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Evaluating blood, gill, and muscle biopsy methods on the behaviour, reproductive success, and survival of a wild freshwater fish

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ABSTRACT

Non-lethal biopsy methods (including blood, gill, and muscle biopsies) have been used to study the health and physiological status of wild fishes. Nonetheless, concerns exist regarding the impact of non-lethal sampling on relevant welfare measures such as behaviour and survival. Here, nesting Smallmouth Bass (*Micropterus dolomieu*) were used as a model species to study in situ how fish respond to non-lethal sampling. Male Smallmouth Bass provide sole parental care and guard well-defined nests for a period of several weeks, providing a unique opportunity to assess behaviour, reproductive success, and survival in the wild. Fish were captured from their nests by angling and subjected to a biopsy (either blood, gill, or muscle), or a combination of all three biopsy methods prior to release. A control group that was captured but not biopsied as well as a non-angled control were also included. Nests were monitored for a period of four weeks or until the parental males either abandoned offspring, died, or raised a brood to independence. Single biopsies, regardless of the biopsy type, were found to have no impact on parental care and survival, but fish that received the combined treatment took longer to return to their nest and displayed a 6.5 times greater likelihood of nest abandonment. Mortality was only observed in fish that received the combined biopsy treatment. As such, this study reveals that it is possible to maintain the welfare status of Smallmouth Bass in the wild by using individual biopsies, thus emphasizing the importance of making careful decisions about which tissues are needed to achieve desired study objectives. This is one of the few studies to assess the behavioural and fitness consequences of increasingly common non-lethal biopsy methods and provides useful information on the relative consequences of different biopsy methods on wild fish.

1. Introduction

Traditional sampling methods for freshwater and marine fish require fish to be lethally sampled to assess fish health and physiological status, contaminant burden, or pathogen load. However, lethal sampling can be detrimental to fish populations, particularly in instances where the species is rare or imperiled, or species with long life histories and late sexual maturity (Rolfhus et al., 2008). Lethal sampling also prevents researchers from studying behaviour and physiology simultaneously, or linking physiology and health to fate (e.g., survival, spawning; Cooke et al., 2016; Jeffries et al., 2021). As a result of these limitations, a variety of non-lethal sampling methods have been developed that allow for the same research topics mentioned above to be addressed by removing only small pieces of tissue from a living specimen – herein referred to as a non-lethal biopsy (Thorstensen et al., 2022).

Given advances in physiology, omics, technology, and fish health, small quantities of tissue can now be used in laboratory assays or microscopy, opening the door for non-lethal biopsy to be used in fish tissue collection. Previous studies have used blood samples taken from the caudal vasculature (Lawrence et al., 2020), gill clips taken from distal ends of gill filaments (McCormick, 1993; Cornwell et al., 2013), or white muscle cores taken using a biopsy punch from the dorsal musculature (Henderson et al., 2016) to assess the physiological and health status of wild fishes. Such techniques are thought to have negligible impact on the post-release survival of the fish (McCormick, 1993; Henderson et al., 2016), however, some studies have indicated that non-lethally sampled fish had higher mortality rates when compared to their non-biopsied counterparts (e.g., Bass et al., 2020). Little is known about the

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post-release behaviour of biopsied fish. In one of the few studies to assess behaviour of biopsied fish in the wild, Cooke et al. (2005) noted small differences in the migration speeds of adult sockeye salmon that had received blood and gill biopsies relative to non-biopsied individuals. If non-lethal biopsy methods are resulting in fitness or performance impairments post-release, the benefits of using non-lethal sampling are largely lost given the impact on fish welfare.

Previous validation studies of non-lethal biopsy have occurred on salmonids (Cooke et al., 2005; Miller et al., 2011) with comparatively little work on warmwater fish species. With a continued emphasis of electronic tagging and tracking studies on wild fish (Hussey et al., 2015), along with the promise of omics, such validation studies will be critical for ensuring that non-lethal biopsy methods do not lead to negative outcomes. To that end, the objective of this study was to evaluate whether non-lethal sampling methods influenced the behaviour and reproductive success of Smallmouth Bass (*Micropterus dolomieu*). Blood, gill, and muscle biopsy were evaluated independently and as a combined treatment to determine their effect on spawning success and nest-guarding behaviour of Smallmouth Bass. We tested the hypothesis that parental care behaviours, survival, and overall nest success will not differ between biopsied fish and their non-sampled counterparts. Male Smallmouth Bass are an effective model species for observing individual-level changes during their nesting season given that they provide sole parental care for a prolonged period. Bass will build a nest and remain guarding the eggs from predation for several weeks, until juveniles develop to the semi-independent stage of free-swimming fry (Philipp et al., 1997; Cooke et al., 2002). During this period, nests (and consequently nesting males) can be individually identified, and routinely monitored for their presence and nest guarding behaviours. Should adult male bass experience significant stress such as extreme temperature fluctuations or predation pressure, there is an increased likelihood of nest abandonment occurring (leading to certain depredation of all zygotes or offspring) (Siepker et al., 2009; Lunn and Steinhart, 2010). As such, the behaviour and reproductive success of bass can be assessed after non-lethal sampling in the wild. With interest in ensuring that research methods do not impact the welfare status of wild animals, this study addresses an important knowledge gap on the welfare and behaviour of fish after non-lethal biopsy.

2. Methods

All research conducted in this study was carried out following the Carleton University Animal Care Protocol (REF 110723).

2.1. Nest Sites and Selection

Smallmouth Bass were captured from their nests via angling on Big Rideau Lake, Ontario, Canada (44.7706°N, 76.2152°W) between May 16th and May 21st, 2021. Snorkel surveys were conducted to identify nests in similar habitats (gravel shoreline, between one to four metres deep), and nests were marked using a nest tag (a section of PVC pipe with a unique ID code). The number of eggs present in a nest was scored from 1 to 5; egg scores have been previously described in Siepker et. al. (2006), with a score of 1 representing few eggs and 5 representing many. As brood size has been determined to impact parental care levels (Lunn and Steinhart, 2010; Zuckerman et al., 2014), only nests with an egg score of 3 or higher were used. The age of eggs was estimated by the diver (based on transparency and presence of fungus), and only nests with new eggs (less than 2 days old) were selected for use in the study.

2.2. Parental Male Capture and Biopsy

Bass were angled from their nests using a single hook with a soft plastic lure (ned rig or dropshot). Hooks were removed from the fish within ten seconds of capture and deeply hooked (i.e., in the gills or esophagus) fish were not used for the study. A diver guarded the nest to prevent nest predation by sunfish species during sampling. Once fish were landed, the hook was removed within ten seconds and a timer was started for two minutes to standardize handling time. Fish were placed in a padded trough filled with fresh lake water, measured for total length (in millimetres), and tagged using an anchor tag for individual identification. Fish smaller than 330 mm were excluded from this study to prevent size discrepancy between treatments. Angled fish were assigned one of five treatments: an angled control, blood, gill, muscle, or a combined biopsy treatment of all three biopsy types (hereafter referred to as 'combined treatment'). A second control treatment of fish that were not angled (referred to as 'un-angled control'), were only observed by a snorkeler in the water, and were included to standardize behaviour and nest success without additional stressors. Total length for fish in the un-angled control group were estimated by the diver using their dive slate (21 ×30 centimeters) for scale. After measurement and tagging, all angled fish were held dorsal side down during the two-minute period, except for during the muscle biopsy which was taken using a 4 mm biopsy punch (Integra Miltex Disposable Biopsy Punch) from the dorsal musculature below the soft dorsal fin. One mL of blood was taken from the caudal vasculature following established procedures (Lawrence et al., 2020), and three to five millimetres of gill filamentous tissue were taken from the distal edges of the third gill arch using nail scissors.

2.3. Nest return time

At the end of the two-minute period, fish were released one boat length (5.5 m) from the nest. A timer was started to record the time it took for the fish to return to the nest, which was observed by the diver. If the fish failed to return after five minutes, testing ceased, and the return time was recorded as greater than five minutes. Previous research has evaluated how different angling stressors (such as durations of air exposure) influence return to nest times where longer times are typically associated with more extreme stressors (Kieffer et al., 1995; Philipp et al., 1997; Hanson et al., 2007). As fish in the un-angled control were not removed from their nest, no return time was collected for fish in this treatment group.

2.4. Parental care

Parental care was assessed 24 h after sampling. A diver re-assessed egg score and recorded whether the male was present or absent from the nest. For present males, an attack score in response to a predator was assessed using a live Bluegill (*Lepomis macrochrius*) caught earlier that day and placed in a 4 L transparent jar with a perforated lid to allow water flow as per Hanson et al. (2007). The jar was placed directly in the nest (avoiding the eggs) by the diver, who then backed off to allow the bass to settle. After the bass had resumed standard nest guarding behaviour, the diver started a timer for 60 s and observed the number of times the bass attacked the jar. If the bass held its mouth open at the jar, the number of seconds were recorded as individual attacks (e.g., three seconds in this position would equal three attacks; Gravel and Cooke, 2009). After 60 s, the jar containing the Bluegill was removed from the nest. Nests were then observed via snorkelling every 3–4 days to assess egg development, and to determine whether the male was still present. Nests were checked until the eggs reached the free-swimming fry stage unless nest abandonment (and subsequent total egg predation) occurred. Mortality was assessed opportunistically by searching for fish that had abandoned their nests within 50 m of their nest sites.

2.5. Statistical analysis

All statistical analysis were preformed using R Studio (version 4.1.2, R Core Team 2021). Total length and egg score were both evaluated using an ANOVA to ensure that average sizes and scores were consistent between treatments. Average attack scores were also compared between treatments using an ANOVA, and a Chi-square test was used to

determine whether there was an association between treatment and nest success. Return to nest time and time of abandonment were both assessed by survival analysis using the *Survival* package (v3.2-13; Therneau 2020). To generate Kaplan-Meier survival curves for return time, fish that failed to return in the 5-minute window were censored and return times (measured in seconds) were compared between treatments. The number of censored fish were also compared between treatments to determine if one treatment was disproportionately excluded from the analysis. A survival curve was also generated to examine when fish abandoned their nests. As nest checks were conducted every 3–4 days, the passage of time was recorded in terms of number of nest checks and not individual days. Multivariate survival analysis (Cox proportional hazard regression) was used to determine what factors were significant predictors of return time or abandonment. Consistent with recent perspectives on the rigidity of statistical significance values in ecological and behavioural research we chose to present our findings as strong evidence of significance in p-values fell between 0.001 and 0.01, moderate evidence if p-values were between 0.01 and 0.05, and weak evidence if p-values were between 0.05 and 0.1. P-values that fell above 0.1 were considered to have little or no evidence of significance (Muff et al., 2022).

3. Results

A total of 138 Smallmouth Bass were used with n = 23 fish per treatment. Total body length of fish and nest egg scores were similar among treatments ($p > 0.05$). Mean attack score did not significantly differ among treatments ($F = 1.878$, $p = 0.102$). Fish that were absent after 24 h when attack scores were generated were excluded from the analysis, but the number of fish excluded was not different among treatments ($\chi^2 = 0.9127$, $p = 0.923$).

There was no evidence that survival curves generated to model return-to-nest time were different from one another ($p = 0.45$). To generate the time-to-event curve, fish that failed to return in the 5-minute window were censored, and the number that failed to return in the five-minute window was compared between treatments. This revealed moderate evidence that the number of fish excluded from analysis differed between biopsy treatments ($\chi^2 = 9.020$, $p = 0.061$, Fig. 1). Proportionally less fish from the combined treatment returned within the five-minute window (12 out of 23 total, compared to a minimum of 17 fish in other treatments).

Egg score, attack score, and treatment were analyzed as covariates in a Cox hazard regression to determine if they impacted return time (Table 1). There is weak evidence that gill biopsies influenced return time ($p = 0.082$), with fish from the gill biopsy treatment on average returning faster than fish from the other treatments. There was little evidence that egg score, attack score, or other biopsy treatments had any impact on return time.

Table 1
Cox proportional hazard regression output for return to nest time.

Characteristic	Hazard Ratio	CI	P-value
Angled	1.51	0.71, 3.17	0.31
Blood	1.24	0.58, 2.64	0.58
Gill	2.05	0.91, 4.63	0.08
Muscle	1.35	0.62, 2.93	0.45
Combined	1.46	0.79, 3.01	0.41
Egg Score	0.93	0.51, 1.69	0.80
Attack Score	1.00	0.98, 1.01	0.70

CR= Confidence Interval

Overall nest success (defined by successful development of free-swimming fry) was measured as a binary response and compared among treatments. A Chi-square test was used to evaluate whether there was an association between non-lethal biopsy treatment and nest success. We found a weak relationship between nest success and treatment ($\chi^2 = 8.604$, $p = 0.126$), however, due to the disparity in nest success between un-angled control fish and fish from the combined treatment (Fig. 2), we did two additional pairwise comparisons. Specifically, we compared nest success between fish in the un-angled control to fish receiving the combined treatment, and fish in the angled control treatment to fish in the combined treatment. There was strong evidence that the combined treatment negatively affected nest success ($\chi^2 = 5.031$, $p = 0.025$) when compared to the un-angled control, however there was no evidence of nest success being impacted when we controlled for the impact of angling ($\chi^2 = 1.493$, $p = 0.222$).

A time-to-event curve was also generated to examine when fish abandoned their nests (Fig. 3). As nest checks were conducted every 3–4 days, the passage of time was recorded in terms of number of nest checks and not individual days. There was strong evidence to suggest that curves generated differed ($p = 0.045$) shown after the first nest check, indicating that treatment effected abandonment throughout the course of the study. Covariates (including egg score and attack score) were analyzed using a Cox hazard regression, and treatment was found to be a strong predictor of abandonment, with fish from the combined treatment being 6.5 times more likely to abandon nests than fish from the other treatment groups ($t = 2.387$, $p = 0.017$, hazard ratio = 6.45, Table 2). There was no evidence that other variables (e.g., egg score, attack score) were directly related to abandonment.

Mortality rate differences were not assessed statistically given that only 3 mortalities were observed during the study. However, all three

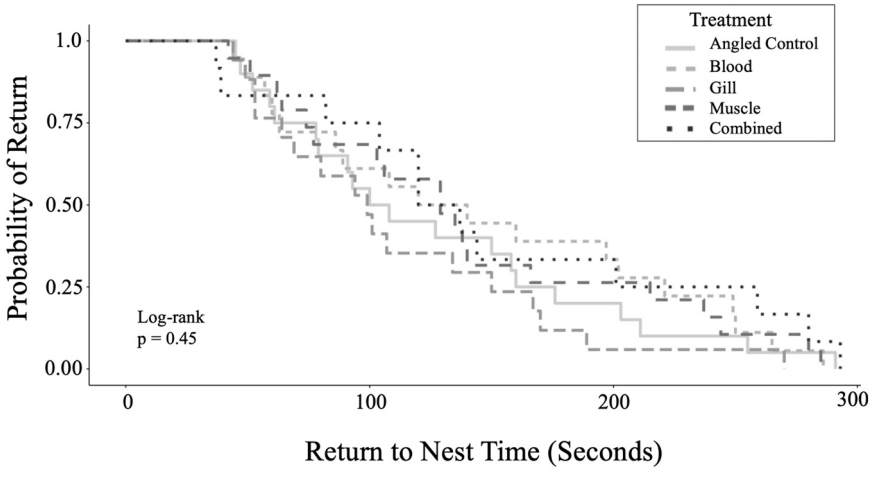

Fig. 1. Kaplan-Meier time-to-event curve for return to nest time in a 5-minute (300 s) monitoring period. Fish from the Un-angled control group are not included as no return time was measured for fish in this treatment group.

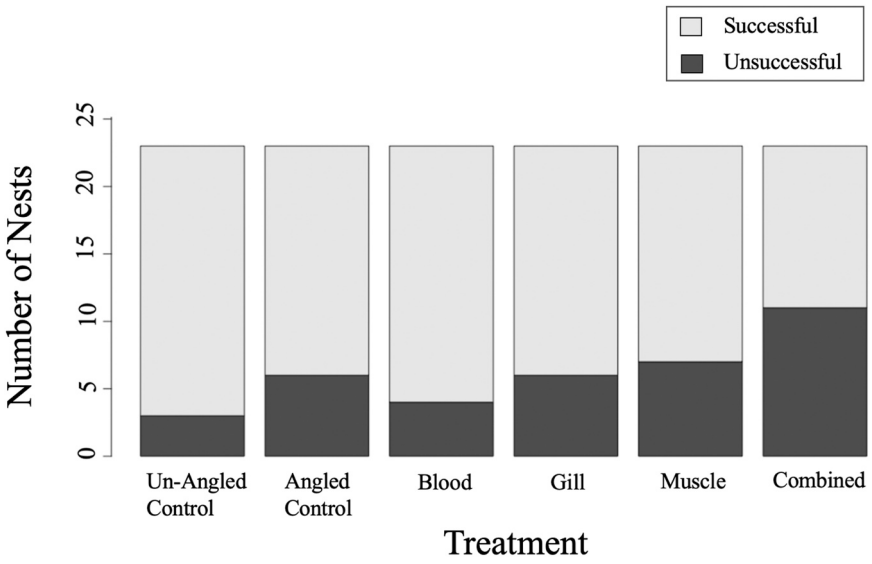

Fig. 2. Number of successful versus unsuccessful nests for male Smallmouth Bass in each non-lethal biopsy treatment (n = 23 nests per treatment). Nest success was determined by eggs hatching and surviving to the free-swimming fry developmental stage.

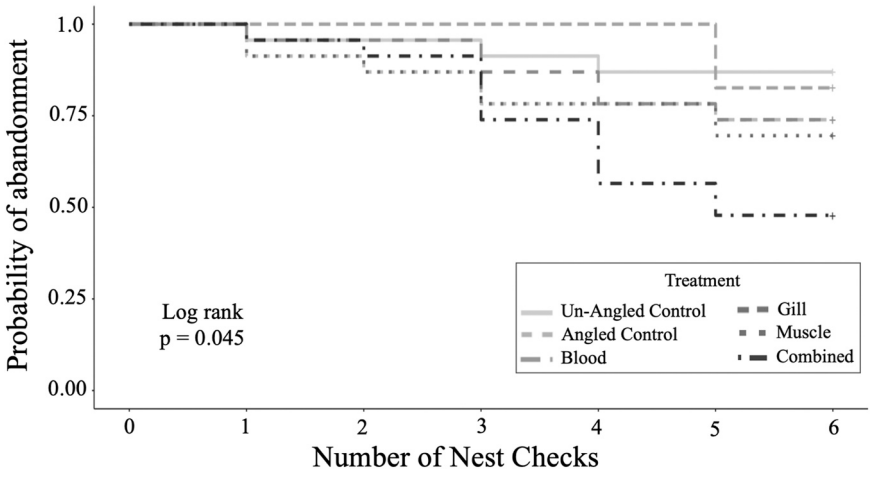

Fig. 3. Kaplan-Meier time-to-event curve for nest abandonment for each treatment. Nest check 0 was the original sampling event, with nest check 1 occurring 24 h after sampling. Nest checks 2–6 occurred every 3–4 days, with all nest checks ceasing after nests had been abandoned or eggs had developed into free-swimming fry.

Table 2
Cox proportional hazard regression output for nest abandonment.

Characteristic	Hazard Ratio	95% CI[1]	P-value
Angled Control	2.05	0.37, 11.41	0.41
Blood	1.73	0.31, 9.51	0.53
Gill	2.58	0.50, 13.32	0.26
Muscle	2.62	0.50, 13.64	0.25
Combined	6.45	1.40, 29.81	0.02
Egg Score	0.87	0.34, 2.24	0.78
Attack Score	0.99	0.96, 1.02	0.37

[1] CI= Confidence Interval

mortalities observed came from the combined treatment group and were recovered less than a week after sampling took place in proximity to the nest sites. External evaluation of the carcasses revealed bruising surrounding the muscle biopsy site, and discolouration throughout the body. Deceased fish were identified by their external tag.

4. Discussion

We were able to assess the consequences of non-lethal biopsy on nesting male Smallmouth Bass over a period of several weeks during parental care. Behaviour immediately after sampling (return to nest time) did not differ between treatments, however more fish from the combined biopsy treatment had to be excluded from the analysis because they did not return to their nest within the five-minute window. Results indicated that sampling using multiple biopsy methods simultaneously leads to impairment in response time after sampling. This could have critical consequences in the wild as responsiveness is a key component in a fish's ability to find shelter, escape predation, or respond to environmental threats (Campbell et al., 2010). The same impairment was not observed in fish from other biopsy treatments; for example, fish that received gill biopsies returned to their nests faster than other biopsy treatments or angled controls.

Previous research has evaluated the consequences of fishing stressors on nesting male bass with prolonged air exposure leading to delays in returning to the nest (Hanson et al., 2007). In our study, fish had minimal exposure to air (i.e., all biopsies were taken with fish held in a water-filled trough). We also had a diver guarding the nest from

predators to minimize nest predation, as decreases in brood size during the male's absence have been associated with greater levels of nest depredation and abandonment (Suski et al., 2003). However, it was not possible to guard nests until all fish returned, hence the implementation of a five-minute cut-off window after which we ceased predator defence. This likely contributed to the higher levels of abandonment noted in fish receiving the combined biopsy treatment, given that we found proportionally more fish from this treatment to not return within the five-minute window. Furthermore, it is well established that bass can assess their brood size throughout the nesting period and are more likely to abandon if there has been significant depredation in brood size (Suski et al., 2003). Although significant depreciation in egg score was not noted between initial nest observations and observations conducted 24-hours later, failure to return in the five-minute window left nests of fish from the combined treatment disproportionately vulnerable to predation when compared to fish from other treatments.

Many of the fish excluded from the return time analysis were found to have returned to their nest by the following day when attack scores were measured. Previous studies (e.g., Philipp et al., 1997; Hanson et al., 2007) found that most bass eventually returned to nests after angling events, although return time increased (often due to fish swimming long and indirect routes) when fish were exposed to additional stressors (e.g., air exposure, displacement), indicating distress or disorientation. In this study, no one treatment was disproportionately absent the following day, and attack scores generated in response to a simulated predation event were consistent among treatments, indicating fish were recovered sufficiently to resume routine parental care duties.

Despite the limited impacts on behaviour 24-hours post sampling, as monitoring continued through egg development an increase in nest abandonment was noted relative to controls, particularly in fish from the combined treatment. Previous studies on bass nest abandonment have concluded that bass trade-off current and future reproductive opportunities (Steinhart and Lunn, 2011). In some instances, it is more optimal to abandon a current brood in favour of future reproductive opportunities, especially if the parental fish is in poor condition such that continued care risks their survival (Lukas, Donald, 1995). Most of the nests abandoned during this study were done so during the egg-sac or swim-up fry stage which would mean total nest destruction (by predators) and zero reproductive success for that individual for that season. As the combined treatment was found to be a strong predictor of abandonment, this indicates that the stress caused by multiple biopsies may have longer term consequences such immune responses or metabolic costs. Consequently, overall nest success was found to be lowest in fish from the combined treatment, whereas single non-lethal biopsies had no impact on nest success.

Undoubtedly, the stress of collection via angling may also have played a role in parental care behaviours exhibited in fish throughout this study, which is particularly apparent when comparing the nest success in the angled and un-angled controls to single and multiple biopsy treatments. Although we attempted to reduce stressors associated with angling by minimizing air exposure, releasing fish close to their nests, and using heavy gear to decrease fight time (all strategies previously identified as being drivers of nesting bass behavioural impacts; e.g., Philipp et al., 1997), nest success was still higher in the un-angled treatment. Single biopsies had comparable nest success rates to fish from the angled control, and success rates were not found to significantly differ between angled and un-angled control fish. However, when nest success was compared between un-angled fish and fish from the combined biopsy treatment, a substantial reduction in overall nest success can be observed. These results suggest that the impact of angling may be seen when multiple other stressors are present; single biopsies, regardless of type may only cause minor increases in overall stress, but the compounding stress of multiple biopsies in combination with angling presumably exceeded a threshold that contributed to abandonment rates and reductions in overall nest success. Such evidence emphasizes the need to continuously work to reduce capture stress levels when working with wild fish.

Overall survival was high throughout the study, with only three observed mortalities. All mortalities came from the combined treatment, although other fish may have died but were not recovered. All fish were tagged with external anchor tags, giving opportunity for other mortalities to be reported. No such reports from the public were made. Given that almost all fish returned to the nest for at least some time suggests that the mortality was not the result of the acute stress of biopsy but rather latent consequences that may have been mediated by disease or a general decline in fish health. Given that bruising was noted around the biopsy sites in the three observed mortalities but not on live fish from the same treatments, there is a high likelihood that substantial inflammation possibly caused by a primary or secondary infection could have contributed to the decline of the individual's health. Moreover, additional handling was needed to obtain all three biopsies which can lead to increased dermal disturbance (Colotelo and Cooke, 2011) and subsequent infection, particularly as skin and mucus act as an essential part of the teleost immune system (Dash et al., 2018). Although we only took a small amount of blood, minor bleeding can also occur at the site of gill and muscle biopsy which could contribute to anemia (Currie et al., 2022). Ubiquitous water mold (i.e., Saprolegnian fungus) is common in warmwater fish that experience injury and/or chronic stress (Xu and Rogers, 1991) and may have contributed to the further decline in fish condition and eventual death of fish exposed to all three biopsies, although fungal growth was not observed at the biopsy sites. Chronic stress caused by biopsy treatments, abiotic factors, or the constant vigilance required for nest defence may have further contributed to a decline in health, particularly as chronic stress has been shown to supress the immune response (Tort, 2011), leaving fish vulnerable to infection.

Results from this experiment indicate that a single non-lethal biopsy can be used safely with negligible impacts on parental care, nesting success, and survival of male Smallmouth Bass. There is however some evidence to suggest that the use of multiple biopsies at once may result in a delayed stress response resulting in nest abandonment. There may be a trade-off between what tissues are needed to answer a given research question and the welfare of fish and as such collected tissues should be chosen carefully and specifically to address desired research outcomes. Given the need to maintain welfare status of individual fish and ensure that research methods do not negatively impact fish populations, this study emphasizes the importance of conducting validation studies of non-lethal biopsy methods using ecologically and biologically relevant endpoints specific to a given species or taxa. With growing interest in linking fish fate to physiological status (e.g., Jeffries et al., 2021), we anticipate a rapid expansion of research involving non-lethal biopsy of fish that are tagged with electronic devices. Research such as we describe here will be essential for developing best practices that maintain fish welfare and ensure that research objectives can be achieved in an effective, ethical, and responsible manner.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Bass, A.L., Stevenson, C.F., Porter, A.D., Rechisky, E.L., Furey, N.B., Healy, S.J., Kanigan, A.M., Lotto, A.G., Welch, D.W., Hinch, S.G., 2020. In situ experimental evaluation of tag burden and gill biopsy reveals survival impacts on migrating juvenile sockeye salmon. Can. J. Fish. Aquat. Sci. 77 (12), 1865–1879. https://doi.org/10.1139/cjfas-2020-0134.

Campbell, M.D., Patino, R., Tolan, J., Strauss, R., Diamond, S.L., 2010. Sublethal effects of catch- and-release fishing: measuring capture stress, fish impairment, and predation risk using a condition index. ICES J. Mar. Sci. 67 (3), 513–521. https://doi.org/10.1093/icesjms/fsp255.

Colotelo, A.H., Cooke, S.J., 2011. Evaluation of common angling-induced sources of epithelial damage for popular freshwater sport fish using fluorescein. Fish. Res. 109 (2), 217–224. https://doi.org/10.1016/j.fishres.2010.12.005.

Cooke, S.J., Schreer, J.F., Wahl, D.H., Philipp, D.P., 2002. Physiological impacts of catch-and-r elease angling practices on Largemouth Bass and Smallmouth Bass, 31. American Fisheries Society Symposium, pp. 489–512.

Cooke, S.J., Crossin, G.T., Patterson, D.A., English, K.K., Hinch, S.G., Young, J.L., Alexander, R.F., Healey, M.C., Van Der Kraak, G., 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. J. Fish. Biol. 67 (5), 1342–1358. https://doi.org/10.1111/j.1095-8649.2005.00830.x.

Cooke, S.J., Martins, E.G., Struthers, D.P., Gutowsky, L.F.G., Power, M., Doka, S.E., Dettmers, J.M., Crook, D.A., Lucas, M.C., Holbrook, C.M., Krueger, C.C., 2016. A moving target— incorporating knowledge of the spatial ecology of fish into the assessment and management of freshwater fish populations. Environ. Monit. Assess. 188 (4), 239. https://doi.org/10.1007/s10661-016-5228-0.

R. Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Cornwell, E.R., Bellmund, C.A., Groocock, G.H., Wong, P.T., Hambury, K.L., Getchell, R. G., Bowser, P.R., 2013. Fin and gill biopsies are effective nonlethal samples for detection of viral hemorrhagic septicemia virus genotype IVb. J. Vet. Diagn. Investig. 25 (2), 203–209. https://doi.org/10.1177/1040638713476865.

Currie, A.R., Cockerill, D., Diez-Padrisa, M., Haining, H., Henriquez, F.L., Quinn, B., 2022. Anemia in salmon aquaculture: Scotland as a case study. Aquaculture 546, 737313. https://doi.org/10.1016/j.aquaculture.2021.737313.

Dash, S., Das, S.K., Samal, J., Thatoi, H.N., 2018. Epidermal mucus, a major determinant in fish health: a review. Iran. J. Vet. Res. 19 (2), 72–81. https://doi.org/10.22099/ijvr.2018.4849.

Gravel, M.A., Cooke, S.J., 2009. Influence of inter-lake variation in natural nest predation pressure on the parental care behaviour of Smallmouth Bass (Micropterus dolomieu. Ethology 115 (6), 608–616. https://doi.org/10.1111/j.1439-0310.2009.01641.x.

Hanson, K.C., Cooke, S.J., Suski, C.D., Philipp, D.P., 2007. Effects of different angling practices on post-release behaviour of nest-guarding male black bass, Micropterus spp. Fish. Manag. Ecol. 14 (2), 141–148. https://doi.org/10.1111/j.1365-2400.2007.00534.x.

Henderson, C.J., Stevens, T.F., Lee, S.Y., 2016. Assessing the suitability of a non-lethal biopsy punch for sampling fish muscle tissue. Fish. Physiol. Biochem. 42 (6), 1521–1526. https://doi.org/10.1007/s10695-016-0237-z.

Hussey, N.E., Kessel, S.T., Aarestrup, K., Cooke, S.J., Cowley, P.D., Fisk, A.T., Harcourt, R.G., Holland, K.N., Iverson, S.J., Kocik, J.F., Flemming, J.E.M., Whoriskey, F.G., 2015. Aquatic animal telemetry: a panoramic window into the underwater world, 1221-1221 Science 348 (6240). https://doi.org/10.1126/science.1255642.

Jeffries, K.M., Teffer, A., Michaleski, S., Bernier, N.J., Heath, D.D., Miller, K.M., 2021. The use of non-lethal sampling for transcriptomics to assess the physiological status of wild fishes. Comp. Biochem. Physiol. B 256, 110629. https://doi.org/10.1016/j.cbpb.2021.110629.

Kieffer, J.D., Kubacki, M.R., Phelan, F.J.S., Philipp, D.P., Tufts, B.L., 1995. Effects of catch-and- release angling on nesting male Smallmouth Bass. Trans. Am. Fish. Soc. 124 (1), 70–76. https://doi.org/10.1577/1548-8659(1995)124%3C0070:EOCARA%3E2.3.CO;2.

Lawrence, M.J., Raby, G.D., Teffer, A.K., Jeffries, K.M., Danylchuk, A.J., Eliason, E.J., Hasler, C.T., Clark, T.D., Cooke, S.J., 2020. Best practices for non-lethal blood sampling of fish via the caudal vasculature. J. Fish. Biol. 97 (1), 4–15. https://doi.org/10.1111/jfb.14339.

Lukas, Joseph A., Donald, J.Orth, 1995. Factors affecting nesting success of smallmouth bass in a regulated virginia stream. Trans. Am. Fish. Soc. 124 (5), 726–735. https://doi.org/10.1577/1548-8659(1995)124<0726:FANSOS>2.3.CO;2.

Lunn, B.D., Steinhart, G.B., 2010. Effect of brood reduction on nest abandonment of Smallmouth Bass. Trans. Am. Fish. Soc. 139 (2), 586–592. https://doi.org/10.1577/T09-060.1.

McCormick, S.D., 1993. Methods for nonlethal gill biopsy and measurement of Na +, K + -ATPase activity. Can. J. Fish. Aquat. Sci. 50 (3), 656–658. https://doi.org/10.1139/f93-075.

Miller, K.M., Li, S., Kaukinen, K.H., Ginther, N., Hammil, E., Curtis, J.M.R., Patterson, D. A., Sierocinski, T., Donnison, L., Pavlidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., Farrell, A.P., 2011. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science 331 (6014), 214–217. https://doi.org/10.1126/science.1196901.

Muff, S., Nilsen, E.B., O'Hara, R.B., Nater, C.R., 2022. Rewriting results sections in the language of evidence. Trends Ecol. Evol. 37 (3), 203–210. https://doi.org/10.1016/j.tree.2021.10.009.

Philipp, D.P., Toline, C.A., Kubacki, M.F., Philipp, D.B.F., Phelan, F.J.S., 1997. The impact of catch-and-release angling on the reproductive success of smallmouth bass and largemouth bass. North Am. J. Fish. Manag., 17(2), 557–567 https://doi.org/10.1577/1548-8675(1997)017<0557:TIOCAR>2.3.CO;2.

Rolfhus, K.R., Sandheinrich, M.B., Wiener, J.G., Bailey, S.W., Thoreson, K.A., Hammerschmidt, C.R., 2008. Analysis of fin clips as a nonlethal method for monitoring mercury in fish. Environ. Sci. Technol. 42 (3), 871–877. https://doi.org/10.1021/es071427.

Siepker, M.J., Cooke, S.J., Wahl, D.H., Philipp, D.P., 2009. Individual reproductive success of Largemouth Bass and Smallmouth Bass subjected to different components of competitive angling events. Trans. Am. Fish. Soc. 138 (4), 818–825. https://doi.org/10.1577/T07-269.1.

Steinhart, G.B., Lunn, B.D., 2011. When and why do Smallmouth Bass abandon their broods? The effects of brood and parental characteristics. Fish. Manag. Ecol. 18 (1), 1–11. https://doi.org/10.1111/j.1365-2400.2010.00758.x.

Suski, C.D., Svec, J.H., Ludden, J.B., Phelan, F.J.S., Philipp, D.P., 2003. The effect of catch-and- release angling on the parental care behavior of male Smallmouth Bass. Trans. Am. Fish. Soc. 132 (2), 210–218. https://doi.org/10.1577/1548-, 8659(2003)132<0210:TEOCAR>2.0.CO;2.

Therneau, T.M. (2020). A Package for Survival Analysis in R. https://CRAN.R- project.org/package=survival.

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. Front. Genet. 13, 795355 https://doi.org/10.3389/fgene.2022.795355.

Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35 (12), 1366–1375. https://doi.org/10.1016/j.dci.2011.07.002.

Xu, D., Rogers, W.A., 1991. Electron microscopy of infection by Saprolegnia spp. in channel catfish. J. Aquat. Anim. Health 3 (1), 63–69. https://doi.org/10.1577/1548-, 8667(1991)003<0063:EMOIBS>2.3.CO;2.

Zuckerman, Z.C., Philipp, D.P., Suski, C.D., 2014. The influence of brood loss on nest abandonment decisions in Largemouth Bass Micropterus salmoides. J. Fish. Biol. 84 (6), 1863–1875. https://doi.org/10.1111/jfb.12404.