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Original Article

Caudal fin as a proxy for dorsal muscle for nutrient enrichment monitoring using stable isotope analysis: the case of *Gerres filamentosus* and *G. oyena* from mangrove creeks of Tanzania

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Abstract
The use of fish dorsal muscles in stable isotope studies, which is an invasive method that results in fish deaths, limits their applicability for rare and endangered fish species, as well as when large sample sizes and replicates are required, prompting research into feasible non-lethal sampling methods. The possibility of employing fin clippings (a non-invasive approach) was investigated as a proxy for dorsal muscle in nutrient pollution monitoring studies using two common mangrove fish species, namely *Gerres filamentosus* and *G. oyena*, which are known to spend their early life stages primarily within mangroves. The dorsal muscles and caudal fin tissues of fish from the mangrove creeks of Kunduchi and Mbegani, Tanzania, were examined for ^{13}C and ^{15}N signatures. Dorsal muscles from Kunduchi (mean SD: ^{13}C = -16.8 ± 2.86, ^{15}N = 9.34 ± 1.15) were more enriched than from Mbegani (mean SD: ^{13}C = -18.60 ± 2.11, ^{15}N = 7.27 ± 1.09), and this enrichment was consistent across the two studied species. Caudal fins indicated similar enrichment trends. Fin tissue stable isotope values explained between 62% and 87% of dorsal muscle ^{13}C and between 89% and 98% of dorsal muscle ^{15}N variability. These findings support the use of fin-clipping as a non-lethal proxy for stable isotope analysis of the studied species for nutrient enrichment, and additional research into non-lethal sampling methods is recommended.

Keywords: mangrove fish, coastal pollution, western Indian Ocean, fin clipping, stable isotopes, non-lethal sampling.

Introduction
Mangroves provide critical habitats for a diverse range of fish species, the majority of which are commercially valuable. They are also important as nurseries for many coral reef fishes (Bradley *et al.*, 2019; Igulu *et al.*, 2014; Kimirei *et al.*, 2016; Lugendo *et al.*, 2006; Lugendo *et al.*, 2007; Nagelkerken *et al.*, 2008), contributing significantly to artisanal coral reef fisheries when assessed at the species level (Kimirei *et al.*, 2013; Nakamura *et al.*, 2008). Nonetheless, mangroves are disappearing globally owing to both natural and human causes (Alongi, 2014; Duke *et al.*, 2007; FAO, 2007), threatening their ability to provide ecosystem goods and services (Abrantes *et al.*, 2019; Guannel *et al.*, 2016; Kimirei *et al.*, 2016).

Most Indo-Pacific mangrove habitats have large tidal ranges, which affect tidal movement of large amounts of seawater and fish between neighbouring habitats, reducing their value as nursery, shelter, and feeding habitats (Faunce and Layman, 2009; Nagelkerken, 2009; Nagelkerken and Velde, 2004) and fisheries (Blaber, 2009). Mangroves may potentially become functionally extinct as a result of degradation, with unexpected consequences for the viability of coastal artisanal fishing, which is a lifeline for many coastal inhabitants (Kimirei *et al.*, 2016; Staehr *et al.*, 2018).

Coastal pollution and eutrophication induced by land-based point and non-point sources are just two of the many threats to mangroves and associated habitats (seagrass beds and coral reefs), particularly on urbanised coasts (Boesch, 2019; Oczkowski *et al.*, 2014; Vikas and Dwarakish, 2015; Xiao *et al.*, 2017). Nutrient input into mangroves and estuaries, as well as other forms of chemical pollution are threatening the mangrove ecosystems and surrounding ecosystems (Asmala *et al.*, 2019; Staehr *et al.*, 2017; Staehr *et al.*, 2018). For example, coastal eutrophication can cause proliferation of harmful algal blooms (HAB) and deoxygenation of coastal waters (Breitburg *et al.*, 2018; Oczkowski *et al.*, 2014), which can lead to the deterioration of ecosystem integrity, loss of critical habitats (coral reefs and seagrasses), and changes in ecological structure (Howarth *et al.*, 2011). Domestic, industrial and agricultural effluents, as well as wastes from aquaculture operations, are example of anthropogenic nitrogen and phosphorus contamination (Lovelock *et al.*, 2009). While mangroves are known to filter nutrients and other forms of pollution, protecting adjacent ecosystems from pollution, excessive pumping of nutrients and pollutants into these wetlands may reach a tipping point, causing die-offs and a critical decimation of their protective and provisioning capacities/services (Selkoe *et al.*, 2015; Serrao-Neumann *et al.*, 2016; Watson *et al.*, 2018). As a result, monitoring nutrient pollution and accumulation, as well as other types of mangrove disturbances, is crucial.

Nutrient analysis and long-term monitoring programs can be used to monitor coastal pollution. Traditional approaches, particularly spectrophotometric analysis, have long been used to assess and monitor nutrient inputs into aquatic ecosystems (Parsons *et al.*, 1984). While this is feasible and can easily document long-term changes in nutrients inputs and accumulation in coastal waters and ecosystems, it may be costly and unsustainable, particularly for resource-poor countries (as it may require regular sample collection), where investment in monitoring programmes may not be a priority. In addition, this technique only reveals the present condition, with the possibility of missing nutrient input events that occurred weeks or months earlier (Gearing, 1991), thus needing regular monitoring. As an alternative, stable isotope analysis may be used in studying nutrient pollution to infer enrichment patterns from a few samples that are relatively easy and cheap to replicate over time (Carmichael *et al.*, 2004; Teichberg *et al.*, 2010).

Although other organisms such as plants, sediment and water samples are also used (Cole *et al.*, 2004; Costanzo *et al.*, 2001; Costanzo *et al.*, 2005; Gritcan *et al.*, 2016; Lugendo and Kimirei, 2021; Savage, 2005), fish and shellfish tissues have been routinely used to examine nitrogen enrichment in coastal waters. Carbon stable isotopes have also been investigated recently to serve the same purpose as nitrogen isotopes (Oczkowski *et al.*, 2020).

Despite the widespread use of fish in ecological studies using the stable isotopes approach, the use of dorsal muscles extracted from fish - an invasive method that results in the death of the specimens, limits their applicability in species of concern such as rare and endangered species, or when large sample sizes and replicates are required. This has prompted research into plausible non-lethal sampling methods that may be used as a proxy for dorsal muscle, with various studies selecting fish fins, fish scales, mucous, liver, plasma, and red blood cells as candidates (Boardman *et al.*, 2022; Church *et al.*, 2009; Hayden *et al.*, 2015; Hayden *et al.*, 2017; Matley *et al.*, 2016; McIntosh and Reid, 2021; Tronquart *et al.*, 2012).

The overarching objective of the current study was to investigate the possibility of using fin clippings as a non-invasive method and a proxy for dorsal muscle in nitrogen pollution monitoring studies in coastal habitats of Tanzania, and the western Indian Ocean (WIO) region in general. This was achieved by comparing the levels of nitrogen and carbon stable isotopes in dorsal muscle and caudal fin tissues of two common mangrove fish species (*Gerres filamentosus* and *G. oyena*). Furthermore, the purpose of this study was to compare the nitrogen enrichment levels of fishes collected from polluted (Kunduchi, Dar es Salaam) and relatively pristine (Mbegani, Bagamoyo) mangrove habitats in Tanzania, and to determine whether or not the nitrogen enrichment in the two species is consistent across the two mangrove areas. There is a dearth of such research in the WIO, but it is particularly lacking in Tanzania.

Materials and methods

Study area

This study was carried out in Tanzanian coastal waters in two mangrove-lined creeks, namely Kunduchi and Mbegani. Kunduchi is located along the coast of Dar es Salaam, about 20 km away from the Dar es Salaam City Centre, and prone to more pollution than Mbegani, which is located along the Bagamoyo coast, about 50 km away from the Dar es Salaam City (Fig. 1). Several species of mangroves occur at Kunduchi with *Avicennia marina*, *Ceriops tagal* and *Rhizophora mucronata* dominating. On the other hand, Mbegani consists of a strip of mangroves (approximately 420 m wide) of mainly *Sonneratia alba*, mixed with *R. mucronata*, *A. marina*, and *Bruguiera gymnorhiza* (Kimirei *et al.*, 2011). Both sites are characterised by large tidal creeks, which never empty completely, even during low spring tides. At the landward side of Mbegani creek, there is a stream (Nyanza River), a potential source of freshwater leading into the mangrove forest during the rainy season. Data were collected here during the dry season.

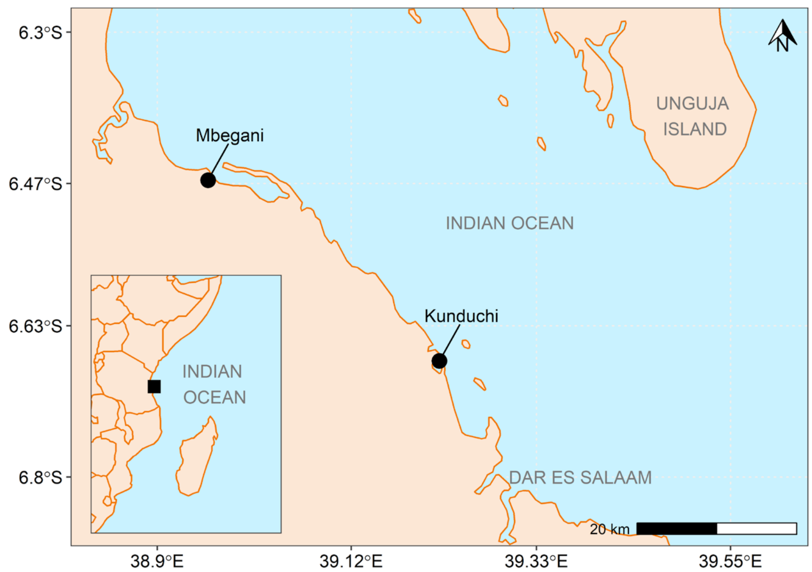

Figure 1. Map showing the study sites.

Sampling design

A total of 425 individual fish were collected using a seine net from the Kunduchi and Mbegani mangrove creeks, during low tide between January and December 2009. In the field, the fish samples were put in a cool box and later frozen at -20 °C pending sorting into species, preparation and analysis.

Sample preparation and stable isotope analysis

The collected fishes were sorted into species, and a total of 23 (6 *Gerres filamentosus* and 17 *G. oyena*) from Mbegani and 24 (4 *G. filamentosus* and 20 *G. oyena*) from Kunduchi were selected. The two species were selected primarily based on their life histories and their presence in the catches from both sites. The two species are known to spend their early lives primarily within mangroves (Mwandya *et al.*, 2009). The size (Total Length) of *G. filamentosus* and *G. oyena* from the two sites ranged between 4.6 and 10 cm, which represents juvenile stages of the two species, respectively, the stage in which they reside in mangroves (Mwandya *et al.*, 2009). A total of 94 tissue samples (47 dorsal muscle tissues and 47 caudal fin tissues) were collected from the two species. Samples were then dried at 70°C for 48 h and ground to a homogeneous powdery mixture. A pre-determined sample of known weight was placed in ultra-pure tin capsules and combusted in a CHN Elemental Analyser from Carlo Erba® (Thermo group), interfaced with a continuous flow isotope ratio mass-spectrometer, the DeltaPlus from Thermo Finnigan, Bremen, Germany, and the stable carbon and nitrogen isotopes of the selected fishes were measured. The reference gasses were calibrated with the International Atomic Energy Agency (IAEA) reference standards, IAEA-N-2 and IAEA-CH-6.

Data analysis

The data ($\delta^{13}C$ and $\delta^{15}N$ values) of fish tissues between sites, species and tissues were tested for normality

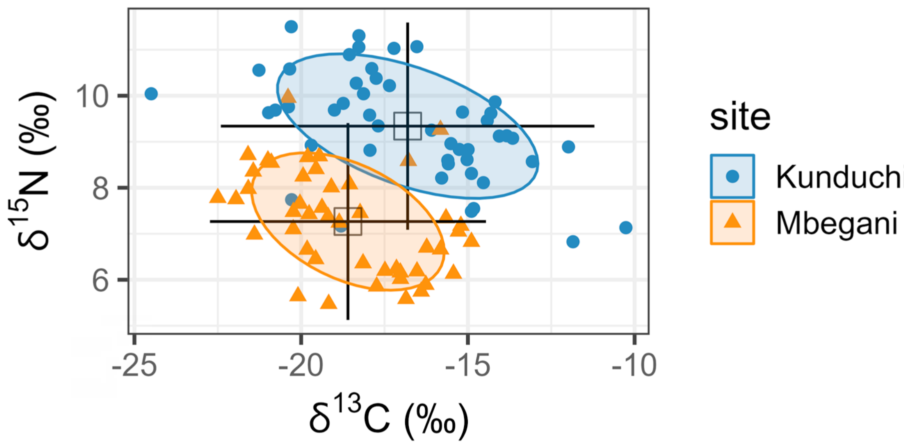

Figure 2. Stable carbon and nitrogen isotope enrichment signatures of fishes collected from Kunduchi (n=48) and Mbegani (n=46). The data for each site include both species and tissue types. Eclipses show the direction of mean values.

using the Shapiro – Wilk test. Measures of central tendencies and descriptive statistics (mean, media, standard deviations, and standard error of mean) were computed and compared across sites, species, and tissues. To test for differences between sites, the carbon and nitrogen stable isotopes data of muscle and fin tissues were pooled across fish species and sites. A non-parametric Wilcoxon Rank Sum test was used to test whether the median of the stable carbon and nitrogen isotope of the combined fish species differed between sites. A two-way ANOVA with interaction was used to test if the enrichment in stable carbon and nitrogen isotopes of *Gerres filamentosus* and *G. oyena* were consistent between the two sites. Comparisons of stable isotopes between tissues for species and sites were performed using the independent samples t-tests on robust location measures (Yuen t-test) for trimmed means (Yuen, 1974). The Yuen test was chosen rather than the student t-test because it is a robust parametric test for the data that violates normal distribution and equal variance rules. Finally, correlation analysis was used to compare stable isotope data of dorsal muscle and fin tissues to elucidate whether fin clips can be used as a proxy for dorsal muscle of the two species for monitoring nutrient pollution in coastal waters of Tanzania. All analyses and plotting were carried out in R programming language (R Core Team, 2020).

Results

Comparison of stable isotopes (δ15N and δ13C) of fishes between sites

The mean stable isotope signatures of the pooled data of both fish species from Kunduchi were more enriched with mean (±SD) δ^{13}C and δ^{15}N values of -16.81 ± -2.86‰ and 9.34 ± 1.15‰, respectively (Fig. 2), than those from Mbegani, which were appreciably depleted both for δ^{13}C (-18.60 ± 2.11) and δ^{15}N (7.27 ± 1.09) (Fig. 2). The stable isotope signatures formed

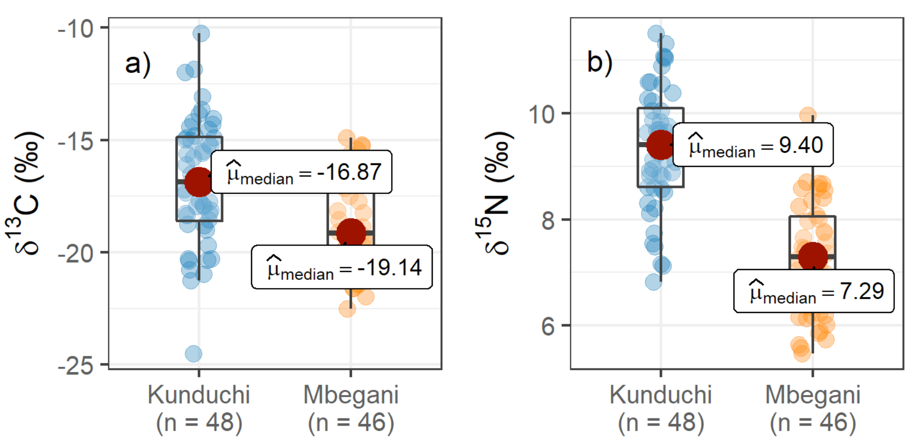

Figure 3. Comparison of a) δ^{13}C and b) δ^{15}N stable isotope signatures of fishes between Kunduchi and Mbegani mangroves.

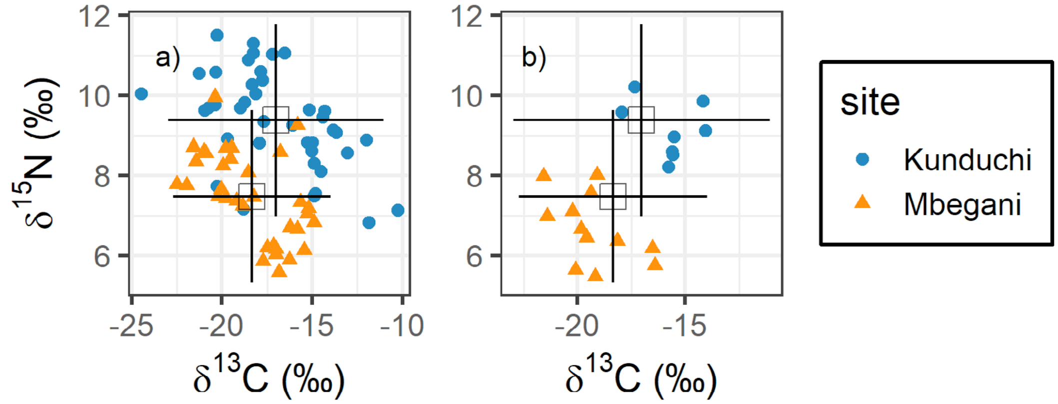

Figure 4. Comparison of stable isotope variation in tissues of a) *G. oyena* (n=74) and b) *G. filamentosus* (n=20) at Kunduchi and Mbegani.

distinct groups as an indication of enrichment status (Fig. 2). The median $\delta^{13}C$ signature was -16.87‰ at Kunduchi while it was -19.14‰ at Mbegani (Wilcoxon rank sum test, W = 1544, $p < 0.05$, Fig. 3a). Similarly, Kunduchi had more enriched $\delta^{15}N$ values than at Mbegani, where the median value of nitrogen isotope was 9.40‰ at Kunduchi and 9.29‰ at Mbegani (W = 1988.5, $p < 0.05$, Fig. 3b).

Comparison between species

The $\delta^{15}N$ and $\delta^{13}C$ signatures of *G. oyena* and *G. filamentosus* were compared to test for the stability of stable isotope enrichment pattern between species without taking tissue types into account. There were no significant differences in stable isotope enrichment between species ($F_{(1,183)}$ = 2.61; p = 0.108) indicating a consistent enrichment pattern between them. However, the $\delta^{15}N$ and $\delta^{13}C$ signatures of the two species were consistently more enriched at Kunduchi than at Mbegani ($F_{(1,183)}$ = 47.60; $p < 0.001$; Fig. 4), indicating that the observed pattern is stable across species and sites. The mean (±SD) of $\delta^{15}N$ and $\delta^{13}C$ signatures for *G. oyena* at Kunduchi were 9.38 ± 1.22 and -17.02 ± 3.04, respectively, while at Mbegani they were 7.47 ± 1.10 and -18.35 ± 2.23, respectively. For *G. filamentosus*, the mean (±SD) of $\delta^{15}N$ and $\delta^{13}C$ signatures for Kunduchi were 9.13 ± 0.70 and -15.75 ± 1.35, respectively, and 6.68 ± 0.87 and -19.29 ± 1.62, respectively, for Mbegani (Fig. 4).

Comparison between muscle and fin tissue

The carbon stable isotope values of muscle tissue (-16.2 ± 2.4‰) were significantly more enriched than those of fin tissue (-16.9 ± 2.1‰) (Yuen t-test, $t_{(65.82)}$ = 2.246, p = 0.028) when samples were pooled for species and sites. The nitrogen stable isotope values, although enriched in muscle tissue (9.56 ± 1.9‰), were barely different from the fin tissue values (8.8 ± 1.9‰) (Yuen t-test, $t_{(65.11)}$ = 1.946, p = 0.056). On an individual species level, the $\delta^{13}C$ and $\delta^{15}N$ values were generally more enriched in muscle than fin tissue for all species (Fig. 5). However, the difference were significant in carbon isotope (Yuen t-test, $t_{(43.23)}$ = 2.452, p = 0.018)

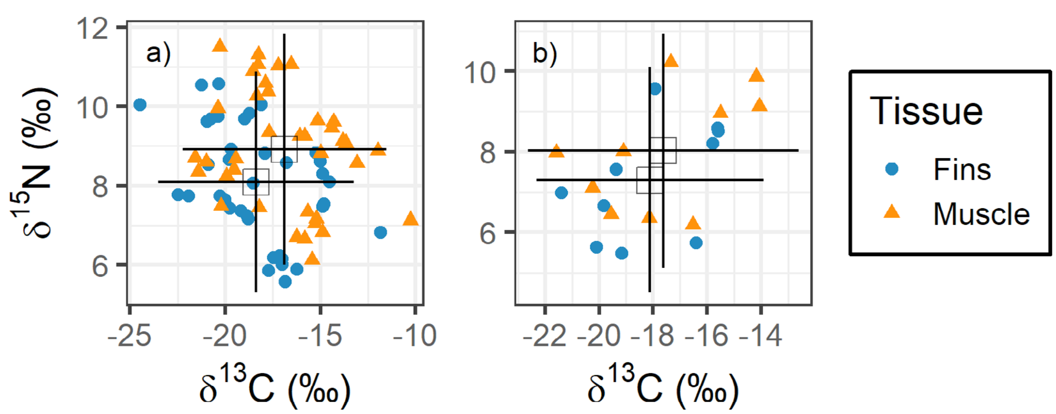

Figure 5. The $\delta^{13}C$ and $\delta^{15}N$ signatures in dorsal muscle and caudal fin tissues of fish species for a) *G. oyena* (n=37 for fin tissues and 37 for muscle tissues) and b) *G. filamentosus* (n=10 for fin tissues and 10 for muscle tissues).

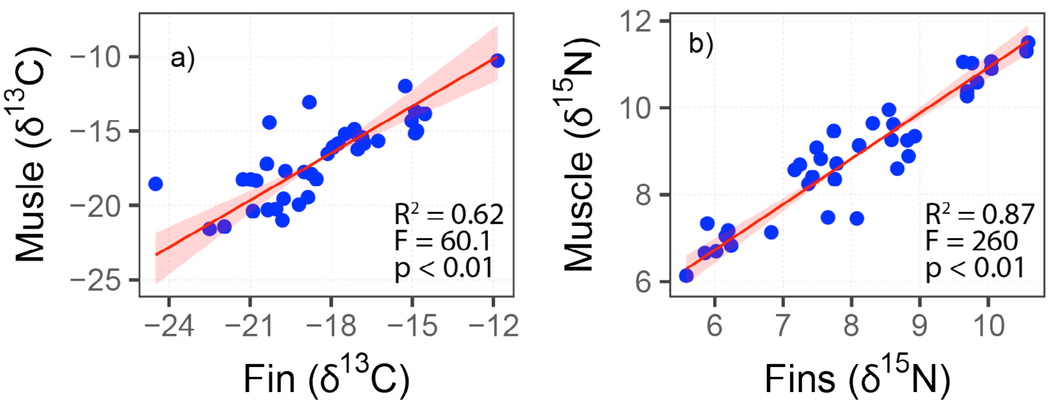

Figure 6. Correlation of a) $\delta^{13}C$ and b) $\delta^{15}N$ signatures between dorsal muscle and caudal fin tissues of *G. oyena* combined for all sites (n=37).

but not for nitrogen ($t_{(43.841)}$ = 1.880, p = 0.067; Fig. 5a). There was no significant difference in values of carbon ($t_{(17.46)}$ = -0.467, p = 0.646) and nitrogen ($t_{(17.97)}$ = -1.119, p = 0.278) stable isotopes between muscle and fin tissues for *G. filamentosus* (Fig. 5b).

Fin-clip as a proxy of muscle δ13C and δ15N

The stable carbon and nitrogen isotopes of *G. oyena* (Fig. 6) and *G. filamentosus* (Fig. 7), combined for both sites, were significantly correlated between dorsal muscle and caudal fin tissues. While $\delta^{13}C$ values of fin tissue explained 62% of stable isotope variations in dorsal muscle tissue of *G. oyena*, $\delta^{15}N$ values explained 87% of the variations (Fig. 6). For *G. filamentosus*, $\delta^{13}C$ values of fin tissue explained 89% of the variations and 98% of variation in $\delta^{15}N$ in the dorsal muscle values (Fig. 7).

Discussion

This study found that stable nitrogen isotope ($\delta^{15}N$) ratios were more enriched in fish samples caught in the Kunduchi mangroves as compared to those collected in Mbegani. While the nitrogen isotope ratio was 10‰ at Kunduchi, and 7‰ at Mbegani, the $\delta^{15}N$ value at Kunduchi was a magnitude higher than the value of 9‰ measured at the same location in 2005 (Kruitwagen *et al.*, 2006). The high $\delta^{15}N$ values at Kunduchi indicate signs of nutrients enrichment (Kruitwagen *et al.*, 2006; Lugendo *et al.*, 2007; McClelland *et al.*, 1997; Samper-Villarreal *et al.*, 2018). Similar $\delta^{15}N$ enrichment observations have been reported in the Mtoni Kijichi mangroves (Kruitwagen *et al.*, 2006; Kruitwagen *et al.*, 2008), where values as high as 13‰ were measured in mudskippers (Kruitwagen *et al.*, 2006), and mangrove snails (Kimirei *et al.*, unpublished data). Both Kunduchi and Mtoni Kijichi mangroves are located in areas with high population densities (NBS, 2013). Also, Mtoni Kijichi receives large quantities of industrial effluents (Kruitwagen *et al.*, 2006; Kruitwagen *et al.*, 2008; Machiwa, 1992; Machiwa, 2010). The Dar es Salaam City has poor waste disposal facilities, which further contributes to the coastal pollution problem (Kimirei *et al.*, 2016).

Like $\delta^{15}N$, the stable carbon isotope ($\delta^{13}C$) values for fish from Kunduchi were slightly enriched when compared to fish from Mbegani. Both *G. oyena* and

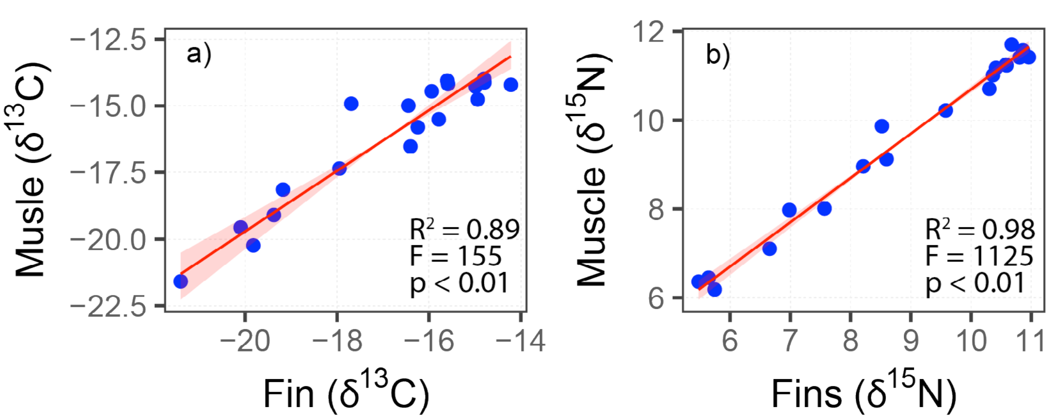

Figure 7. Corelation of a) $\delta^{13}C$ and b) $\delta^{15}N$ signatures between dorsal muscle and caudal fin tissues of *G. filamentosus* combined for all sites (n = 20).

G. filamentosus are generalists (Mavuti *et al.*, 2004), and share various carbon sources at Kunduchi, as indicated by the highly mixed $\delta^{13}C$ signatures (see Fig. 3a). However, the $\delta^{13}C$ signatures were different between *G. oyena* and *G. filamentosus* at Mbegani, with $\delta^{13}C$ isotopes enriched in *G. oyena* in both muscle and fin tissues (see Fig. 3b). These results may indicate that the two species have significantly different carbon sources at Mbegani, although they have been reported to feed in similar environments (Lugendo *et al.*, 2007).

When compared to Mbegani, the fishes collected from the Kunduchi mangrove creek appear to have a substantially higher level of nutrient enrichment, which is indicated by the high $\delta^{15}N$ ratio. While Kunduchi was considered relatively pristine in other studies (Kruitwagen *et al.*, 2008), this study indicates that during the sampling time, it was becoming increasingly polluted (Jiang *et al.*, 2019; McClelland and Valiela, 1998; McClelland *et al.*, 1997). The increased enrichment in Kunduchi may be due to increasing human population, industrial and domestic effluents, and urban agriculture, which utilize inorganic fertilizers to boost production. Mangroves are increasingly being polluted (Machiwa, 1992; Machiwa, 2010), perturbed and cleared (Ajai and Chauhan, 2017), which decimates the value they play in terms of ecosystem services (Kimirei *et al.*, 2016). While the data analysed in the current study are based on samples collected a decade ago, it has significance in assessing the role of population growth and anthropogenic pollution on nutrients pollution in mangroves (see Lugendo and Kimirei, 2021).

On an individual species-level, $\delta^{15}N$ values were always more enriched in muscle than in fin tissues while the opposite was true for the $\delta^{13}C$ values which were higher in fins than in muscles. Similar observations were made for *Oncorhynchus tshawytscha* and *O. mykiss* (Sanderson *et al.*, 2009). While it is beyond the scope of this study, the differences in isotopic enrichment between muscle and fin tissues may be explained by the abundance of both essential and non-essential amino acids for $\delta^{15}N$, and lipids for $\delta^{13}C$ (Pinnegar and Polunin, 1999; Sanderson *et al.*, 2009). Nonetheless, it was found that both carbon and nitrogen stable isotope signatures were highly correlated between muscle and fin tissues of the fish species examined. The nitrogen isotopes of fins explained >80% of the variations in stable isotope values of muscle tissue. These findings indicate that fin-clipping can be used as a reliable non-lethal method for stable isotope analysis (SIA) of nitrogen enrichment for the studied fish species. Fin-clipping has been found to be especially useful in monitoring endangered species (Jardine *et al.*, 2011; Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Valladares and Planas, 2012), as well as in situations requiring large sample sizes and replications (Sanderson *et al.*, 2009). Stable isotope analysis as a tool for monitoring nitrogen enrichment in coastal waters should be especially useful in countries with minimal financial resources to run long-term monitoring campaigns.

The findings of this study support the use of fin-clipping as a non-lethal proxy for stable isotope analysis for the fish species under consideration. Furthermore, this study found that the stable nitrogen and carbon isotope signatures of fish collected from Kunduchi mangrove creeks were more enriched than those collected from Mbegani, and this enrichment was consistent across the two studied species. The results of this study, the first in the WIO region to examine the non-lethal collection of fish tissues for use in stable isotope studies, are positive, and should encourage the use of fin clipping as an alternative to the extraction of dorsal muscle tissues in stable isotope studies involving fishes. It is also recommended that more research is done on non-lethal sampling techniques to include more species and tissue types.

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