly used in fish microbiome studies compared to other animal taxa, such as primates and birds, where fecal or cloacal sampling is more often used (Björk et al., 2022; Risely et al., 2017; Waite et al., 2012). Several studies on humans assessed rectal swabs' effectiveness in characterizing the hindgut microbiome, compared to colon biopsy and/or fecal samples (Araújo-Pérez et al., 2012; Bassis et al., 2017; Budding et al., 2014). This has occurred for other vertebrate taxa as well, including bird fecal versus cloacal sampling (Videvall et al., 2018), bat fecal versus intestinal sampling (Ingala et al., 2018), and most recently, fish fecal versus intestinal sampling (Nyholm et al., 2022). The general conclusion is that different sampling methodology captures different parts of the microbiome and should be carefully considered when formulating research questions. However, despite these differences, non-lethal sampling is still highly prevalent among higher vertebrate classes and suggests that lethal sampling in fish microbiome research may be overused and associated opportunities that come from non-lethal sampling missed.

Fish welfare should be prioritized when planning microbiome studies, and lethal sampling should be conducted only when absolutely necessary (i.e., development of robust non-lethal measures would represent a major animal welfare refinement). However, beyond animal welfare arguments, there are research opportunities that arise when we are able to resample individual fish over time and to link individual-level microbial communities with ecological activities such as behavior, reproductive success, or survival. The purpose of this perspective article is to highlight some of the opportunities non-lethal microbiome sampling of fish offers, including the ability to work on rare/threatened species, the ability to combine microbiome sampling with other methods (e.g., biotelemetry, biologging) and end points (e.g., behavior, reproductive success, survival), as well as the ability to do serial sampling on the same individuals across space and time. Non-lethal microbiome sampling also has its challenges and limitations, which will also be discussed. Our hope is that this paper will stimulate additional validation studies that will determine the contexts in which non-lethal sampling is effective.

2 | NON-LETHAL SAMPLING METHODS

Four main sampling methods are used for non-lethal sampling of fish microbiomes (Figure 1). Fecal sampling is the most common of these and involves simply collecting the feces of an animal. This can be done on scuba/snorkeling underwater by following a fish until it defecates (Smriga et al., 2010), or fish can be temporarily removed and feces manually expressed by applying gentle pressure along the ventral abdominal wall toward the anus (Eichmiller et al., 2016). Fecal sampling is advantageous as it collects a generous amount of sample, often much more than the minimum requirement for DNA extraction kits, which allows for some redundancy. A disadvantage of fecal sampling is that it collects only fecal matter, which contains bacteria primarily associated with the digesta (allochthonous microbiota; Ringø & Birkbeck, 1999). The bacteria associated with the intestinal mucosa (autochthonous bacteria) are largely missed by sampling using this method (Romero et al., 2014).

Hindgut swabbing, where a swab is inserted through the anus and rotated along the intestinal walls of the hindgut (Figure 2), is more invasive than collecting fecal matter but offers the advantage of collecting autochthonous bacteria associated with the intestinal mucosa, as well as bacteria associated with the digesta. In theory, it offers a more complete picture of the hindgut microbiota. Hindgut swabbing has not been used substantively in fish studies. However, it is fairly common practice among bird and reptile studies, where cloacal swabbing is used as a proxy for the colon or fecal microbiota (Dewar et al., 2013, 2014; Martin et al., 2010; Stanley et al., 2015; Videvall et al., 2018). However, in practice, method papers have found mixed results regarding the validity of this proxy. Videvall et al. (2018) compared

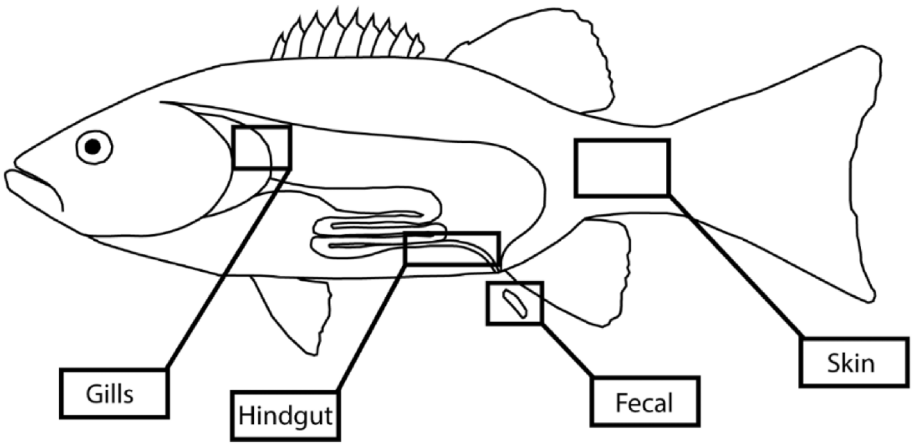

FIGURE 1 The four main non-lethal fish microbiome sample types.

FIGURE 2 Example of hindgut swabbing on a common white sucker (*Catostomus commersonii*).

the microbiota composition between fecal and cloacal sampling in juvenile ostriches (*Struthio camelus*) and found that fecal samples better represented the bacterial community of the colon than did cloacal swabs. Further, a previous study by Videvall et al. (2017) found that cloacal swabs had lower repeatability compared to fecal samples, and this was likely due to the low-biomass nature of swab sampling. Low initial DNA concentration introduces stochasticity, depending on what bacterial taxa are initially amplified (Videvall et al., 2017).

Gill biopsy and gill swabbing are microbiome samples taken from the gill mucus layer and/or tissue. The gills are open to the external environment, which makes them an important site for pathogen entry, and are immunologically active organs (Secombes & Wang, 2012). This makes them a good option if the interest is in examining gill microbial communities and the presence of disease, as they have been found to reflect disease states, such as chronic gastroenteritis (Legrand et al., 2018). However, there are limited studies examining gill microbiomes, especially using non-lethal methods such as gill swabbing (Dunn et al., 2020; Legrand et al., 2018). One study to date has compared gill biopsy and swabbing in Atlantic salmon (*Salmo salar*) and found a divergence in microbial communities obtained using the two sampling strategies (Clinton et al., 2021). Overall, gill swabs were preferable as they isolated a more diverse microbial community and did not have as many issues with host DNA. However, biopsies recovered more cryptic community membership and may be more suitable for subsurface or intracellular microbes (Clinton et al., 2021). Small, non-lethal gill biopsies are routinely used for other molecular techniques, such as transcriptomics (Drenner et al., 2018; Jeffries et al., 2014).

The final non-lethal sampling method is skin swabbing. The skin of fish is mucosal and exposed to the external environment (Gomez & Primm, 2021); this makes it one of the easiest non-lethal methods. As such, there is a wide variety of studies that examined the skin microbiota of several fish species, including both wild and aquaculture species (Boutin et al., 2014; Pratte et al., 2018). Sampling typically involves using a swab to sample the skin and mucosa on the lateral side from the back of the operculum to the caudal peduncle, along the lateral line (Uren Webster et al., 2018). There is a risk of disrupting this protective layer, which could lead to disease. Catfish skin microbiomes disrupted by potassium permanganate were found to have increased mortality from the pathogenic bacteria *Flavobacterium columnare*, which causes columnaris disease (Mohammed & Arias, 2015). Despite the relative ease of using this sampling method non-lethally, many studies still lethally sample for skin microbiome research (Chiarello et al., 2018; Lowrey et al., 2015). This may be due, in part, to the capture method (e.g., by speargun; Chiarello et al., 2018) or because more invasive samples are being taken in concert (Uren Webster et al., 2018).

3 | OPPORTUNITIES WITH NON-LETHAL MICROBIOME SAMPLING

Non-lethal sampling provides an opportunity to sample rare or threatened populations and species that would otherwise be unattainable due to legal protections or conservation concerns. Having a better understanding of host–microbiome associations and the functional role microbes play in host health and fitness can aid in the conservation of imperiled species (Zhu et al., 2021). Many threatened species must also contend with anthropogenic disturbances such as habitat degradation and pollution (Arthington et al., 2016), along with climate change factors such as elevated temperatures or changes in salinity (Portner & Peck, 2010), which would also negatively impact their microbiome, potentially leading to reduced host fitness and survival that could further depress population numbers or prevent recovery (Zhu et al., 2021). Conservation reintroduction programmes could also benefit from understanding optimal host–microbe associations to maximize fitness after releasing captive individuals back into the wild (Zhu et al., 2021), as is commonly done for terrestrial organisms (Bahrndorff et al., 2016; West et al., 2019). This is relevant to fish hatcheries that use captive breeding as a means to conserve, reintroduce, or supplement populations in the wild (Rytwinski et al., 2021). Prerelease conditioning of the gut microbial community through diet training was attempted in captive-bred endangered Yangtze sturgeon (*Acipenser dabryanus*) prior to release to increase postrelease survival and fitness (Yang et al., 2020). This is a promising area of research (see Jin Song et al., 2019) that would benefit significantly from more research effort.

Another opportunity provided by non-lethal sampling is that it can be integrated with movement research, such as telemetry and mark-recapture, to provide insight into a fish's behavior and associated microbiome. For a full review of non-lethal sampling and fish movement research in freshwater fishes, see Thorstensen et al. (2022). In the context of fish movement ecology, non-lethal sampling is necessary as you need to see what the fish are doing after you sample their microbiota to answer your proposed research questions. This has relevance to both migration behavior and reproductive behavior studies. Most salmonid migration microbiome studies to date lethally

sample fish and provide characterizations of the gut microbiome at different stages of their migration or life cycle using cross-sectional population-based analyses (Element et al., 2020a; Element et al., 2020b; Le et al., 2020; Liu et al., 2022; Llewellyn et al., 2016; Tosin et al., 2020), rather than individual-based analysis. However, if host–microbiota research maintains that the commensal microbiome increases host survival and fitness, then there should be relevant studies examining fish under these circumstances. Fish migration and spawning offer an excellent opportunity to intrinsically test these hypotheses. Spawning migration runs are arduous and physiologically challenging endeavors, especially among semelparous anadromous species, such as sockeye salmon (*Oncorhynchus nerka*), that rely on endogenous energy reserves to fuel their journey (Brett, 1995). Many fish die before reaching the spawning grounds, due to the depletion of energy reserves and disease, among other reasons (Hinch et al., 2006). Differential survival among a migratory population would be a prime example to study correlations between successful migrants and the gut, skin, or gill microbiomes. Taking it a step further, spawning success as a proxy for fitness among female Pacific salmon can easily be established based on the presence or absence of eggs in the abdominal cavity after death on the spawning grounds and could be correlated to microbiome composition and diversity. Currently, no research studies to our knowledge utilize non-lethal sampling for wild fish behaviors, such as migration. It is, however, a commonly used method in avian migration studies, where fecal samples are taken at bird stopover points along their migration route (Lewis et al., 2016; Risely et al., 2017; Skeen et al., 2021).

A final opportunity provided by non-lethal sampling is the ability to collect time series data, which provide invaluable insight into how microbiome dynamics change over time. It is particularly well studied in humans, where in-depth research has shown how dynamic microbial communities are during the first years of life (Koenig et al., 2011) and even on shorter time scales, such as after infections (Hoffmann et al., 2009) or antibiotic treatments (Peterfreund et al., 2012). Time series data have also been studied in wild animal populations, particularly in primates (Björk et al., 2022; Murillo et al., 2022), but also in birds (Skeen et al., 2021). For example, Björk et al. (2022) provided an extensive gut microbial time series from wild baboons and found that despite synchronizing forces in baboon populations (e.g., shared environments and diets), hosts still retained highly idiosyncratic gut microbiomes. Both the studies of primates and birds have important implications in terms of linking microbial dynamics to health outcomes and are, therefore, a topic of interest. Within fish species, microbiome time series data are important in the aquaculture industry, where health outcomes are also closely monitored in association with microbial dynamics, as well as the effect of different feeding regimes and other pertinent metrics. Although repeat fecal microbiome sampling of aquaculture fish does occur (Neuman et al., 2016; Zarkasi et al., 2014), lethal sampling is still largely used where fish are lethally sampled at different time points to examine how microbiomes change over time in response to different treatment regimes (Payne et al., 2022; Ringø et al., 2006). Microbiome time series data were also examined using captive clownfish and anemones in a tank experiment, using non-lethal skin mucus swabs to sample the skin microbiome of fish to see how it changes before, during, and after association with an anemone (Pratte et al., 2018); however, time series microbiome studies are rare in wild fish species. We identified two studies that assessed temporal variability (among other drivers) of the gut or mucosal microbiota in wild rabbit fish (*Siganus guttatus*; Le et al., 2020) and Pacific chub mackerel (*Scomber japonicus*; Minich et al., 2020). However, these were cross-sectional studies, and fish were killed to collect microbiome samples. That being said, we could not find any studies that non-lethally sampled wild fish microbiomes at more than one time point for temporal analysis of the microbiome. One could argue that it is difficult to recapture the same individual fish in aqueous environments. There are circumstances that would make this task easier. For instance, iteroparous fish that spawn annually could be externally tagged and non-lethally sampled for microbiome analysis over multiple years. On a shorter time frame, some fish species, such as smallmouth bass (*Micropterus dolomieu*), undertake paternal care during the spawning period and exhibit nest and brood-guarding behaviors for up to 4 weeks, until offspring are self-sufficient (Cooke et al., 2002). This would also provide an excellent means to examine fish microbiomes in relation to fitness end points and should be further investigated.

4 | CHALLENGES WITH NON-LETHAL MICROBIOME SAMPLING

The greatest challenge concerning non-lethal microbiome sampling is the low biomass often obtained when taking swabs of different fish body compartments. Low-biomass samples typically have lower repeatability (same results from replicates of the same sample) than higher biomass samples (such as feces; Videvall et al., 2017). This is because low-biomass samples have low initial template DNA concentrations, which increases the likelihood of stochastic noise generated during PCR amplification prior to sequencing (Erb-Downward et al., 2020; Videvall et al., 2017). Further, any small amount of contamination during the sampling stage and/or the DNA extraction stage can result in over-amplification during PCR, which can critically impact downstream analyses and result in erroneous interpretations (Eisenhofer et al., 2019; Salter et al., 2014). To mitigate this issue, using both positive and negative controls can help recognize contamination signals, so that they can then be excluded from the final dataset (Kennedy et al., 2023). Contamination can also be removed during the analysis phase using software packages, such as *decontam*, which removes more abundant contaminants (Davis et al., 2018).

Another challenge in tandem with low-biomass samples is the presence of PCR inhibitors. Inhibitors comprise a variety of organic and inorganic substances and can come from a biological origin (such as the biological materials being sampled) or be introduced during sample processing or DNA extraction (Schrader et al., 2012). Inhibitors function by interfering with cell lysis during DNA extraction, degrading nucleic acid, or inhibiting the amplification of nucleic acids during the PCR process (Wilson, 1997). This has downstream effects on the final sequencing libraries produced and overall microbial diversity characterized. Until recently, the majority of gut microbiome optimization

method papers have been centered on mammals (Blekhman et al., 2016; Choo et al., 2015; Jin Song et al., 2016) and have targeted protocols to remove PCR inhibitors. Despite an influx of fish gut microbiota research, fish microbiome optimization has received comparatively less attention (Talwar et al., 2018). Further, fish gut samples have very different chemical and enzymatic profiles, which may result in differing degrees of PCR inhibition (Hildonen et al., 2019). Fish gill samples also prove to be rich in PCR inhibitors; however, gill biopsies are likely more problematic than gill swabs due to being a blood-rich tissue (Clokie et al., 2022). Inhibitors can also be introduced during sample preservation and storage. A comparison study on different storage methods (immediate freezing, 96% ethanol, RNAlater, and DNA/RNA shield) for gut microbiome samples from rainbow trout (*Oncorhynchus mykiss*) showed that different methods were associated with different degrees of PCR inhibition and highlighted the importance of these types of optimization studies when exploring new species systems (Hildonen et al., 2019). The authors found that RNAlater-stored mucosal samples had the lowest levels of inhibition. However, 96% ethanol was the preferred storage method for rainbow trout gut microbiome samples as it yielded higher amounts of DNA, and DNA sequencing libraries were of sufficient quality (Hildonen et al. 2019). However, when working with wild species at remote field sites, especially if air travel is required, some sample storage methods, such as ethanol, are not viable if you plan to fly commercially.

As mentioned previously, a great opportunity provided by non-lethal sampling is the ability to take multiple samples in time series experiments. However, more research needs to be conducted to determine the impact, if any, and the magnitude of the impact, that repeat sampling has on the microbiome. This would be particularly relevant to skin and gill swabs, where a thick mucus layer is present, and the disruption of this protective barrier could potentially allow an alternate microbiome to become established, leading to dysbiosis and disease in the host. To our knowledge, only one study has attempted non-lethal repeat sampling of individual fish. Pratte et al. (2018) examined captive clownfish skin mucosal microbial communities before, during, and after association with sea anemones. However, they did not report on the potentially disruptive effects of repeat sampling (Pratte et al., 2018). More methodological studies are needed to examine if repeat sampling of the mucosal microbiome creates a confounding factor in temporal studies.

5 | CONCLUSIONS

Research has shown the microbiome to be highly dynamic, with large inter- and intra-individual variation. Lethal sampling for microbiome analysis offers only a snapshot of what is present at that specific moment in time. Transitioning to non-lethal sampling can help provide a more in-depth assessment of host–microbe associations as they relate to fitness end points and behaviors, both across spatial and temporal scales. Several decades ago, the same discussions occurred in the context of animal physiology where there was a desire to move away from lethal sampling in an effort to understand the physiological basis for individual variation in animal fitness and behavior (Bennett, 1987; Spicer & Gaston, 1999). Today, non-lethal sampling enables physiologists to assess the mechanistic physiological basis for variation in fish performance (e.g., Chapman et al., 2021; Cooke et al., 2006), and we submit that the same opportunities exist for non-lethal microbiome studies. Being able to do the same with microbiomes would not only reduce animal welfare and population impacts on fish but also create novel opportunities to link microbiome communities of fish hosts to the behavior and survival of individuals across space and time (e.g., life-stages, seasons). Currently, temporal trends are often assessed in fish via lethally sampled cross-sectional population studies to examine how the microbiota change over development stages or time. Indeed, lethal sampling efforts constrain our ability to understand the mechanistic ecological consequences of variation in microbiome communities in the wild such that transitioning to non-lethal sampling will open new frontiers in ecological and microbial research. Moving forward, more fish-specific comparison studies of lethal versus non-lethal microbiome sampling methods would add value to the literature base and provide evidence that non-lethal sampling has merit. Further refinement of non-lethal methods and validation studies of these methods would also be beneficial. There has been some movement in the past few years toward fish-specific microbiome method evaluations (see Clinton et al., 2021; Clokie et al., 2022; Hildonen et al., 2019; Nyholm et al., 2022). Ideally, the research community will converge on a set of best practices that provide repeatable and reproducible results for different fish body compartments for microbiome analysis. In addition, there is a need to create a robust methodological pipeline for non-lethal microbiome research, from sample collection to data analysis, so that comparisons can be made across studies and meta-analyses and systematic reviews can be conducted to provide more concrete evidence for host–microbe associations and their benefits to host health and fitness in fish research.

AUTHOR CONTRIBUTIONS

Lisa Kelly, Christopher Yost, and Steven Cooke contributed to manuscript design and conception. Lisa Kelly prepared the manuscript. All authors contributed to editing the manuscript.

ACKNOWLEDGMENTS

We thank Emily Standen, Alex Wong, and Brian Leydet for their helpful feedback on this manuscript. Lisa Kelly was supported by a Queen Elizabeth II Graduate Scholarship in Science and Technology and an Ontario Graduate Scholarship. Steven Cooke and Christopher Yost were supported by the Canada Research Chairs Program and the Natural Sciences and Engineering Research Council of Canada. Cooke is further supported by GenFish and Genome Canada.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest declared in this article.

ORCID

Lisa A. Kelly https://orcid.org/0000-0003-2830-6493

REFERENCES

Araújo-Pérez, F., Mccoy, A. N., Okechukwu, C., Carroll, I. M., Smith, K. M., Jeremiah, K., Sandler, R. S., Asher, G. N., & Keku, T. O. (2012).

Differences in microbial signatures between rectal mucosal biopsies and rectal swabs. *Gut Microbes*, *3*(6), 530–535. https://doi.org/10.4161/gmic.22157

Arthington, A. H., Dulvy, N. K., Gladstone, W., & Winfield, I. A. N. J. (2016). Fish conservation in freshwater and marine realms: Status, threats and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*, 838–857. https://doi.org/10.1002/aqc.2712

Bahrndorff, S., Alemu, T., Alemneh, T., & Lund Nielsen, J. (2016). The microbiome of animals: Implications for conservation biology. *International Journal of Genomics*, *2016*, 5304028. https://doi.org/10.1155/2016/5304028

Bassis, C. M., Moore, N. M., Lolans, K., Seekatz, A. M., Weinstein, R. A., Young, V. B., & Hayden, M. K. (2017). Comparison of stool versus rectal swab samples and storage conditions on bacterial community profiles. *BMC Microbiology*, *17*(1), 78. https://doi.org/10.1186/s12866-017-0983-9

Bennett, A. (1987). Interindividual variability: An underutilized resource. In M. E. Feder, A. F. Bennett, W. W. Burggren, & R. B. Huey (Eds.), *New directions in ecological physiology* (pp. 147–169). Cambridge University Press.

Björk, J. R., Dasari, M. R., Roche, K., Grieneisen, L., Gould, T. J., Grenier, J., Yotova, V., Gottel, N., Jansen, D., Gesquiere, L. R., Gordon, J. B., Learn, N. H., Wango, T. L., Mututua, R. S., Warutere, J. K., Siodi, L., Mukherjee, S., Barreiro, L. B., Alberts, S. C., … Archie, E. (2022). Synchrony and idiosyncrasy in the gut microbiome of wild baboons. *Nature Ecology & Evolution*, *6*, 955–964. https://doi.org/10.1038/s41559-022-01773-4

Blekhman, R., Tang, K., Archie, E. A., Barreiro, L. B., Johnson, Z. P., Wilson, M. E., Kohn, J., Yuan, M., Gesquiere, L., Grieneisen, L. E., & Tung, J. (2016). Common methods for fecal sample storage in field studies yield consistent signatures of individual identity in microbiome sequencing data. *Scientific Reports*, *6*, 31519. https://doi.org/10.1038/srep31519

Boutin, S., Sauvage, C., Bernatchez, L., Audet, C., & Derome, N. (2014). Inter individual variations of the fish skin microbiota: Host genetics basis of mutualism? *PLoS ONE*, *9*(7), 1–17. https://doi.org/10.1371/journal.pone.0102649

Brett, J. (1995). Energetics. In C. Groot, L. Margolis, & W. Clarke (Eds.), *Physiological ecology of Pacific salmon* (pp. 3–68). University of British Columbia Press.

Budding, A. E., Grasman, M. E., Eck, A., Bogaards, J. A., Vandenbroucke-Grauls, C. M. J. E., Van Bodegraven, A. A., & Savelkoul, P. H. M. (2014). Rectal swabs for analysis of the intestinal microbiota. *PLoS ONE*, *9*(7), 5–12. https://doi.org/10.1371/journal.pone.0101344

Chapman, J. M., Lennox, R. J., Twardek, W. M., Teffer, A. K., Robertson, M. J., Miller, K. M., & Cooke, S. J. (2021). Serial sampling reveals temperature associated response in transcription profiles and shifts in condition and infectious agent communities in wild Atlantic salmon. *Freshwater Biology*, *66*, 2086–2104. https://doi.org/10.1111/fwb.13817

Chiarello, M., Auguet, J. C., Bettarel, Y., Bouvier, C., Claverie, T., Graham, N. A. J., Rieuvilleneuve, F., Sucré, E., Bouvier, T., & Villéger, S. (2018). Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome*, *6*, 147. https://doi.org/10.1186/s40168-018-0530-4

Choo, J. M., Leong, L. E. X., & Rogers, G. B. (2015). Sample storage conditions significantly influence faecal microbiome profiles. *Scientific Reports*, *5*, 1–10. https://doi.org/10.1038/srep16350

Clinton, M., Wyness, A. J., Martin, S. A. M., Brierley, A. S., & Ferrier, D. E. K. (2021). Sampling the fish gill microbiome: A comparison of tissue biopsies and swabs. *BMC Microbiology*, *21*, 313. https://doi.org/10.1186/s12866-021-02374-0

Clokie, B. G. J., Elsheshtawy, A., Albalat, A., Nylund, A., Beveridge, A., Payne, C., & MacKenzie, S. (2022). Optimization of low-biomass sample collection and quantitative PCR-based titration impact 16S rRNA microbiome resolution. *Microbiology Spectrum*, *10*(6), e0225522.

Cooke, S. J., Hinch, S. G., Crossin, G. T., Patterson, D. A., English, K. K., Healey, M. C., Shrimpton, J. M., Van Der Kraak, G., & Farrell, A. P. (2006). Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. *Ecology*, *87*(6), 1575–1586.

Cooke, S. J., Philipp, D. P., & Weatherhead, P. J. (2002). Parental care patterns and energetics of smallmouth bass (*Micropterus dolomieu*) and largemouth bass (Micropterus salmoides) monitored with activity transmitters. *Canadian Journal of Zoology*, *80*, 756–770. https://doi.org/10.1139/Z02-048

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*, 226. https://doi.org/10.1186/s40168-018-0605-2

Dewar, M. L., Arnould, J. P. Y., Dann, P., Trathan, P., Groscolas, R., & Smith, S. (2013). Interspecific variations in the gastrointestinal microbiota in penguins. *Microbiology*, *2*(1), 195–204. https://doi.org/10.1002/mbo3.66

Dewar, M. L., Arnould, J. P. Y., Krause, L., Dann, P., & Smith, S. C. (2014). Interspecific variations in the faecal microbiota of Procellariiform seabirds. *FEMS Microbiology Ecology*, *89*, 47–55. https://doi.org/10.1111/1574-6941.12332

Drenner, S. M., Hinch, S. G., Furey, N. B., Clark, T. D., Li, S., Ming, T., Jeffries, K. M., Patterson, D. A., Cooke, S. J., Robichaud, D., Welch, D. W., Farrell, A. P., & Miller, K. M. (2018). Transcriptome patterns and blood physiology associated with homing success of sockeye salmon during their final stage of marine migration. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*, 1511–1524.

Dunn, C. D., Campbell, L. J., Wallace, E. M., Danylchuk, A. J., Cooke, S. J., Shultz, A. D., Black, B. D., Brownscombe, J. W., Griffin, L. P., Philipp, D. P., Adams, A. J., & Goldberg, T. L. (2020). Bacterial communities on the gills of bonefish (Albula vulpes) in the Florida Keys and The Bahamas show spatial structure and differential abundance of disease-associated bacteria. *Marine Biology*, *167*(6), 1–11. https://doi.org/10.1007/s00227-020-03698-7

Eichmiller, J. J., Hamilton, M. J., Staley, C., Sadowsky, M. J., & Sorensen, P. W. (2016). Environment shapes the fecal microbiome of invasive carp species. *Microbiome*, *4*, 44. https://doi.org/10.1186/s40168-016-0190-1

Eisenhofer, R., Minich, J. J., Marotz, C., Cooper, A., Knight, R., & Weyrich, L. S. (2019). Contamination in low microbial biomass microbiome studies: Issues and recommendations. *Trends in Microbiology*, *27*(2), 105–117. https://doi.org/10.1016/j.tim.2018.11.003

Element, G., Engel, K., Neufeld, J. D., Casselman, J. M., van de Coeverden Groot, P., Greer, C. W., & Walker, V. K. (2020a). Seasonal habitat drives intestinal microbiome composition in anadromous Arctic char (Salvelinus alpinus). *Environmental Microbiology*, *22*(8), 3112–3125. https://doi.org/10.1111/1462-2920.12735

Element, G., Engel, K., Neufeld, J. D., Casselman, J. M., van de Coeverden Groot, P., & Walker, V. K. (2020b). Distinct intestinal microbial communities of two sympatric anadromous Arctic salmonids and the effects of migration and feeding. *Arctic Science*, *7*(3), 634–654.

Erb-Downward, J. R., Falkowski, N. R., D'Souza, J. C., McCloskey, L. M., McDonald, R. A., Brown, C. A., Shedden, K., Dickson, R. P., Freeman, C. M., Stringer, K. A., Foxman, B., Huffnagle, G. B., Curtis, J. L., & Adar, S. D. (2020). Critical relevance of stochastic effects on low-bacterial-biomass 16S rRNA gene analysis. *MBio*, *11*, e00258-20.

Gajardo, K., Rodiles, A., Kortner, T. M., Krogdahl, Å., Bakke, A. M., Merrifield, D. L., & Sørum, H. (2016). A high-resolution map of the gut microbiota in Atlantic salmon (Salmo salar): A basis for comparative gut microbial research. *Scientific Reports*, *6*, 30893. https://doi.org/10.1038/srep30893

Gomez, J. A., & Primm, T. P. (2021). A slimy business: The future of fish skin microbiome studies. *Microbial Ecology*, *82*, 275–287.

Hildonen, M., Kodama, M., Puetz, L. C., Gilbert, M. T. P., & Limborg, M. T. (2019). A comparison of storage methods for gut microbiome studies

in teleosts: Insights from rainbow trout (Oncorhynchus mykiss). *Journal of Microbiological Methods*, *160*, 42–48. https://doi.org/10.1016/j.mimet.2019.03.010

Hinch, S. G., Cooke, S. J., Healey, M. C., & Farrell, A. P. (2006). Behavioural physiology of fish migrations: Salmon as a model approach. In K. A. Sloman, R. W. Wilson, & S. Balshine (Eds.), *Fish Physiology* (pp. 239–295). Elsevier Inc.

Hoffmann, C., Hill, D. A., Minkah, N., Kirn, T., Troy, A., Artis, D., & Bushman, F. (2009). Community-wide response of the gut microbiota to enteropathogenic *Citrobacter rodentium* infection revealed by deep sequencing. *Infection and Immunity*, *77*(10), 4668–4678. https://doi.org/10.1128/IAI.00493-09

Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Speer, K. A., & Perkins, S. L. (2018). Comparing microbiome sampling methods in a wild mammal: Fecal and intestinal samples record different signals of host ecology, evolution. *Frontiers in Microbiology*, *9*, 803. https://doi.org/10.3389/fmicb.2018.00803

Jeffries, K. E. N. M., Hinch, S. G., Gale, M. K., Clark, T. D., Lotto, A. G., Casselman, M. T., Li, S., Rechisky, E. L., Porter, A. D., Welch, D. W., & Killer, K. M. (2014). Immune response genes and pathogen presence predict migration survival in wild salmon smolts. *Molecular Ecology*, *23*, 5803–5815. https://doi.org/10.1111/mec.12980

Jin Song, S., Amir, A., Metcalf, J. L., Amato, K. R., Zech, X. Z., Humphrey, G., & Knight, R. (2016). Preservation methods differ in fecal microbiome stability, affecting suitability for field studies. *mSystems*, *1*(3), e00021-16. https://doi.org/10.1128/mSystems.00021-16.Editor

Jin Song, S., Woodhams, D. C., Martino, C., Allaband, C., Mu, A., Javorschi-Miller-Montgomery, S., Suchodolski, J. S., & Knight, R. (2019). Engineering the microbiome for animal health and conservation. *Experimental Biology and Medicine*, *244*, 494–504. https://doi.org/10.1177/1535370219830075

Kennedy, K. M., de Goffau, M. C., Perez-Muñoz, M. E., Arrieta, M., Bäckhed, F., Bork, P., Braun, T., Bushman, F. D., Dore, J., de Vos, W. M., Earl, A. M., Eisen, J. A., Elovitz, M. A., Ganal-Vonarburg, S. C., Gänzle, M. G., Garrett, W. S., Hall, L. J., Hornef, M. W., Huttenhower, C., … Walter, J. (2023). Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature*, *613*, 639–649. https://doi.org/10.1038/s41586-022-05546-8

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T., & Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *PNAS*, *108*(suppl. 1), 4578–4585. https://doi.org/10.1073/pnas.1000081107

Le, D., Nguyen, P., Nguyen, D., Dierckens, K., Boon, N., Lacoere, T., Kerckhof, F.-M., De Vrieze, J., Vadstein, O., & Bossier, P. (2020). Gut microbiota of migrating wild rabbit fish (*Siganus guttatus*) larvae have low spatial and temporal variability. *Microbial Ecology*, *79*, 539–551.

Legrand, T. P. R. A., Catalano, S. R., Wos-Oxley, M. L., Stephens, F., Landos, M., Bansemer, M. S., Stone, D. A. J., Qin, J. G., & Oxley, A. P. A. (2018). The inner workings of the outer surface: Skin and gill microbiota as indicators of changing gut health in yellowtail kingfish. *Frontiers in Microbiology*, *8*(JAN), 1–17. https://doi.org/10.3389/fmicb.2017.02664

Legrand, T. P. R. A., Wynne, J. W., Weyrich, L. S., & Oxley, A. P. A. (2020). A microbial sea of possibilities: Current knowledge and prospects for an improved understanding of the fish microbiome. *Reviews in Aquaculture*, *12*, 1101–1134. https://doi.org/10.1111/raq.12375

Lewis, W. B., Moore, F. R., & Wang, S. (2016). Characterization of the gut microbiota of migratory passerines during stopover along the northern coast of the Gulf of Mexico. *Journal of Avian Biology*, *47*(5), 659–668. https://doi.org/10.1111/jav.00954

Liu, Y., Li, Y., Li, J., Zhou, Q., & Li, X. (2022). Gut microbiome analyses of wild migratory freshwater fish (*Megalobrama terminalis*) through geographic isolation. *Frontiers in Microbiology*, *13*, 858454. https://doi.org/10.3389/fmicb.2022.858454

Llewellyn, M. S., Boutin, S., Hoseinifar, S. H., & Derome, N. (2014). Teleost microbiomes: The state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology*, *5*, 1–17. https://doi.org/10.3389/fmicb.2014.00207

Llewellyn, M. S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G. R., Creer, S., & Derome, N. (2016). The biogeography of the Atlantic salmon (Salmo salar) gut microbiome. *The ISME Journal*, *10*, 1280–1284. https://doi.org/10.1038/ismej.2015.189

Lowrey, L., Woodhams, D. C., Tacchi, L., & Salinas, I. (2015). Topographical mapping of the rainbow trout (Oncorhynchus mykiss) microbiome reveals a diverse bacterial community with antifungal properties in the skin. *Applied and Environmental Microbiology*, *81*(19), 6915–6925. https://doi.org/10.1128/AEM.01826-15

Lyons, P. P., Turnbull, J. F., Dawson, K. A., & Crumlish, M. (2017). Exploring the microbial diversity of the distal intestinal lumen and mucosa of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum) using next generation sequencing (NGS). *Aquaculture Research*, *48*(1), 77–91. https://doi.org/10.1111/are.12863

Martin, M. O., Gilman, F. R., & Weiss, S. L. (2010). Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, Sceloporus virgatus. *Symbiosis*, *51*, 97–105. https://doi.org/10.1007/s13199-010-0078-y

Minich, J. J., Petrus, S., Michael, J. D., Michael, T. P., Knight, R., & Allen, E. E. (2020). Temporal, environmental, and biological drivers of the mucosal microbiome in a wild marine fish, Scomber Japonicus. *mSphere*, *5*, e00401–e00420.

Mohammed, H. H., & Arias, C. R. (2015). Potassium permanganate elicits a shift of the external fish microbiome and increases host susceptibility to columnaris disease. *Veterinary Research*, *46*, 82. https://doi.org/10.1186/s13567-015-0215-y

Murillo, T., Schneider, D., Fichtel, C., & Daniel, R. (2022). Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs. *ISME Communications*, *2*(1), 3. https://doi.org/10.1038/s43705-021-00086-0

Neuman, C., Hatje, E., Zarkasi, K. Z., Smullen, R., Bowman, J. P., & Katouli, M. (2016). The effect of diet and environmental temperature on the faecal microbiota of farmed Tasmanian Atlantic Salmon (Salmo salar L.). *Aquaculture Research*, *47*(2), 660–672. https://doi.org/10.1111/are.12522

Nyholm, L., Odriozola, I., Bideguren, G. M., Aizpurua, O., & Alberdi, A. (2022). Gut microbiota differences between paired intestinal wall and digesta samples in three small species of fish. *PeerJ*, *10*, e12992. https://doi.org/10.7717/peerj.12992

Payne, C. J., Turnbull, J. F., Mackenzie, S., & Crumlish, M. (2022). The effect of oxytetracycline treatment on the gut microbiome community dynamics in rainbow trout (*Oncorhynchus mykiss*) over time. *Aquaculture*, *560*, 738559. https://doi.org/10.1016/j.aquaculture.2022.738559

Peterfreund, G. L., Vandivier, L. E., Sinha, R., Marozsan, A. J., Olson, W. C., Zhu, J., & Bushman, F. D. (2012). Succession in the gut microbiome following antibiotic and antibody therapies for Clostridium difficile. *PLoS ONE*, *7*(10), e46966. https://doi.org/10.1371/journal.pone.0046966

Portner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, *77*, 1745–1779. https://doi.org/10.1111/j.1095-8649.2010.02783.x

Pratte, Z. A., Patin, N. V., McWhirt, M. E., Caughman, A. M., Parris, D. J., & Stewart, F. J. (2018). Association with a sea anemone alters the skin microbiome of clownfish. *Coral Reefs*, *37*(4), 1119–1125. https://doi.org/10.1007/s00338-018-01750-z

Ringø, E., & Birkbeck, T. H. (1999). Intestinal microflora of fish larvae and fry. *Aquaculture Research*, *30*(2), 73–93. https://doi.org/10.1046/j.1365-2109.1999.00302.x

Ringø, E., Sperstad, S., Myklebust, R., Mayhew, T. M., & Olsen, R. E. (2006). The effect of dietary inulin on aerobic bacteria associated with hindgut of Arctic charr (*Salvelinus alpinus* L.). *Aquaculture Research*, *37*(9), 891–897. https://doi.org/10.1111/j.1365-2109.2006.01509.x

Risely, A., Waite, D., Ujvari, B., Klaassen, M., & Hoye, B. (2017). Gut microbiota of a long-distance migrant demonstrates resistance against environmental microbe incursions. *Molecular Ecology*, *26*, 5842–5854. https://doi.org/10.1111/mec.14326

Romero, J., Ringø, E., & Merrifield, D. L. (2014). The gut microbiota of fish. In D. Merrifield, & E. Ringø (Eds.), *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics* (pp. 75–100). John Wiley & Sons, Ltd.

Rytwinski, T., Kelly, L. A., Donaldson, L. A., Taylor, J. J., Smith, A., Drake, D. A. R., Martel, A. L., Geist, J., Morris, T. J., George, A. L., Dextrase, A. J., Bennett, J. R., & Cooke, S. J. (2021). What evidence exists for evaluating the effectiveness of conservation-oriented captive breeding and release programs for imperilled freshwater fishes and mussels? *Canadian Journal of Fisheries and Aquatic Sciences*, *78*(9), 1332–1346.

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner, P., Parkhill, J., Loman, N. J., & Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*, 87.

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors–Occurrence, properties and removal. *Journal of Applied Microbiology*, *113*, 1014–1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x

Secombes, C., & Wang, T. (2012). The innate and adaptive immune system of fish. In B. Austin (Ed.), *Infectious disease in aquaculture: Prevention and control* (pp. 3–68). Woodhead Publishing.

Skeen, H. R., Cooper, N. W., Hackett, S. J., Bates, J. M., & Marra, P. P. (2021). Repeated sampling of individuals reveals impact of tropical and temperate habitats on microbiota of a migratory bird. *Molecular Ecology*, *30*, 5900–5916. https://doi.org/10.1111/mec.16170

Smriga, S., Sandin, S. A., & Azam, F. (2010). Abundance, diversity, and activity of microbial assemblages associated with coral reef fish guts and feces. *FEMS Microbiology Ecology*, *73*(1), 31–42. https://doi.org/10.1111/j.1574-6941.2010.00879.x

Spicer, J., & Gaston, K. (1999). *Physiological diversity and its ecological implications*. Blackwell Scientific Publications.

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews. Microbiology*, *9*, 279–290. https://doi.org/10.1038/nrmicro2540

Stanley, D., Geier, M. S., Chen, H., Hughes, R. J., & Moore, R. J. (2015). Comparison of fecal and cecal microbiotas reveals qualitative similarities but quantitative differences. *BMC Microbiology*, *15*, 51. https://doi.org/10.1186/s12866-015-0388-6

Talwar, C., Nagar, S., Lal, R., & Negi, R. K. (2018). Fish gut microbiome: Current approaches and future perspectives. *Indian Journal of Microbiology*, *58*(4), 397–414. https://doi.org/10.1007/s12088-018-0760-y

Thorstensen, M. J., Vandervelde, C. A., Bugg, W. S., Michaleski, S., Vo, L., Mackey, T. E., Lawrence, M. J., & Jeffries, K. M. (2022). Non-lethal sampling supports integrative movement research in freshwater fish. *Frontiers in Genetics*, *13*, 795355. https://doi.org/10.3389/fgene.2022.795355

Tosin, V., Noor, A., Nurul, A., Danish-daniel, A. M., Sunday, A., Abolmunafi, A. B., Isaiah, K., & Ariffin, A. (2020). Microbiota composition data for wild and captive bluestreak cleaner wrasse *Labroides dimidiatus* (Valenciennes, 1839). *Data in Brief*, *32*, 106120. https://doi.org/10.1016/j.dib.2020.106120

Uren Webster, T. M., Consuegra, S., Hitchings, M., & Garcia de Leaniz, C. (2018). Interpopulation variation in the Atlantic salmon microbiome reflects environmental and genetic diversity. *Applied and Environmental Microbiology*, *84*, e00691-18. https://doi.org/10.1128/AEM.000691-18

Videvall, E., Strandh, M., Engelbrecht, A., Cloete, S., & Cornwallis, C. K. (2017). Direct PCR offers a fast and reliable alternative to conventional DNA isolation methods for gut microbiomes. *mSystems*, *2*, e00132-17.

Videvall, E., Strandh, M., Engelbrecht, A., Cloete, S., & Cornwallis, C. K. (2018). Measuring the gut microbiome in birds: Comparison of faecal and cloacal sampling. *Molecular Ecology Resources*, *18*(3), 424–434. https://doi.org/10.1111/1755-0998.12744

Waite, D. W., Deines, P., & Taylor, M. W. (2012). Gut microbiome of the critically endangered New Zealand parrot, the kakapo (Strigops habroptilus). *PLoS One*, *7*(4), 1–10. https://doi.org/10.1371/journal.pone.0035803

West, A. G., Waite, D. W., Deines, P., Bourne, D. G., Digby, A., McKenzie, V. J., & Taylor, M. W. (2019). The microbiome in threatened species conservation. *Biological Conservation*, *229*, 85–98. https://doi.org/10.1016/j.biocon.2018.11.016

Wilson, I. G. (1997). Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology*, *63*(10), 3741–3751.

Yang, H., Leng, X., Du, H., Luo, J., Wu, J., & Wei, Q. (2020). Adjusting the prerelease gut microbial community by diet training to improve the postrelease fitness of captive-bred Acipenser dabryanus. *Frontiers in Microbiology*, *11*, 488. https://doi.org/10.3389/fmicb.2020.00488

Zarkasi, K. Z., Abell, G. C. J., Taylor, R. S., Neuman, C., Hatje, E., Tamplin, M. L., Katouli, M., & Bowman, J. P. (2014). Pyrosequencing-based characterization of gastrointestinal bacteria of Atlantic salmon (*Salmo salar* L.) within a commercial mariculture system. *Journal of Applied Microbiology*, *117*(1), 18–27. https://doi.org/10.1111/jam.12514

Zhu, L., Wang, J., & Bahrndorff, S. (2021). Editorial: The wildlife gut microbiome and its implication for conservation biology. *Frontiers in Microbiology*, *12*, 697499. https://doi.org/10.3389/fmicb.2021.697499

How to cite this article: Kelly, L. A., Yost, C. K., & Cooke, S. J. (2024). Opportunities and challenges with transitioning to non-lethal sampling of wild fish for microbiome research. *Journal of Fish Biology*, 1–8. https://doi.org/10.1111/jfb.15650