A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes

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Abstract

Despite prior studies showing good agreement between fin and muscle isotope ratios in temperate fishes, the non-lethal method of fin sampling has yet to become a standard technique in isotopic food web studies, and the relationship between the two tissues has never been tested in the tropics. We hypothesized that fin and muscle $\delta^{13}C$ and $\delta^{15}N$ would be strongly correlated in tropical fishes, thus allowing non-lethal sampling of these species. To test this hypothesis, we analysed fin and muscle from 174 tropical fishes representing 27 species from the Mitchell River, Queensland, Australia. Fin tissue was a strong predictor of muscle tissue $\delta^{13}C$ ($r^2 = 0.89$ for all species) and was slightly enriched in ^{13}C (0.9‰), consistent with studies on temperate species. Fin tissue was a poorer predictor of muscle tissue $\delta^{15}N$ ($r^2 = 0.56$ for all species) but the mean difference between the tissues was small (<0.1‰). Differences were smallest in the largest fish, possibly because the elemental composition (%N) of

fin more closely resembled that of muscle. These measurements provide more impetus for increased use of fin tissue as a non-destructive means of testing hypotheses about fish food webs in the tropics and elsewhere.

Introduction

Stable isotope analysis (SIA) is now a standard method of answering questions about the feeding ecology of fishes (Araujo-Lima *et al*. 1986; Cherel *et al*. 2010). The use of SIA has led to numerous insights about invasive species (Vander Zanden *et al*. 1999), the provision of energy to higher trophic levels in aquatic food webs (Forsberg *et al*. 1993) and the fundamental niche of organisms (Layman *et al*. 2007). However, the massive growth in popularity of SIA in aquatic ecology means that every year thousands of fishes are sampled. The ideal tissue to obtain accurate dietary information using SIA is white muscle because it is homogenous, accurately reflects the isotopic composition of diet and has a moderate turnover rate of several months (Pinnegar and Polunin 1999, Dalerum and Angerbjorn 2005). This sampling is typically destructive and therefore could have impacts on the local population viability of less common species.

There are both ethical and epistemological reasons for minimizing the impact of sampling on the population under study. Many non-lethal approaches are now commonplace in molecular ecology (e.g. Brodeur *et al*. 2008; Masci *et al*. 2008), field studies of proximate body composition (Cox and Hartman 2005), and contaminants research (Baker *et al*. 2004; Gremillion *et al*. 2005; Rolfhus *et al*. 2008). Those who use SIA have, to date, been slow to embrace non-destructive alternatives to muscle (Church *et al*. 2009), despite studies that illustrate comparability of results when using

readily available non-lethal tissues such as fins (Kelly *et al*. 2006; Sanderson *et al*. 2009; Hanisch *et al*. 2010).

Currently, fin tissue is occasionally used in studies using SIA of temperate fishes (McCarthy and Waldron 2000; Finlay *et al*. 2002; Rasmussen *et al*. 2009). However, SIA of fin tissue has not been applied to tropical fishes. Tropical fishes live in warm, productive waters (Davies *et al*. 2008), and have diverse diets (Winemiller 1990; Pusey *et al*. 2004) and rapid growth rates which may influence their isotopic composition, relative tissue turnover rates and fractionation among tissues (Martinez del Rio *et al*. 2009). The purpose of this study was to determine the suitability of fin tissue as a proxy for muscle in isotopic studies of tropical fishes. We analysed fin and muscle $\delta^{13}C$ and $\delta^{15}N$ from a range of fish species common in floodplain rivers of northern Australia. Based on earlier work in temperate species (Kelly *et al*. 2006; Sanderson *et al*. 2009; Hanisch *et al*. 2010), we hypothesized that fin and muscle isotopic fractionation would be similar. We predicted strong correlations in isotope ratios between fin and muscle, and where differences existed between the tissues, we aimed to provide appropriate correction factors allowing their reciprocal use in future food web studies in the tropics.

Methods

The Mitchell River, Queensland (15.22º S, 141.61º E) is located in the wet-dry tropics and has a monsoonal climate leading to distinct wet (December to March) and dry (May to October) seasons. A total of 174 samples of fish from 27 species were collected by backpack and boat-mounted electrofishing and gill nets at 29 sites in the Mitchell River in May, June and October 2008 and February 2009 and 2010 (Table 1). Fish were euthanized by severing the spinal cord. The sites sampled in 2008 are

74 located throughout the catchment (maximum distance between sites > 400 km), while
75 samples in 2009 and 2010 came from two locations in the lower floodplain delta.
76 Multiple sites were sampled in an attempt to obtain a broad range of isotopic values
77 associated with different source baselines, and include waterholes and 100-m reaches
78 of small streams and large rivers. All samples were stored on ice and frozen within
79 one day of collection.
80 Isotope ratios of carbon and nitrogen and elemental composition in fin and
81 muscle tissue were measured in all 174 samples, but only seven had adequate
82 replication to perform taxon-specific regressions (Table 2). In instances where two
83 species were closely related and had similar dietary habits, data were combined to
84 increase sample sizes. This included sleepy cod (*Oxyeleotris lineolatus*) and giant
85 gudgeon (*O. selhemi*), bony bream (*Nematalosa erebi*) and gizzard shad (*N. come*),
86 and two species of fork-tailed catfish (*Neoarius graeffei* and *N. paucus*).
87 Two species of fish (spangled perch *Leiopotherapon unicolor*, and
88 rainbowfish *Melanotaenia splendida*) were collected from a subset of sites that
89 spanned the catchment (rainbowfish, n = 5 sites; spangled perch, n = 3 sites) at the
90 beginning (May) and the end (October) of the dry season in 2008 and both fin and
91 muscle tissues were measured for isotope ratios. Initial and re-sampling of the two
92 tissues after this period of approximately five months was a proxy for determining
93 their relative C and N isotopic turnover rates. This is because a shift in isotopic
94 composition of the diet over that time period would result in different C and N isotope
95 ratios if the two tissues had different turnover rates.
96 In the laboratory, white muscle tissue was dissected from above the lateral line
97 and fin tissue was taken from the caudal or anal fin. Tissues were dried for 48h at 60
98 ^{o}C. Muscle tissue was ground into a powder and fin clips were cut to appropriate

99 weights with a scalpel. Samples were then combusted in an EA 3000 elemental
100 analyser (Eurovector, Milan, Italy) and sample gases delivered to an Isoprime mass
101 spectrometer (GV Instruments, Manchester, UK) for isotope analysis of C and N.
102 Working standards were liquids calibrated against IAEA CH6, CH7, N1 and N2. A
103 single sample of fish tissue (muscle from spangled perch) analysed repeatedly to
104 measure precision over time yielded $\delta^{13}C$ = -21.9 ± 0.2‰ S.D. and $\delta^{15}N$ = 5.5 ± 0.4‰
105 S.D. (n = 29). High and variable tissue lipid content can cause $\delta^{13}C$ values to deviate
106 from that expected from diet; however, because C/N of both fin and muscle was less
107 than 4, indicative of low-lipid tissue, $\delta^{13}C$ data were not corrected for lipids (Logan et
108 al. 2008).
109 Data were analyzed using NCSS software (Kaysville, UT). We used
110 regressions of fin vs muscle $\delta^{13}C$ and $\delta^{15}N$ to estimate the efficacy of fin in predicting
111 muscle isotope ratios for all fish combined, and separately for those taxa for which we
112 had sufficient data ($n \geq 10$, barramundi *Lates calcarifer*, *Nematalosa* spp., longtom
113 *Strongylura krefftii*, rainbowfish, *Oxyeleotris* sp., *Neoarius* spp., and spangled perch).
114 We placed the degree of error introduced when using fin tissue instead of muscle in
115 the context of analytical error (Jardine and Cunjak 2005) by comparing the standard
116 deviation observed when comparing the two tissues (fin vs muscle) versus that
117 observed when random samples (either fin, n = 18, or muscle, n = 85) were analysed
118 in duplicate. We determined whether correction factors were necessary by running a
119 t-test on muscle $\delta^{13}C$ and $\delta^{15}N$ vs fin $\delta^{13}C$ and $\delta^{15}N$ to determine if fin and muscle
120 isotope ratios were significantly different. Prior to analysis, these data were checked
121 for non-normality (skewness and kurtosis) to ensure the appropriateness of parametric
122 tests. For the seasonal analysis, we subtracted isotope ratios observed in the late dry
123 sample from those observed in the early dry sample, and used linear regression to

124 determine if the two tissues showed similar patterns over this time period. To
125 evaluate and determine possible reasons for isotopic differences between tissues and
126 in assisting future workers in determining the most appropriate weights for analysis,
127 we regressed elemental composition and fin-muscle isotope ratios against body size
128 (log transformed fork length). We then grouped large and small fish according to a
129 semi-arbitrary fork length of 200 mm, and used a two-sample t-test to determine if
130 fin-muscle was different between the two size classes.
131
132 **Results**
133 Muscle and fin $\delta^{13}C$ were strongly correlated in all species ($r^2 = 0.91$, $p <$
134 0.001, n = 174, Fig. 1, Table 2). The average difference in $\delta^{13}C$ between fin and
135 muscle for all fish was 0.9 ± 1.1‰ S.D. ($t_{173} = 10.70$, $p < 0.001$). All of the taxa with
136 sufficient data also had fin $\delta^{13}C >$ muscle $\delta^{13}C$ when analysed individually ($t > 2.65$, p
137 < 0.01) with the exception of *Nematalosa* spp. ($t_{18} = 1.38$, $p = 0.093$, Table 2).
138 Random samples of fin or muscle analysed in duplicate yielded a mean difference of
139 0.0 ± 0.5‰ S.D. (n = 103, Fig. 1); because the SD associated with analysis of fin vs.
140 muscle is 1.1‰, this illustrates that the use of fin in place of muscle results in an
141 additional error of approximately 0.6‰ above analytical error.
142 Muscle and fin $\delta^{15}N$ were less well-correlated ($r^2 = 0.56$ for all species, $p <$
143 0.001, n = 174, Fig. 1, Table 2). The average difference between fin and muscle $\delta^{15}N$
144 was 0.0 ± 1.2‰ S.D. ($t_{173} = 0.15$, $p = 0.440$). However, individual species varied in
145 their fin-muscle relationships, with some having significantly higher $\delta^{15}N$ values in
146 fins (spangled perch and *Oxyeleotris* sp.: $t > 6.4$, $p < 0.001$), others having more ^{15}N
147 in muscle (rainbowfish and *Neoarius* sp.: $t < -2.6$, $p < 0.015$) and the remainder
148 having no difference between the tissues (barramundi, *Nematalosa* spp., and longtom:

-1.1 < t < 1.4, p > 0.100) (Table 2). Despite small variation in the fin-muscle relationship, due largely to the limited range in $\delta^{15}N$ tested, regressions of fin vs muscle yielded poor fits for many of the species (e.g. *Oxyeleotris* sp. $\delta^{15}N$ range in muscle < 2‰, r^2 fin vs muscle = 0.08, mean difference = 1.2 ± 0.5‰ S.D., Table 2). Random samples of fin or muscle tissue analysed in duplicate yielded a mean difference of 0.0 ± 0.5‰ S.D. (n = 103, Fig. 1), illustrating that the use of fin in place of muscle resulted in an additional error of approximately 0.7‰ above analytical error.

The change in $\delta^{13}C$ of fin tissue of rainbowfish and spangled perch between the early and late dry seasons was comparable to that observed in muscle (r^2 = 0.94, n = 8, p < 0.001), but shifts in $\delta^{15}N$ were poorly correlated (r^2 = 0.22, n = 8, p = 0.243) (Fig. 2). Within a site, changes of up to 4‰ were observed in both tissues for $\delta^{13}C$, and positive shifts in muscle $\delta^{13}C$ were accompanied by positive shifts in fin $\delta^{13}C$. $\delta^{15}N$ values in both tissues changed very little over the time period (≤ 1‰).

Elemental composition differed between the two tissues (Table 1). While C/N was similar between fin (3.6 ± 0.3 S.D.) and muscle (3.6 ± 0.3 S.D.), %C and %N were lower in fin (%C = 27.4 ± 7.2% S.D., %N = 7.8 ± 2.4% S.D.) compared with muscle (%C = 46.9 ± 3.4% S.D., %N = 13.4 ± 1.1% S.D.). In fin tissue, smaller individuals had lower %C and %N compared to larger individuals (%C: r^2 = 0.23, p < 0.001, %N: r^2 = 0.30, p < 0.001, n = 157, Fig. 3), while muscle %C and %N changed less with increasing body size (%C: r^2 = 0.09, p < 0.001, %N: r^2 = 0.03, p = 0.024, n = 157, Fig. 3).

There was little evidence for size-related changes in the relationship between fin and muscle isotope ratios. Differences between fin and muscle were weakly related to size for all fish ($\delta^{13}C$, r^2 = 0.02, p = 0.100; $\delta^{15}N$, r^2 = 0.03, p = 0.025, n =

157, Fig. 4) and when species were considered independently (only spangled perch $\delta^{13}C$ had a slope significantly different than zero, all others $r^2 < 0.28$, $p > 0.05$). However, larger species such as barramundi and longtom had smaller differences between fin and muscle $\delta^{15}N$ than smaller-bodied species such as spangled perch and rainbowfish (Fig. 4). When categorized into large (>200 mm, n = 65) and small (<200 mm, n = 92) size groups, the absolute difference in $\delta^{15}N$ was significantly greater in small fish (1.0 ± 0.7‰ S.D.) compared to large fish (0.8 ± 0.7‰ S.D.) ($t_{153} = -2.50$, $p = 0.013$), but $\delta^{13}C$ differences between fin and muscle were equal in small and large fish ($t_{153} = 0.79$, $p = 0.375$).

Discussion

Tropical vs. temperate fishes

Fin tissue of tropical fishes was slightly enriched in ^{13}C but highly correlated with muscle $\delta^{13}C$, consistent with earlier studies on temperate species (Kelly *et al*. 2006; Sanderson *et al*. 2009; Hanisch *et al*. 2010). This suggests that fin can be easily substituted for muscle in stable C isotope studies and thereby reduce impacts on fish populations under study. The correction necessary to equate fin with muscle for the tropical species reported here is 0.9‰, which compares well with values observed for temperate species, mainly salmonids, that showed differences between -0.3 and 1.6‰ (McCarthy and Waldron 2000; Jardine *et al*. 2005; Kelly *et al*. 2006; Sanderson *et al*. 2009; Hanisch *et al*. 2010) and with laboratory-reared bass and catfish that had equilibrium values for fin that were 1.3‰ and 1.8‰ higher in fin compared to muscle (Suzuki *et al*. 2005, German and Miles 2010). These values are also lower than the differences in $\delta^{13}C$ between muscle and another non-lethal tissue, fish scales, that differs from muscle by between 2‰ and 3‰ (Kelly *et al*. 2006).

While $\delta^{15}N$ in fin tissue was less well-correlated with that of muscle, the average difference between the tissues was negligible (<0.1‰). Other studies on temperate species have found mean differences that range from -1.2‰ to 0.7‰ (McCarthy and Waldron 2000; Jardine *et al*. 2005; Kelly *et al*. 2006; Sanderson *et al*. 2009; Hanisch *et al*. 2010) and laboratory-reared bass and catfish had equilibrium fin $\delta^{15}N$ values that were 0.2‰ and 1.0‰ higher than muscle (Suzuki *et al*. 2005, German and Miles 2010). Given the lack of difference in fin and muscle $\delta^{15}N$ for the tropical species reported here, we recommend that $\delta^{15}N$ data derived from fin be used in place of muscle without a correction factor but with the caveat that some error (approximately 0.7‰) is incorporated into the use of fin tissue in this manner.

The variability in the difference in $\delta^{15}N$ between fin and muscle appears to decline with increasing body size, providing a rationale for using non-lethal fin sampling on larger fish. Large fish tend to be older and less abundant, making non-lethal sampling more desirable. One consideration for those wishing to substitute fin for muscle in studies with $\delta^{15}N$ is that the typical natural variation in $\delta^{15}N$ in aquatic food webs is much smaller than that of $\delta^{13}C$ (approximately 15‰ for the former versus 30‰ for the latter, Fig. 1). Although the "noise" is similar for both elements (1 standard deviation was 1.1‰ for C and 1.2‰ for N), the "signal" is far greater for $\delta^{13}C$. Therefore, the error in the fin-muscle relationship for N will likely have larger consequences in the interpretation of data from fin tissue in food web studies on fish. This degree of error (0.6‰ to 0.7‰) introduced by using fin tissue is, however, small relative to other sources of variation in isotopic food web studies (Jardine *et al*. 2006), such as baseline $\delta^{15}N$ variation (range = 12‰ across sites, Cabana and Rasmussen 1996) and the variation of trophic fractionation reported in studies that compiled literature data (e.g. 3.4 ± 1.0‰ S.D., range = 6‰, Post 2002; 2.0 ± 1.8‰ S.D., range

= 7‰, McCutchan *et al*. 2003; 2.5 ± 1.3‰ S.D., range = 9‰, Vanderklift and Ponsard 2003). Responsible users will therefore avoid using fins to detect extremely subtle differences in $\delta^{15}N$ (e.g. within trophic levels) or ensure that sample sizes are adequate to account for the additional variation imposed when fins are used in place of muscle.

Seasonal changes

The similarity in seasonal changes between muscle and fin $\delta^{13}C$ suggests that turnover rates of the two tissues may be comparable. Earlier work found that half-lives of fin and muscle were equivalent at 19 to 26 days for $\delta^{13}C$ and $\delta^{15}N$ in rapidly growing Japanese temperate bass (*Lateolabrax japonicus*) (Suzuki *et al*. 2005). However, recent work in slow-growing blue cod (*Parapercis colias*) showed turnover in fin tissue that was slower than whole blood and blood fractions (plasma and cellular); the latter tissues had half-lives ranging from 205 to 359 days (Suring and Wing 2009). These observations suggest that in slow-growing adult fish with low food consumption rates, turnover will be slow and differ among tissues, while fast-growing juvenile and sub-adult fish will have rapid turnover rates that are driven largely by growth and differ little among tissues. For example, the bass measured by Suzuki *et al*. (2005) were approximately ten times larger at the end of the 100-day experiment, whereas the cod in Suring and Wing (2009) had only gained approximately 20% mass after more than 150 days of experimentation. Fish with this latter type of growth trajectory will therefore have tissues that reflect the last year or more of feeding (Hesslein *et al*. 1993) rather than the days to weeks observed for rapidly growing juvenile fish (Herzka and Holt 2000). We can therefore rule out differences in turnover rate as the likely cause of the poor agreement between tissues

249 in some species, as the largest fish that were most likely to show differences based on
250 turnover rates actually had most similar values in the current study.
251 Other non-lethal tissues may have similar steady-state diet-tissue fractionation
252 as muscle but their turnover rates differ. For example, mucus harvested from trout
253 (*Oncorhynchus mykiss*) differed from muscle by only 1.5‰ for $\delta^{15}N$ and 0.5‰ for
254 $\delta^{13}C$, but half-lives were far shorter in mucus (27 to 42 days) compared with muscle
255 (76 to 193 days) (Church *et al*. 2009), meaning that rather than being interchangeable,
256 these tissues are complementary (i.e. they can provide simultaneous measures of diet
257 on different time scales). Blood plasma had similar $\delta^{13}C$ and $\delta^{15}N$ to muscle
258 (differences < 0.7‰, German and Miles 2010) but plasma turnover is known to occur
259 much more rapidly than muscle (Dalerum and Angerbjorn 2005). Blood cells,
260 however, also have similar steady-state values to muscle (German and Miles 2010)
261 and are more likely to have equivalent turnover rates making them suitable surrogates.
262 Likewise, muscle biopsies (Baker *et al*. 2004) clearly can provide the tissue needed
263 for isotope work and avoid any error introduced by using alternative tissues.
264 However, the likelihood of infection associated with a biopsy is likely to be much
265 higher than removal of fin tissue because the latter requires no actual skin penetration.
266
267 Methodological considerations
268 The amount of tissue required to obtain isotope data from a fin is relatively
269 minimal, with a 5 mm x 5 mm aliquot (approximately the cross-sectional area of a
270 pencil) being more than sufficient to provide enough C and N for analysis (T. Jardine,
271 pers. obs.). However, given the low carbon and nitrogen composition of this tissue,
272 larger amounts than those used for muscle are needed at the point of enclosing the
273 dried tissue into tin capsules for mass spectrometry. Despite similar C/N, fin contains

roughly half the carbon and nitrogen of muscle (Table 1), so an approximate doubling of weight is recommended to ensure that target gas peaks are within the working range of the mass spectrometer. Most mass spectrometers require between 0.2 and 1 milligrams of animal tissue, so the needed weight for fin tissue would range between 0.4 and 2 milligrams, corresponding to approximately 2 to 10 milligrams wet weight (conservatively assuming 80% moisture). The lower %C and %N in fin tissue likely reflects a higher proportion of bone in fin tissue, particularly in smaller fish. Besides carbonate, bone is composed largely of hydroxylapatite that contains oxygen, hydrogen, calcium and phosphorus but no carbon or nitrogen, thus yielding lower percentages of C and N in tissues with a large proportion of bone. Furthermore, the majority of carbon in bony structures of fish (e.g. otoliths) is derived from dissolved inorganic carbon (DIC) of surrounding water rather than from food (Solomon *et al*. 2006); this DIC is enriched in ^{13}C relative to available food sources (McConnaughey *et al*. 1997). Thus, a higher proportion of bone in fin, relative to muscle, would explain the slightly higher $\delta^{13}C$ for the former tissue, as the contribution from isotopically light DIC in bone leads to enrichment in ^{13}C relative to other tissues (e.g. Dube *et al*. 2005). Acid-washing samples to remove carbonates could therefore reduce the difference in $\delta^{13}C$ between fin tissue and muscle tissue, but this would require further testing to ensure there are no effects on $\delta^{15}N$ (Bunn *et al*. 1995).

While we did not systematically test for differences among the two fin types analysed here (caudal and anal), there is little reason to suspect that the choice of fin should impact results. The greater consideration will be around the effects of fin clipping on behaviour and survival of fish and the mass required for analysis (Sanderson *et al*. 2009). While fin clipping procedures may cause an immediate physiological stress response in fish due to handling, recent longer-term studies on

299 numerous fish species have detected no significant impact on key aspects of fish
300 biology and behaviour, including growth, survival, movement and sexual maturation,
301 as a consequence of fin clipping (e.g. Pratt and Fox 2002; Vander Haegen *et al*. 2005;
302 Champagne *et al*. 2008). Fin regeneration is often observed following clipping, but
303 fins that are completely removed may not regrow (Thompson and Blankenship 1997).
304 It is therefore recommended that if possible, partial fin clips be taken, distal to the
305 body, to promote fin regeneration and minimise possible infection. Since an estimated
306 minimum fin sample of 25 mm^2 is required for C and N analysis, fin size will limit the
307 application of fin clips as an alternative to muscle for smaller individuals and fish
308 species. In this study, fish with fork lengths greater than 200 mm yielded partial fin
309 clips of a size suitable for analysis that were removed with no obvious effect on the
310 fish (T. Jardine, pers. obs.). Sanderson *et al*. (2009) found that fish as small as 50 mm
311 fork length yielded caudal fin clips of sufficient size for analysis. Smaller fishes often
312 require the complete removal of a fin (e.g. adipose) to provide adequate tissue for
313 analysis. However, laboratory techniques are available to increase the sensitivity of
314 mass spectrometers (e.g. Hanson and Sommer 2007), allowing smaller masses to be
315 analysed with acceptable accuracy and precision; this could generate reliable data for
316 fin clips smaller than 25 mm^2 in the instance of a small-bodied but rare or endangered
317 fish (e.g. some species of rainbowfish *Melanotaenia* spp.).
318 The substitution of fin tissue in place of muscle in stable isotope studies will
319 undoubtedly reduce the impact of sampling on tropical aquatic systems. In a recent
320 large-scale food web study, approximately 500 large-bodied fishes including the
321 threatened freshwater sawfish *Pristis microdon,* were sampled non-lethally from two
322 tropical Australian river systems using the approach described here (T. Jardine,
323 unpublished data). The strong correlations of fin with muscle for $\delta^{13}C$ and, to a lesser

extent, $\delta^{15}N$ were derived from a low sample size (10-45 individuals) and from spatially distinct sample sites (up to 400 km distant), for each species, and demonstrates that high statistical confidence of fin as a predictor of muscle $\delta^{13}C$ and $\delta^{15}N$ for fish species and assemblages may be achieved from low levels of non-localised destructive sampling. In the interest of conservation, clearly there must be a move away from destructive sampling as much as possible, except in those circumstances when other important biological information is being acquired (e.g. proximate body composition, examination of internal organs). By minimising lethal techniques, researchers can avoid having undesired effects on the system, particularly in those instances where the same locations are being sampled repeatedly and possible population level consequences may result from removal of individuals for research purposes.

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Table 1 Mean (1 S.D.) fork length for fish species from floodplain rivers in northern Australia, and elemental composition (percent carbon and nitrogen and C/N) in fin tissue versus muscle. Collection dates are wet (W, February 2009 and 2010) and dry (D, May/June and October 2008).

Species Name	Common name	Collection	n	Fork length	%C fin	%N fin	C/N fin	%C muscle	%N muscle	C/N muscle
Amniataba percoides	Barred grunter	D	1	42	27.0	7.6	3.6	40.9	10.4	3.9
Anoxypristis cuspidatus	Pointed sawfish	W	1	850	46.8	15.3	3.1	41.7	14.7	2.8
Carcharhinus leucas	Bull shark	W	1	1160	27.6	8.7	3.5	39.1	13.8	3.2
Eleutheronema tetradactylum	Threadfin salmon	W	3	406 (24)	28.9 (4.3)	8.2 (1.2)	3.5 (0.0)	44.0 (1.7)	13.6 (0.4)	3.2 (0.0)
Glossamia aprion	Mouth almighty	D	2	N/A	17.0, 20.5	5.1, 5.6	3.3, 3.7	48.5, 49.2	15.3, 15.1	3.2, 3.3
Hephaestus carbo	Coal grunter	D	1	182	39.2	11.0	3.6	46.7	10.9	4.3
Hephaestus fuliginosus	Sooty grunter	D	1	52	25.2	6.7	3.7	45.0	13.4	3.3
Lates calcarifer	Barramundi	D,W	18	413 (94)	37.1 (4.7)	10.6 (1.1)	3.5 (0.1)	46.7 (3.0)	13.7 (0.6)	3.4 (0.2)
Leiopotherapon unicolor	Spangled perch	D	37	81 (26)	27.7 (4.2)	7.8 (1.1)	3.6 (0.2)	47.6 (3.4)	13.1 (0.9)	3.6 (0.2)
Megalops cyprinoides	Tarpon	D	1	206	22.4	6.4	3.5	41.3	12.5	3.3
Melanotaenia splendida	Rainbowfish	D	45	65 (16)	20.5 (3.1)	5.3 (0.7)	3.9 (0.2)	48.7 (6.8)	13.5 (2.2)	3.6 (0.3)
Nematalosa come	Gizzard shad	W	8	239 (56)	27.8 (3.9)	8.3 (1.3)	3.4 (0.2)	45.8 (1.8)	13.0 (0.8)	3.5 (0.4)
Nematalosa erebi	Bony bream	D	11	148 (57)	24.7 (3.8)	7.0 (0.9)	3.5 (0.2)	48.3 (2.9)	13.5 (1.0)	3.6 (0.2)
Neoarius graeffei	Blue catfish	W	9	248 (44)	40.8 (2.5)	12.2 (1.1)	3.3 (0.2)	46.2 (2.2)	13.5 (0.8)	3.4 (0.1)
Neoarius paucus	Shovelnose catfish	W	1	316	43.6	12.4	3.5	44.0	13.6	3.2
Neoarius sp.	Fork-tailed catfish	D	1	185	29.4	8.1	3.6	45.7	14.0	3.3
Nibea squamosa	Drum	W	1	260	28.6	8.2	3.5	42.3	13.1	3.2
Oxyeleotris lineolatus	Sleepy cod	D	8	202 (20)	29.1 (5.1)	8.2 (1.3)	3.5 (0.2)	46.8 (2.3)	14.3 (0.4)	3.3 (0.1)

Oxyeleotris selheimi	Giant gudgeon	D	4	217 (30)	26.0 (6.2)	7.8 (2.2)	3.3 (0.2)	48.0 (3.7)	14.2 (1.0)	3.4 (0.2)
Polydactylus sheridani	Threadfin salmon	W	1	540	31.8	9.7	3.3	45.0	14.1	3.2
Pomadasys argenteus	Grunter	W	1	490	32.5	12.6	2.6	40.4	12.1	3.3
Pristis zijsron	Longcomb sawfish	W	1	1580	33.7	11.0	3.1	41.7	13.8	3.0
Scleropages jardinii	Saratoga	D	1	219	33.6	12.3	2.7	45.2	14.1	3.2
Scomberoides commersonnianus	Queenfish	W	2	500, 550	23.8, 32.5	6.9, 10.2	3.5, 3.2	44.5, 42.1	13.5, 13.3	3.3, 3.2
Scortum ogilbyi	Gulf grunter	D	2	159, 193	30.5, 23.0	9.4, 7.2	3.2, 3.2	44.9, 48.9	11.4, 12.0	3.9, 4.1
Strongylura krefftii	Longtom	D,W	11	286 (148)	25.6 (5.0)	7.4 (1.6)	3.5 (0.2)	47.0 (2.0)	13.9 (0.7)	3.4 (0.2)
Family Carangidae	Skippy	W	1	275	26.7	8.0	3.4	47.3	14.7	3.2

Table 2 Best-fit regressions of $\delta^{13}C$ and $\delta^{15}N$ in fin tissue versus muscle tissue for six common fish species from floodplain rivers in northern Australia. "All fishes" represents all 27 species pooled.

Taxon	n	Best-fit Equation	r^2	p	Mean (fin - muscle)
Lates calcarifer	18	Muscle $\delta^{13}C$ = 0.67 * Fin $\delta^{13}C$ - 7.40	0.59	<0.001	0.5 ± 0.6
Leiopotherapon unicolor	37	Muscle $\delta^{13}C$ = 0.79 * Fin $\delta^{13}C$ - 5.31	0.83	<0.001	0.5 ± 1.2
Melanotaenia splendida	45	Muscle $\delta^{13}C$ = 0.90 * Fin $\delta^{13}C$ - 3.28	0.78	<0.001	0.9 ± 0.9
Nematalosa spp.	19	Muscle $\delta^{13}C$ = 0.98 * Fin $\delta^{13}C$ - 0.93	0.92	<0.001	0.4 ± 1.3
Neoarius spp.	11	Muscle $\delta^{13}C$ = 0.54 * Fin $\delta^{13}C$ - 10.30	0.66	0.002	1.5 ± 0.8
Oxyeleotris spp.	12	Muscle $\delta^{13}C$ = 0.84 * Fin $\delta^{13}C$ - 4.50	0.90	<0.001	1.3 ± 0.8
Strongylura krefftii	11	Muscle $\delta^{13}C$ = 0.88 * Fin $\delta^{13}C$ - 3.99	0.96	<0.001	1.1 ± 1.0
All fish	174	Muscle $\delta^{13}C$ = 0.89 * Fin $\delta^{13}C$ - 3.27	0.91	<0.001	0.9 ± 1.0

Taxon	n	Best-fit Equation	r^2	p	Mean (fin - muscle)
Lates calcarifer	18	Muscle $\delta^{15}N$ = 0.83 * Fin $\delta^{15}N$ + 1.71	0.40	0.005	0.0 ± 0.5
Leiopotherapon unicolor	37	Muscle $\delta^{15}N$ = 0.71 * Fin $\delta^{15}N$ + 1.70	0.65	<0.001	1.1 ± 1.0
Melanotaenia splendida	45	Muscle $\delta^{15}N$ = 0.49 * Fin $\delta^{15}N$ + 5.05	0.21	0.001	-0.7 ± 1.3
Nematalosa spp.	19	Muscle $\delta^{15}N$ = 1.06 * Fin $\delta^{15}N$ - 0.29	0.82	<0.001	-0.2 ± 0.8
Neoarius spp.	11	Muscle $\delta^{15}N$ = 0.27 * Fin $\delta^{15}N$ + 7.81	0.20	0.172	-0.5 ± 0.6
Oxyeleotris spp.	12	Muscle $\delta^{15}N$ = 0.36 * Fin $\delta^{15}N$ + 4.91	0.10	0.317	1.2 ± 0.5
Strongylura krefftii	11	Muscle $\delta^{15}N$ = 0.63 * Fin $\delta^{15}N$ + 4.18	0.56	0.008	-0.3 ± 0.8
All fish	174	Muscle $\delta^{15}N$ = 0.81 * Fin $\delta^{15}N$ + 1.73	0.56	<0.001	0.0 ± 1.2

Figure captions

Figure 1. Fin and muscle $\delta^{13}C$ (A) and $\delta^{15}N$ (B) for tropical fishes from the Mitchell River, North Queensland, Australia (open circles). To illustrate analytical error, 'x' = random muscle samples from a larger dataset analysed in duplicate, and '+' = random fin samples analysed in duplicate.

Figure 2. Seasonal change in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) in fin and muscle tissue from two common species (rainbowfish – open squares, and spangled perch – shaded triangles) sampled at the beginning and end of the annual dry season in the Mitchell River, North Queensland, Australia.

Figure 3. Fish body size versus percent carbon (%C, A) and nitrogen (%N, B) for fin (open diamonds, hatched line) and muscle (solid circles, solid line) of tropical fishes from the Mitchell River, North Queensland, Australia.

Figure 4. Fish body size versus the difference in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) between fin and muscle for barramundi (solid circles), longtom (open circles), *Oxyeleotris* sp. (shaded squares), rainbowfish (open squares), spangled perch (shaded triangles), *Nematalosa* sp. (solid diamonds), *Neoarius* sp. (open triangles) and other tropical fishes (plus signs) from the Mitchell River, North Queensland, Australia.