

Assessing the suitability of a non-lethal biopsy punch for sampling fish muscle tissue

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Abstract Samples for the analysis of stable isotopes, genetics and other tissue sampling methods of small fish are often taken via lethal techniques. The aim of this study was to determine the suitability of a non-lethal sampling method for removing muscle tissue from fish under 30 cm total length (TL). A 4-mm biopsy punch was used to remove muscle tissue from two different species, *Lutjanus russelli* (Lutjanidae) and *Lethrinus laticaudis* (Lethrinidae). Several scales were removed from the sampling location, and then the biopsy punch was inserted to remove the muscle tissue. Immediately following this, a mixture of Fish Bandage™ and three drops of Betadine™ antiseptic solution was applied to the wound to reduce the chance of infection. The biopsy punch removes an average of 8 mg of muscle tissue from the fish, more than is required for stable isotope and genetic analyses (1 mg). The condition of fish subjected to our three treatments, biopsied fish, a handling controls and a tank control, was compared via gill beat counts at the time of biopsy and 3, 6, 9, 12 and 24 h after treatment. Overall, no instances of mortality were recorded across the study for all species and all treatments. This method has been found to be a suitable non-lethal method in the removal of muscle tissue from these two

fish species, potentially also other species under 30 cm TL, by eliminating mortality and minimising stress responses in sampled fishes.

Keywords Biopsy · Fish · Non-lethal tissue sampling · Stable isotope analysis · Genetic analysis

Introduction

Tissue sampling has become an important method for understanding how different animals interact within an ecosystem. The tissue sampling of fish has predominantly focused on the genetic structure of different populations (e.g. Williams et al. 2015), the feeding habits of different species (e.g. Davis et al. 2014), assessing where an individual is placed within a food web (e.g. Boecklen et al. 2011) or assessing how much mercury may be present in the tissue of some individuals (e.g. Baker et al. 2004). While tissue sampling is important for furthering our understanding of fish ecology, large quantities of individuals each year are killed for small amounts of muscle tissue (Jardine et al. 2011). This has resulted in an increase in studies investigating non-lethal sampling methods to collect tissue samples from a range of different species (Busst et al. 2015; Cano-Rocabayera et al. 2015; Smith et al. 2015).

The effects of non-lethal sampling have been investigated on cetaceans, birds, reptiles, sea horses

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and even large fish species (in particular, several shark species) (Daly and Smale 2013; Hammerschlag and Sulikowski 2011). Non-lethal sampling of fin clips has been shown to be effective in some species, with *Hippocampus guttulatus* having a good correlation between fin tissue and muscle tissue for stable isotope analysis (Valladares and Planas 2012). However, some non-lethal methods for collecting tissue samples provide inconsistent results in application such as stable isotope analysis. For example, Jardine et al. (2011) found that the collection of fin clips for stable isotope analysis was effective for certain elements such as carbon, but there was a poor correlation between muscle and fin clip nitrogen isotopes. These inconsistencies may compromise reliability of studies on changes in trophic position and feeding habits (Davis et al. 2014; Hussey et al. 2012), thereby suggesting that a range of non-lethal sampling methods are usable; however, there are cases where muscle tissue is the only option.

Non-lethal methods of tissue collection are particularly important for studies requiring large numbers of specimens (Robbins 2006). For example, Evans (2008) used a biopsy probe that was attached to the end of a speargun shaft and recorded an approximately 80 % success rate in tissue collection of fish larger than 30 cm TL. While this method is considered an effective way of collecting tissue samples, it is not suitable for smaller fish species. Similarly, methods for sampling cetacean tissues have been developed over the last 40 years, using a crossbow to take the biopsy (Noren and Mocklin 2012); however, neither of these is usable for smaller fish. The development of non-lethal techniques for muscle tissue sampling suitable for a wide range of fish species and sizes is therefore an essential step to further conserve fish communities, while also gathering important information on the biology, ecology, habitat use and contaminant loads of different species (Daly and Smale 2013), especially in marine protected areas or for endangered species where destructive sampling is obviously undesirable.

Non-lethal sampling is often associated with acute levels of stress for the organisms being sampled, resulting in likely higher mortality upon release as high levels of stress may result in death (Davis 2010). Biological indicators such as ventilation rate (measured in fish as gill beats per minute) have been shown to be effective indicators of acute stress (Barreto and

Volpato 2011; Bonga 1997). Ventilation rates are directly linked to an individual's metabolic rate and general fitness and are therefore a suitable measure of an individual's response to a stressful event (Barreto and Volpato 2011). Ventilation rates are easily quantified and non-invasive, thereby reducing confounding stressful encounters (Bell et al. 2010) and are one of the first indicators that increases in response to a stressful situation (Barreto and Volpato 2004). Given the suitability of ventilation rates for assessing stress, it is a suitable method to determine the stress experienced by fish that have undergone a biopsy punch to remove a portion of muscle tissue.

Therefore, the overall aims of this study were to determine:

1. If levels of mortality are higher in biopsied fish than in non-biopsied fish,
2. If stress levels (as measured by gill beats per minute) differ between biopsied and non-biopsied fish, and
3. If the biopsy punch method for muscle tissue sampling is suitable for field use.

Materials and methods

Moses perch, *Lutjanus russelli* (Bleeker, 1849) and the Grass Emperor, *Lethrinus laticaudis* (Alleyne and Macleay, 1877) were collected from Moreton Bay (27°S, 153°E), Queensland, Australia. Thirty individuals from each species were caught by rod and reel and transported to the Moreton Bay Research Station where they were placed in open-circuit aquaria. The time between when fish were captured and being placed in individual aquaria never exceeded 2 h, with fish in the interim placed in an aerated bucket. Individual fish were placed in separate 50-L glass aquaria (30 cm × 20 cm × 30 cm). The total length (TL) of all individuals was measured to the nearest millimetre using a measuring tape. *L. russelli* ranged from 110 mm to 200 mm in total length and was on average 158 ± 18.9 mm (SD) total length. *L. laticaudis* was on average 140 ± 17.1 mm and ranged from 105 to 170 mm total length. Fish were not acclimated to laboratory conditions prior to the commencement of the experiment so that the effect of the biopsy could be measured at a time point when they are still stressed due to capture and handling.

In this study, we will be using a medical-grade biopsy punch (Kai Medical, see Fig. 1) that has been used predominantly in dermatological applications in the past. The biopsy punch has a stainless steel sharp cutting edge. This style of biopsy punch has been also used in veterinary science as a tool for removing tissue. For the biopsy treatment, three or four scales were removed with forceps from approximately 2 cm below the leading edge of dorsal fin. Fish were restrained by hand (with latex gloves) on a soft, rubber surface covered in water to prevent drying out and effects of hand acids. A 4-mm-diameter biopsy punch was inserted approximately 3 cm into the muscle of the fish by gently twisting and the tissue removed and placed in a micro-tube to be frozen. Once the biopsied muscle tissue was removed, two wound treatments were applied. Firstly, Fish Bandage™ was inserted in the wound to reduce the chance of infection. Fish Bandage™ is a non-toxic, non-allergenic cellulose-based powder that forms a clear viscous gel when in contact with water and can treat skin ulcers and bind open wounds in fish when combined with an antiseptic. Secondly, three drops of Betadine™ antiseptic solution were applied to the surface of the Fish Bandage™ powder to further reduce the chance of infection. This procedure takes less than one minute, and fish were returned to their aquaria immediately.

In addition, ten handling controls were completed for each species. Here, fish were handled in exactly the same way as the biopsied fish (fish were removed from the tank and placed on a soft rubber surface for the same period of time as biopsied fish), except that the biopsy punch was not used and the skin not broken. These individuals were used to account for the effects of handling and laboratory conditions. The final ten fish were left in the aquaria throughout to provide reference stress levels without any handling (tank controls).

Gill rates were measured for all individuals from all treatments at the time of the biopsy and then 3, 6, 9, 12 and 24 h after the biopsy had been taken. After this, the biopsied fish were moved to a 12,000-L tank (3 m diameter × 1.6 m deep, open-circuit flow through system). Fish remained in this tank over the following 4 weeks to monitor the wound healing. Prior to release, all individuals were visually assessed for any damage to the skin or infections that may have arisen throughout the biopsy sampling. Mortality was assessed at the end point of the study.

The dependent variable ‘gill rate’ was analysed using a repeated measures linear mixed model (LMM) in SPSS (SPSS Released 2013). Three factors were used: species (two levels: *L. russelli* and *L. laticaudis*), treatment (three levels: biopsied fish, tank control and handling control) and time (random, repeated measure

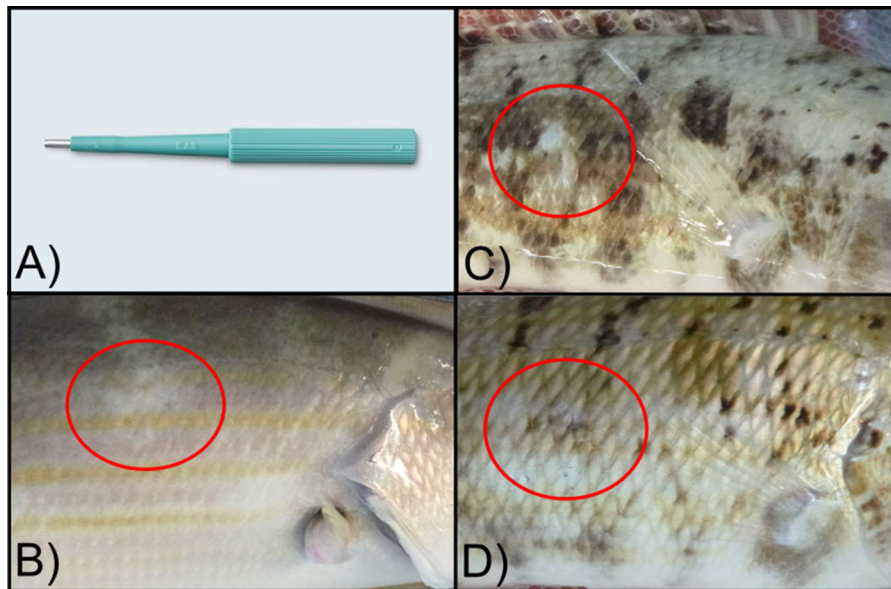


Fig. 1 **a** Kai Medical biopsy punch used in the study has a 4-mm-diameter cutting edge; **b** the wound, highlighted by the red circle, of a *Lutjanis russellii* after a 4-week period; **c**, **d** the biopsy punch wound after 4 weeks of two different *Lethrinus laticaudis* individuals. (Color figure online)

with six levels: 0, 3, 6, 9, 12 and 24 h from biopsy). Fish total length was initially included as a covariate in the analysis to assess potential effects of size; however, was found to be non-significant and subsequently removed. Gill rate was transformed to meet assumptions using a natural log (ln) transformation. A range of models were investigated, and the best model was obtained by comparing various goodness-of-fit statistics ($-2 \log$ likelihood, AIC and BIC). When significant differences were found, estimated marginal means were used to identify which means differed.

Results

The removal of muscle tissue for the biopsy sample had no effect on survival, with all fish surviving for the entire period of the study. The biopsy punch was able to remove on average 8 mg of muscle tissue from each individual. We found no significant differences in gill beat rates among biopsied fish, handling controls or tank controls. However, gill beat rates did vary through time and between fish species (Table 1; Fig. 2). This could be seen with a significant interaction between species and time ($df = 5$, $F = 3.573$, $p = 0.004$) that showed that at the time of biopsy and 3 h afterwards, gill rate was higher for both species. The following four sampling times all had a significantly lower gill rate. Biopsy wound sites for all individuals were covered with an epidermis layer within 1 week, and scales had regrown within 2 weeks. Figure 1b–d show the biopsied sites on different individuals after 2 weeks.

Gill rates for biopsied fish showed a similar pattern to those of both controls, and a lack of significant

Table 1 Three-way linear mixed model for analysing differences in stress levels (as measured by gill beat rates) for time after biopsy, fish species and treatment type

Source	Numerator <i>df</i>	<i>F</i>	Sig.
Species	1	15.835	<0.001
Treatment	2	1.698	0.192
Treatment × species	2	83.163	0.168
Time	5	1.682	<0.001
Time × species	5	3.573	0.004
Time × treatment	10	1.639	0.101
Time × treatment × species	10	0.738	0.688

difference indicates that a majority of the stress is caused by the captive state of the fish and that it is more stressful for the fish to be caught using rod and reel and then it is to undergo the biopsy. *L. russelli* had a consistently higher gill rate than *L. laticaudis* at all times except at the initial time of biopsy. The significant difference at the time level shows that the effects of the biopsy are only influencing the stress levels of the fish for the first 3 h of the study. While it is expected that there would be differences in gill beats after the removal of the biopsy, the lack of treatment effect suggests that this method may be suitable.

Discussion

For a non-lethal method to be suitable, it must (1) minimise impacts and stress on individual animals; (2) remove the correct amount of tissue; (3) prevent long-

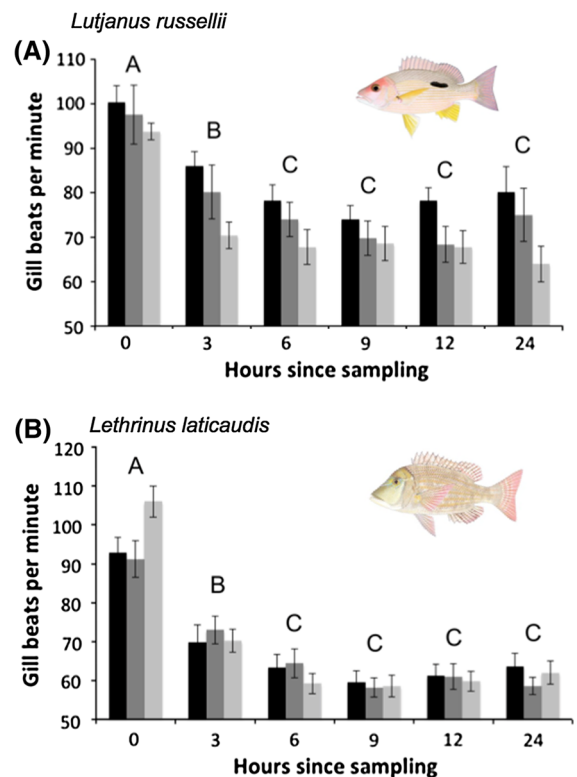


Fig. 2 Changes in gill rates per minute over the length of the trials for **a** Moses perch, *Lutjanus russelli* and **b** Grass Emperor, *Lethrinus laticaudis*. Black indicates the biopsied fish, dark grey indicates handling controls (not biopsied), and light grey indicates tank controls (not handled). Different letters above each time period indicate significant differences in the means

term injury; and (4) be quick and efficient (Baker et al. 2004). The method described in this study removes at least 8 mg dry mass of muscle tissue, meaning it removes more than is required for stable isotope and genetic analyses (1 mg). Gill beat rates were seen to stabilise 6 h after the biopsy, with differences only seen in the initial two sampling times. Our method resulted in zero mortality for both species, with all individuals being under 30 cm TL (minimum of 11.0 cm and mean of 14.9 cm). This method would therefore be suitable for individuals under the 30-cm limit found in Evans (2008). However, applicability to individuals under 10 cm is unknown, and further testing into this would be necessary. The short time taken to complete this procedure and the lack of long-term effect on gill rate also contribute to the suitability of this method for field studies. Note that we did not evaluate the use of local anaesthesia, which in practised hands would probably not add more than a few seconds to handling time. Our main aims surrounded the effect of the biopsy punch alone, and we have shown that even without the use of anaesthesia there is no long-term effect on the gill rate of individual fish or mortality.

Many studies on the effects of non-lethal sampling have focused on the suitability of fin tissue, scales or other portions of the fish for different chemical analysis. However, many have found issues with the efficiency of these methods to give similar measurements to that of muscle tissue (Jardine et al. 2011; Willis et al. 2013). While these studies have shown that there are non-lethal methods suitable for sampling fish, they also outline that there are obvious differences in the turnover rates of different tissues, creating issues such as ambiguous trophic position (Cabana and Rasmussen 1996) or feeding habit (Davis et al. 2014). By creating a cost effective and simple method for the removal of muscle tissue from fish, a majority of these problems will be negated. This method also supports the removal of fish muscle tissue from individuals that are as small as 11 cm, smaller than those in other biopsy removal studies (Baker et al. 2004).

We have demonstrated that this method has only a limited and short-duration effect on fish stress and that it does not result in mortality. We therefore suggest that this method would be suitable to use in the field as part of a catch and release sampling programme. We propose that biopsies could be performed in the field using the techniques trialled here, with the fish being

released immediately following application of the Fish Bandage and antiseptic liquid. We also propose that during the biopsy procedure that the fish be handled with wet hands in order to reduce any loss of the mucus layer of the fish (Hannan et al. 2015). The increase in the number of studies and management programmes using tissue sampling to monitor fish populations suggests that a non-lethal method of collecting muscle tissue is required. Depending on the type of sampling required, the method presented here would be highly suitable, especially for studies conducted in protected areas or on endangered species, where destructive fish removal is undesirable.

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References

- Baker R, Blanchfield P, Paterson M, Flett R, Wesson L (2004) Evaluation of nonlethal methods for the analysis of mercury in fish tissue. *Trans Am Fish Soc* 133(3):568–576
- Barreto RE, Volpato GL (2004) Caution for using ventilatory frequency as an indicator of stress in fish. *Behav Process* 66(1):43–51
- Barreto RE, Volpato GL (2011) Ventilation rates indicate stress-coping styles in Nile tilapia. *J Biosci* 36(5):851–855
- Bell AM, Henderson L, Huntingford FA (2010) Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. *J Comp Physiol B* 180(2):211–220
- Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. *Annu Rev Ecol Evol Syst* 42:411–440
- Bonga SW (1997) The stress response in fish. *Physiol Rev* 77(3):591–625
- Busst GMA, Basic T, Britton JR (2015) Stable isotope signatures and trophic-step fractionation factors of fish tissues collected as non-lethal surrogates of dorsal muscle. *Rapid Commun Mass Spectrom: RCM* 29(16):1535–1544
- Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proc Natl Acad Sci* 93(20):10844–10847
- Cano-Rocabayera O, Maceda-Veiga A, de Sostoa A (2015) Fish fins and scales as non-lethally sampled tissues for stable isotope analysis in five fish species of north-eastern Spain. *Environ Biol Fishes* 98(3):925–932
- Daly R, Smale M (2013) Evaluation of an underwater biopsy probe for collecting tissue samples from bull sharks *Carcharhinus leucas*. *Afr J Mar Sci* 35(1):129–132

- Davis MW (2010) Fish stress and mortality can be predicted using reflex impairment. *Fish Fish* 11(1):1–11
- Davis JP, Pitt KA, Fry B, Olds AD, Connolly RM (2014) Seascape-scale trophic links for fish on inshore coral reefs. *Coral Reefs* 33(4):897–907
- Evans R (2008) Assessment of an underwater biopsy probe for collecting teleost fish tissue samples. *Mar Ecol Prog Ser* 368:305–308
- Hammerschlag N, Sulikowski J (2011) Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. *Endanger Species Res* 14:135–140
- Hannan KD, Zuckerman ZC, Haak CR, Shultz AD (2015) Impacts of sun protection on feeding behavior and mucus removal of bonfish, *Albula vulpes*. *Environ Biol Fishes* 98(11):2297–2304
- Hussey NE, MacNeil MA, Olin JA, McMeans BC, Kinney MJ, Chapman DD, Fisk AT (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *J Fish Biol* 80(5):1449–1484
- Jardine TD, Hunt RJ, Pusey BJ, Bunn SE (2011) A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes. *Mar Freshw Res* 62(1):83–90
- Noren DP, Mocklin JA (2012) Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. *Mar Mamm Sci* 28(1):154–199
- Robbins WD (2006) Evaluation of two underwater biopsy probes for in situ collection of shark tissue samples. *Mar Ecol Prog Ser* 310:213–217
- Smith A, Marty J, Power M (2015) Non-lethal sampling of lake sturgeon for stable isotope analysis: comparing pectoral fin-clip and dorsal muscle for use in trophic studies. *J Great Lakes Res* 41(1):292–297
- SPSS (2013) IBM SPSS statistics for windows. Version 22.0. IBM Corp., Armonk
- Valladares S, Planas M (2012) Non-lethal dorsal fin sampling for stable isotope analysis in seahorses. *Aquat Ecol* 46(3):363–370
- Williams SM, Holmes BJ, Pepperell JG (2015) The novel application of non-lethal citizen science tissue sampling in recreational fisheries. *PLoS One* 10(9):e0135743
- Willis TJ, Sweeting CJ, Bury SJ, Handley SJ, Brown JCS, Freeman DJ, Cairney DG, Page MJ (2013) Matching and mismatching stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) ratios in fin and muscle tissue among fish species: a critical review. *Mar Biol* 160(7):1633–1644