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# Diet of invasive pikeperch *Sander lucioperca*: developing non-destructive tissue sampling for stable isotope analysis with comparisons to stomach contents analysis

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**Abstract** – Impact assessments of invasive piscivorous fishes usually rely on dietary analyses to quantify their predation pressure on prey communities. Stomach contents analysis (SCA), typically a destructive sampling method, is frequently used for this. However, many invasive piscivores are exploited by catch-and-release sport angling, with destructive sampling often not feasible. Stable isotope analysis (SIA) provides an alternative dietary analysis tool to SCA, with use of fin tissue, scales and/or epidermal mucus potentially enabling its non-destructive application. Here, the diet of a population of pikeperch *Sander lucioperca*, an invasive sport fish to Great Britain, was investigated by applying SIA to a range of tissues. Testing SI data of dorsal muscle (destructive sampling) versus fin, scale and mucus (non-destructive sampling) revealed highly significant relationships, indicating that the tissues collected non-destructively can be reliably applied to pikeperch diet assessments. Application of these SI data to Bayesian mixing models predicted that as *S. lucioperca* length increased, their diet shifted from macro-invertebrates to fish. Although similar ontogenetic patterns were evident in SCA, this was inhibited by 54% of fish having empty stomachs. Nevertheless, SCA revealed that as *S. lucioperca* length increased, their prey size significantly increased. However, the prey: predator length ratios ranged between 0.08 and 0.38, indicating most prey were relatively small. These results suggest that when non-destructive sampling is required for dietary analyses of sport fishes, SIA can be applied using fin, scales and/ or mucus. However, where destructive sampling has been completed, SCA provides complementary dietary insights, especially in relation to prey size.

**Keywords:** Bayesian mixing models / gut contents / piscivory / trophic

**Résumé – Régime alimentaire du sandre *Sander lucioperca* : mise au point d'un échantillonnage non destructif des tissus pour l'analyse des isotopes stables et comparaisons avec l'analyse du contenu de l'estomac.** Les évaluations de l'impact des poissons piscivores exotiques s'appuient généralement sur des analyses alimentaires pour quantifier la pression de prédation qu'ils exercent sur les communautés de proies. L'analyse du contenu stomacal (SCA), généralement une méthode d'échantillonnage destructive, est fréquemment utilisée à cette fin. Cependant, de nombreux piscivores exotiques sont exploités par la pêche sportive avec remise à l'eau, l'échantillonnage destructif étant souvent impossible. L'analyse des isotopes stables (SIA) est un outil d'analyse diététique alternatif à l'SCA, l'utilisation de tissus de nageoire, d'écailles et/ou de mucus épidermique permettant potentiellement son application non destructive. Ici, le régime alimentaire d'une population de sandre *S. lucioperca*, un poisson de pêche sportive envahissant, a été étudié en appliquant le SIA à une gamme de tissus. L'analyse des données du SI du muscle dorsal (échantillonnage destructif) par rapport aux nageoires, à l'écaille et au mucus (échantillonnage non destructif) a révélé des relations très significatives, indiquant que les tissus recueillis de façon non destructive peuvent être appliqués de façon fiable aux évaluations du régime alimentaire du sandre. L'application de ces données du SI aux modèles de mélange bayésiens a estimé qu'à mesure que la longueur de *S. lucioperca* augmentait, leur alimentation passait des macro-invertébrés aux poissons. Bien que des schémas ontogénétiques similaires aient été observés chez les SCA, ils ont été limités par 54% des poissons ayant l'estomac vide. Néanmoins, SCA a révélé qu'à mesure que la longueur de *S. lucioperca* augmentait, la taille de leurs proies augmentait considérablement. Cependant, le rapport proie : longueur des prédateurs variait entre 0,08 et 0,38, ce qui

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indique que la plupart des proies étaient relativement petites. Ces résultats suggèrent que lorsqu'un échantillonnage non destructif est nécessaire pour l'analyse du régime alimentaire de poissons de pêche sportive exotiques, le SIA peut être appliquée en utilisant des nageoires, des écailles et/ou du mucus. Cependant, lorsque l'échantillonnage destructif a été effectué, l'ACS fournit des informations complémentaires sur l'alimentation, en particulier en ce qui concerne la taille des proies.

**Mots-clés :** modèles bayésiens de mélange / carbone / contenu intestinal / azote / piscivorie / écologie trophique

## 1 Introduction

Piscivorous fishes play an important role in regulating the structure of aquatic food-webs (Woodward and Hildrew, 2002). They can exert substantial top-down forces on prey communities, potentially initiating trophic cascades (Brett and Goldman, 1996; Pace *et al.*, 1999; Drenner and Hambright, 2002). Alien piscivorous fishes that are introduced to enhance sport angling, such as largemouth bass *Micropterus salmoides* and peacock bass *Cichla* spp., also exert substantial top-down forces on prey fish communities, resulting in impacts including reduced prey abundances and decreased species diversity (Gratwicke and Marshall 2001; Pelicice and Agostinho, 2009). As the diets of piscivorous fishes tend to involve strong ontogenetic changes via increasing gape sizes (Zhao *et al.*, 2014), the strength of trophic cascades can be strongly influenced by the resultant dietary shifts (Sato and Watanabe, 2014). Thus, an important step in the assessments of the ecological impacts of invasive piscivores is analyses of their diet composition, including assessing ontogenetic shifts in their prey selection.

Dietary assessments of piscivorous fishes are often reliant on stomach contents analysis (SCA) (Sandlund *et al.*, 2016). Whilst providing information on diet composition of individual fish, the method usually utilises relatively large numbers of fish to maximise statistical power and to assist understandings of dietary patterns over time and space (Cortés, 1997). For piscivores such as Northern pike (*Esox lucius*) and pikeperch (*S. lucioperca*), an inherent issue in stomach contents analysis is that many of the fish often have empty stomachs, resulting in a paucity of dietary data from the sampled population. Piscivorous fishes in general and particularly those that consume prey whole have higher proportions of empty stomachs compared to lower trophic level fishes (Arrington *et al.*, 2002), with feeding frequency thought to decrease through the consumption of energy-rich food items (Bowen *et al.*, 1995). These methodological issues can potentially be overcome by using complementary dietary assessment methods, such as stable isotope analysis (SIA) (Cucherousset *et al.*, 2012; Jensen *et al.*, 2012). Indeed, in a study where an average of 36% of *E. lucius* had empty stomachs across 16 populations, stable isotope analysis showed no trophic position differences between fish with and without prey items in their stomachs, or between piscivorous and invertebrate feeders determined through stomach content analysis (Paradis *et al.*, 2008), indicating opportunistic rather than specialist invertebrate feeding strategies. Therefore, integrative studies may often show that SCA and SIA provide contrasting dietary information due to, for example, differences in the temporal scales of the methods (*i.e.* short-term SCA versus long-term SIA diet assessments),

(Locke *et al.*, 2013; Busst and Britton, 2017), but these differences can provide insights where disintegrated studies cannot.

The issues of sacrificing relatively large numbers of piscivorous fish to satisfy the requirements needed for stomach contents analysis is also problematic when these fish have high values within sport angling. For example, catch and release angling (C&R) is increasingly applied to sport fishing for species such as *M. salmoides*, *S. lucioperca* and *Cichla* spp. Mortalities associated with C&R can be minimised via use of best practice angling techniques and fish handling codes (Siepker *et al.*, 2007; Arlinghaus and Hallermann, 2007; Cook *et al.*, 2015, Bower *et al.*, 2016). Consequently, dietary assessments for piscivorous sport fishes based on destructive sampling are increasingly at odds with their fishery management and angling practises, even where the fishes are invasive (Hickley and Chare, 2004). Indeed, the fishery value of invasive fishes are increasingly recognised (Gozlan, 2008), especially when their populations are in large open systems in which their population management is inherently difficult (Britton *et al.*, 2011; Britton and Orsi, 2012).

Pikeperch *S. lucioperca* is a large-bodied piscivorous freshwater fish whose native range in Europe extends from Germany in the West to Central Russia in the East (Maitland, 2004). The species has been introduced outside of this range, into countries such as France, Spain and Great Britain (Elvira and Almodóvar, 2001; Kopp *et al.*, 2009), often with the primary purpose of increasing sport angling opportunities (Hickley and Chare, 2004). Following their initial introduction into Britain in 1878, there was a series of translocations of *S. lucioperca* into waters in Eastern England during the 1960s (Wheeler and Maitland, 1973). It was these releases that resulted in their invasion of river catchments across Eastern, Central and Southern England (Linfield and Rickards, 1979; Hickley, 1986; Copp *et al.*, 2003). Whilst there were initial concerns on their impacts on prey populations, the species is now considered as an important angler target species in many fisheries (Hickley and Chare, 2004). Consequently, whilst studies on their diet previously utilised stomach contents analyses (*e.g.* Smith *et al.*, 1997; Schulze *et al.*, 2006), methods based on stable isotope analysis might now be more preferable (Kopp *et al.*, 2009), especially where tissues can be utilised that can be collected non-lethally (Britton and Busst, 2018).

The diet of the *S. lucioperca* has been well studied both within their native and non-native ranges (*e.g.* Campbell 1992; Keskinen and Marjomäki 2004; Pérez-Bote and Roso 2012; Didenko and Gurbyk 2016). They are generally considered to be piscivorous within their first year of life (Mittelbach and Persson 1998), although this switch to piscivory can become delayed if individuals do not reach a size advantage over prey (Persson and Brönmark 2002) or if suitable prey fish are

unavailable (Ginter *et al.*, 2011). While *S. lucioperca* diet comprises of fish across a range of size classes, they can also be cannibalistic, with this acting as an important regulatory force (Mehner *et al.*, 1996; Frankiewicz *et al.*, 1999; Lappalainen *et al.*, 2006). Individual *S. lucioperca* will also consume macro-invertebrates, with these prey items most frequently encountered in the diets of smaller individuals (Hansson *et al.*, 1997; Argillier *et al.*, 2012).

The application of SIA using multiple tissues in conjunction with SCA enables the dietary habits of the target population to be assessed across difference timescales. SCA provides 'snapshot' dietary information (Cortés, 1997). By contrast, SIA provides longer term dietary perspectives, with the timescale dependent on the analysed tissue (Fry, 2006; Newsome *et al.*, 2007; Martínez del Rio *et al.*, 2009). The aim of this study was thus to use *S. lucioperca* as a model fish exploited by C&R sport angling to assess how stable isotope analysis can be applied to assess their diet in relation to using tissues that are collected non-destructively. Objectives were to (1) quantify the relationships of the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ between dorsal muscle and three tissues that can be collected non-lethally; (2) utilise the stable isotope data to predict the diet composition of a *S. lucioperca* population using Bayesian mixing models (Stock *et al.*, 2018); and (3) complete stomach contents analyses on the *S. lucioperca* population and assess the results in the context of the dietary predictions from the mixing models.

## 2 Materials and methods

### 2.1 Sample collection

The *S. lucioperca* population of the Grand Union Canal, Northamptonshire, in Central England was sampled by boat-mounted electric fishing ('boom-boat', power supplied by a 2 kVA generator) in April 2017. This canal is generally of 15 m maximum width and depth rarely exceeds 2 m. A series of locks overcome changes in the gradient of the surrounding land. Small-bodied cyprinid fishes are dominant in the fish community, especially roach *Rutilus rutilus*. Pikeperch have been present in the canal for at least 30 years (Hickley, 1986). A total of 180 individuals were captured by electric fishing that ranged in fork length (to nearest mm) between 169 and 551 mm (mean ± 95% CI; 355 ± 14 mm) and weight between 48 and 1924 g (mean ± 95% CI; 561 ± 71 g). Following their capture, the fish were euthanised and held on ice while being transferred to the laboratory where they were processed immediately.

### 2.2 Stable isotope analysis

Of the 180 sampled *S. lucioperca*, a sub-sample of 19 were processed for stable isotope analysis using fish from across the length range (mean ± 95% CI; 323 ± 54 mm). Following their measurement, the tissues that were sampled from each fish were dorsal muscle, pelvic fin tissue, scales and epidermal mucus. The epidermal mucus was collected by scraping the dorsal surface of each fish with a cover slip, with the sample then cleaned with forceps as per Maruyama *et al.* (2015) and transferred to a sample tube. This method was used in preference to the filtration method of Church *et al.* (2009), as it

was demonstrated to result in reduced error (Maruyama *et al.*, 2017). Scales were collected from the body area between the dorsal fin and the lateral line. Scale decalcification was not performed prior to isotopic analysis, since the removal of inorganic carbonates has been shown to have no significant effect on scale $\delta^{13}C$ and $\delta^{15}N$ values (Sinnatamby *et al.*, 2007; Ventura and Jeppesen, 2010; Woodcock and Walther, 2014). Preparation thus focused on cleaning scales with distilled water prior to removing the outer portion of the scale for SIA, ensuring the tissue analysed was from recent growth only (~1 year) (Hutchinson and Trueman, 2006; Bašić and Britton, 2015). A selection of all prey fish species (dorsal muscle only) and macroinvertebrates (*cf*. Stomach content analysis) were also prepared for stable isotope analysis recovered through dissection and removal of prey from the stomachs. These samples were based only on individual animals that were recovered in good condition, *i.e.* those very recently ingested, with negligible digestion and that were identifiable to species level. All samples were then dried at 60 °C for 48 h.

The samples were then analysed at the Cornell Isotope Laboratory, New York, USA, where they were ground to powder, weighed precisely to approximately 1000 µg and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Verification of accuracy was against internationally known reference material and accuracy and precision of the sample runs was tested every 10 samples using a standard animal sample (mink). Delta (δ) isotope ratios were expressed as units per mil (‰). Analytical precision of the $\delta^{15}N$ and $\delta^{13}C$ sample runs was estimated at 0.42 and 0.15‰ respectively. Lipid correction was not necessary as C:N ratios indicated very low lipid content (Post *et al.*, 2007).

### 2.3 Tissue comparisons

The significance of differences in the stable isotope ratios between the tissues were tested using pair-wise *t*-tests. Simple linear regression models tested the significance of the relationship between mucus and muscle, fin and muscle and scale and muscle for $\delta^{13}C$ and $\delta^{15}N$ isotope values. Models were run both with and without fish length. The best fitting model was chosen using regression statistics and the lowest value of Akaike's Information Criteria (AIC). Statistical analysis and graphical outputs were performed using R (R Core Team, 2018, version 3.4.3).

### 2.4 Bayesian mixing models

The stable isotope data were analysed to assess the effect of tissue type on fish diet predictions, including after conversion of the stable isotope data of the non-lethal tissues to dorsal muscle (as the standard tissue used in fish isotope studies). The primary tool for these analyses was the use of Bayesian mixing models (Phillips *et al.*, 2014) allowing for predictions of the relative proportions of the putative prey resources that contributed to the diet of *S. lucioperca* for each tissue both before and after their conversion to dorsal muscle values. The models were run in the package 'Mixing Models for Stable Isotope Analysis in R' (MixSIAR; Parnell *et al.*, 2013;

**Table 1.** Number of individuals, tissue specific carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable-isotope ratios (Mean ± SD) indicating variation in isotope values between tissues.

| Tissue | $n$ | $\delta^{13}$C‰ | Range | $\delta^{15}$N‰ | Range |
|---|---|---|---|---|---|
| Muscle | 19 | −31.68 ± 0.77 | −32.90 to −30.36 | 21.26 ± 1.03 | 19.15 to 22.94 |
| Mucus | 19 | −32.23 ± 1.04 | −34.15 to −30.84 | 20.88 ± 1.03 | 18.95 to 22.39 |
| Fin | 19 | −31.15 ± 1.13 | −33.38 to −29.13 | 21.27 ± 1.10 | 19.15 to 22.84 |
| Scale | 19 | −28.67 ± 0.70 | −29.68 to −27.37 | 19.99 ± 1.11 | 17.61 to 21.80 |

Stock *et al.*, 2018). All models were run using normal run length (chain length: 100,000 iterations with burn-in of 50,000, with posterior thinning (thin: 50) and 3 chains). Model diagnostics were based on Gelman-Rubin and Geweke, with sufficient convergence to accept the results (Stock and Semmens, 2016).

Five mixing models were run that covered the use of the *S. lucioperca* (as the consumer) stable isotope data from (1) dorsal muscle, (2) epidermal mucus, (3) scales, (4) epidermal mucus data converted to dorsal muscle values (using the linear models for $\delta^{13}$C and $\delta^{15}$N mucus to muscle), and (5) scale data converted to dorsal muscle values (using the linear models for $\delta^{13}$C and $\delta^{15}$N scale to muscle). The putative prey (source) data used within the mixing models was constant across all models, except for model (3) where fish muscle isotope data were converted to scale data based on conversion factors in Busst *et al.* (2015) to ensure consistency in predictions by accounting for differences in isotope values between the tissues of source and consumer. Dietary contributions were predicted by splitting the fish into two size classes, <350 and >350 mm, with distinctions made between the two groupings based on (1) the likelihood of sexual maturity at above approximately 350 mm (Lappalainen *et al.*, 2003) and (2) differences in the contribution of prey items to the diet of individuals in each size class from stomach content analysis (*cf.* results).

In the mixing models, the isotopic fractionation values between the prey resources and *S. lucioperca* were varied according to the *S. lucioperca* tissue being used. For muscle and mucus, values were chosen based on standards proposed by Post (2002): $\delta^{15}$N 3.4 ± 0.5‰; $\delta^{13}$C 1 ± 0.5‰. For scales, the fractionation factors used were $\delta^{15}$N = 2.58 ± 1‰ and $\delta^{13}$C = 2.78 ± 1‰), based on the standards of Post (2002) but with correction for scales using the mean differences from three studies comparing fractionation between muscle and scale tissue ($\Delta^{15}$N −0.82‰, $\Delta^{13}$C 1.78‰) (Heady and Moore, 2013; Busst and Britton, 2015; Busst *et al.*, 2015).

Reported outputs of the models were overall estimated posterior density contributions to diet given as summary statistics; mean, standard deviation and 95% confidence limits. Posterior density plots for each model are given in supplementary material.

## 2.5 Stomach content analysis

*S. lucioperca* were measured (fork length, nearest mm) and weighed (nearest g), and then dissected and their stomach contents removed. Prey items from stomach contents were identified to their lowest possible taxonomic level, total stomach fullness (% in volume) was assessed, as was the

contribution of each prey item to overall fullness. For subsequent analyses, stomach contents were categorised into three groupings consisting of (1) 'Cyprinidae' including roach *Rutilis rutils*, common bream *Abramis brama* and gudgeon *Gobio gobio*; (2) 'Percidae' including perch *Perca fluviatilis* and ruffe *Gymnocephalus cernua*; and (3) 'Invertebrates' where macro-invertebrates were identified to family level, and included Gammaridae, Chironomidae and Mysidae.

The contribution of each diet category was expressed as percentages in terms of frequency of occurrence and prey-specific abundance. Frequency of occurrence (% $F_i$) of a given prey type was defined as the number of stomachs in which that prey occurred, expressed as a frequency of the total number of stomachs in which prey were present (Costello, 1990). For prey-specific abundance, prey-type contribution was first estimated in proportion to overall stomach fullness (in volume). The proportional fullness contribution of each diet category was then expressed as percentage prey-specific abundance (% $P_i$):

$$\% \, P_i = \left( \frac{\sum F_i}{\sum F_t} \right) \times 100,$$

where $P_i$ was the prey-specific abundance of prey $i$, $F_i$ was the stomach content fullness for diet category $i$ and $F_t$ was the total stomach fullness in only those predators with prey $i$ in their stomach (Amundsen *et al.*, 1996). In addition, the fork length (mm) of each prey item was also taken to assess changes in prey use patterns with increasing body length of *S. lucioperca* using regression analysis (as prey: predator length ratios). Dietary contribution was predicted for size classes <350 and >350 mm as per Bayesian mixing models.

## 3 Results

### 3.1 Relationship of $\delta^{13}$C and $\delta^{15}$N values between *S. lucioperca* tissues

There was a significant difference in the $\delta^{13}$C values between scale and all other tissues (Tab. 1, Fig. 1), where scale was significantly enriched in $\delta^{13}$C relative to muscle (*t*-test, $t = 12.6$, $P < 0.001$), mucus (*t*-test, $t = 12.4$, $P < 0.001$) and fin (*t*-test, $t = 8.1$, $P < 0.001$). Although not significantly different, mucus was depleted in $\delta^{13}$C relative to muscle (−0.55‰; *t*-test, $t = -1.8$, $P = 0.07$), whilst fin was enriched in $\delta^{13}$C relative to muscle (+0.53‰; *t*-test, $t = 1.6$, $P = 0.10$). For $\delta^{15}$N, significant differences were also evident between scale and all other tissues (Tab. 1, Fig. 1), with scale depleted in $\delta^{15}$N relative to muscle (−1.25‰; *t*-test, $t = -3.6$, $P < 0.001$), mucus (*t*-test, $t = -2.5$, $P < 0.001$) and fin (*t*-test, $t = -3.5$,
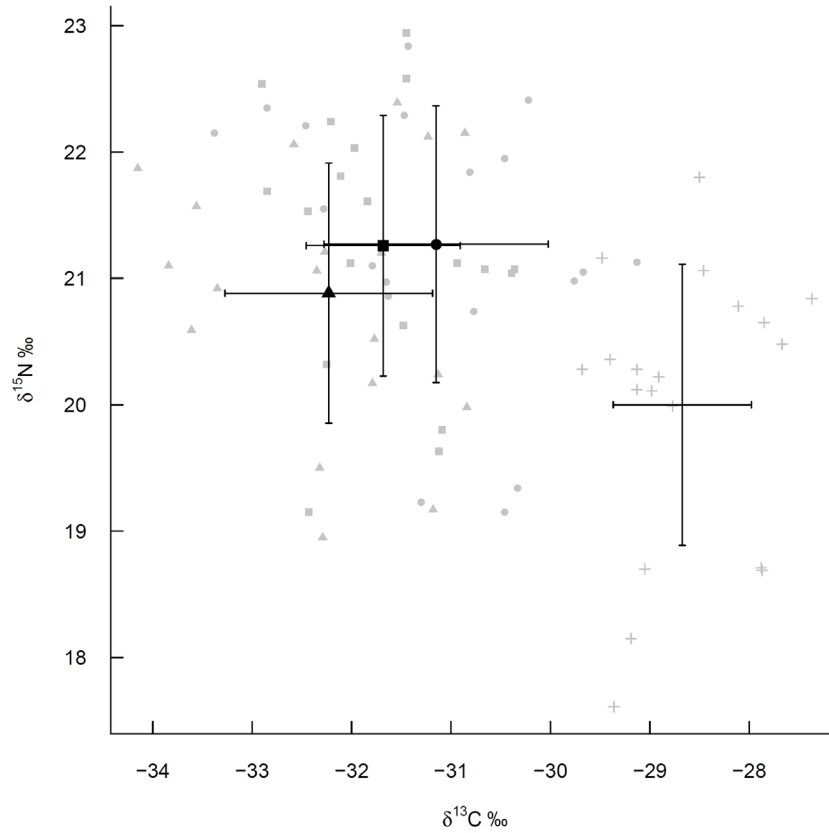
**Fig. 1.** Stable isotope bi-plot of $\delta^{13}C$ versus $\delta^{15}N$ showing individual (light grey) and mean (black) values for all tissue types (■ muscle; ▲ mucus; ● fin; + scale), where error bars represent standard deviation.

**Table 2.** Linear regression statistics for the relationship between dorsal muscle stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and those of epidermal mucus, fin and scales collected from *S. lucioperca*.

| Isotope | Relationship | $R^2$ | $F$ | $P$ | $a$ | $b_1$(95% CI) | $b_2$(95% CI) | AIC |
|---|---|---|---|---|---|---|---|---|
| $\delta^{13}C$ | Muscle/Mucus | 0.63 | (DF1,17) 32.18 | <0.001 | −12.34 | 0.60 (0.37, 0.82) | | 29.02 |
| | Muscle/Mucus + length | 0.75 | (DF 2,16) 28.32 | <0.001 | −22.64 | 0.31(0.04, 0.58) | 0.003 (0.001, 0.006) | 22.45 |
| | Muscle/Fin | 0.80 | (DF 1,17) 74.45 | <0.001 | −12.37 | 0.62 (0.47, 0.77) | | 17.23 |
| | Muscle/Fin + length | 0.82 | (DF 2,16) 42.34 | <0.001 | −18.04 | 0.46 (0.20, 0.71) | 0.001 (−0.001, 0.004) | 16.25 |
| | Muscle/Scale | 0.86 | (DF 1,17) 111.9 | <0.001 | −1.90 | 1.04 (0.83, 1.25) | | 10.71 |
| | Muscle/ Scale + length | 0.86 | (DF 2,16) 56.4 | <0.001 | −6.69 | 0.88 (0.49, 1.27) | 0.001 (−0.001, 0.003) | 11.57 |
| $\delta^{15}N$ | Muscle/Mucus | 0.28 | (DF 1,17) 8.152 | 0.01 | 9.35 | 0.57 (0.15, 0.99) | | 52.60 |
| | Muscle/Mucus + length | 0.51 | (DF 2,16) 10.32 | 0.001 | 8.14 | 0.70 (0.33, 1.05) | −0.004 (−0.007, −0.001) | 46.29 |
| | Muscle/Fin | 0.75 | (DF 1,17) 56.5 | <0.001 | 3.71 | 0.83 (0.59, 1.06) | | 32.22 |
| | Muscle/Fin + length | 0.75 | (DF 2,16) 28.18 | <0.001 | 4.57 | 0.79 (0.55, 1.04) | −0.001 (−0.003, 0.001) | 33.36 |
| | Muscle/Scale | 0.69 | (DF 1,17) 41.42 | <0.001 | 5.65 | 0.78 (0.52, 1.04) | | 36.58 |
| | Muscle/Scale + length | 0.88 | (DF 2,16) 72.21 | <0.001 | 5.88 | 0.82 (0.67, 0.99) | −0.004 (−0.005, −0.002) | 18.24 |

$P < 0.001$). There was no significant difference in $\delta^{15}N$ between muscle and mucus (+0.38, *t*-test, $t = 1.1$, $P = 0.27$) or between muscle and fin (−0.01; *t*-test, $t = −0.1$, $P = 0.97$).

Significant relationships were found between *S. lucioperca* muscle isotope values ($\delta^{13}C$ and $\delta^{15}N$) and all other tissue types (Tab. 2, Fig. 2). Including length in the models improved their fit in all cases (according to AIC and regression statistics; Tab. 2). This is likely explained by the significant increase in $\delta^{13}C$ with increasing fish length (Fig. 3; muscle, $R^2 = 0.68$; $F_{(1,17)} = 39.2$; $P < 0.001$; mucus, $R^2 = 0.52$; $F_{(1,17)} = 20.1$; $P < 0.001$; fin, $R^2 = 0.66$; $F_{(1,17)} = 35.4$; $P < 0.001$; scale, $R^2 = 0.70$; $F_{(1,17)} = 42.91$; $P < 0.001$). Consequently, length was retained in the regression analyses across all tissue/isotope conversions for consistency. There was no relationship between $\delta^{15}N$ and fish length (muscle, $R^2 = 0.06$; $F_{(1,17)} = 2.12$; $P = 0.16$; mucus, $R^2 = 0.01$; $F_{(1,17)} = 1.12$; $P = 0.31$; fin, $R^2 = 0.02$; $F_{(1,17)} = 1.32$; $P = 0.27$; scale, $R^2 = 0.01$; $F_{(1,17)} = 0.25$; $P = 0.62$).
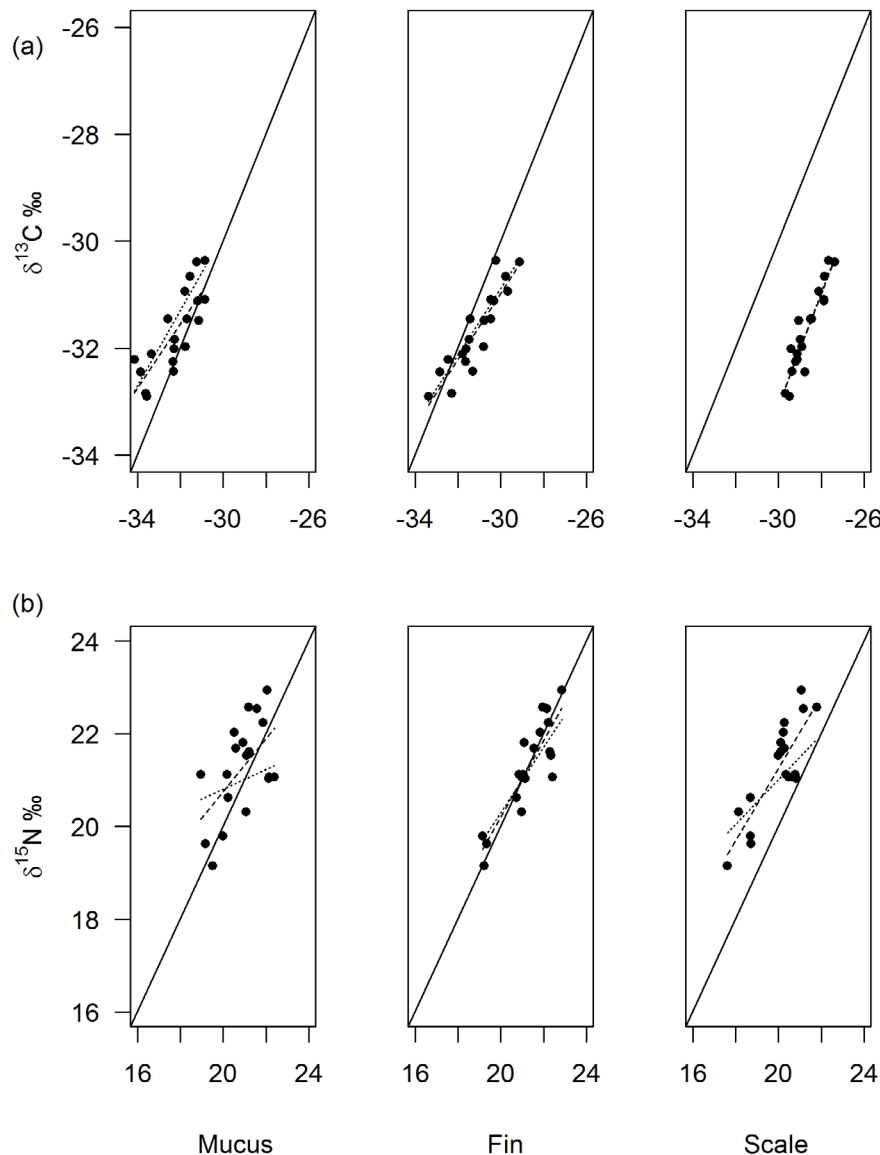
**Fig. 2.** Linear relationships of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ dorsal muscle versus epidermal mucus ('mucus'), fin and scale, where the dotted line represents the relationship with length included in the model and the dashed line represents the relationship with length excluded.

### 3.2 Stable isotope mixing models

The mixing models predicted the diet category 'invertebrates' to be the most important item to the diet of *S. lucioperca* <350 mm, followed by Cyprinidae and then Percidae (Tab. 3). This result was consistent across all models (Tab. 3, Fig. 4). For *S. lucioperca* >350 mm, Cyprinidae had the greatest predicted contribution to *S. lucioperca* diet, followed by invertebrates and then Percidae (Tab. 3, Fig. 4).

The difference in mean dietary contribution predictions across size classes between model 1 (muscle) and all other models was lowest for model 5 (scale data converted to dorsal muscle values) (Tab. 3, Fig. 4). Differences were greatest between model 1 (muscle) and model 2 (mucus) for mean dietary contribution predictions in size class <350 mm and for Percidae in size class >350 mm, whereas differences were greatest between model 1 (muscle) and model 4 (epidermal

mucus data converted to dorsal muscle values) for Cyprinidae and Invertebrates in size class >350 mm (Tab. 3, Fig. 4).

### 3.3 Stomach contents analysis

Of the 180 sampled *S. lucioperca*, 98 had empty stomachs (54%). Of the 82 fish with items in the stomach, analyses revealed that as *S. lucioperca* body size increased, the size of their prey significantly increased (Cyprinidae: $R^2 = 0.41$, $F_{(1,65)} = 46.48$, $P < 0.01$; Percidae: $R^2 = 0.43$, $F_{(1,6)} = 6.28$, $P = 0.05$) (Fig. 5a). Between the two fish prey groups, there was no significant difference in their sizes (ANOVA $F_{1,72} = 0.35$, $P = 0.56$). Regarding prey: predator length ratios, these ratios generally decreased as *S. lucioperca* body size increased, although the relationships were not significant (Cyprinidae: $R^2 = 0.03$, $F_{(1,65)} = 2.23$, $P = 0.14$; Percidae:
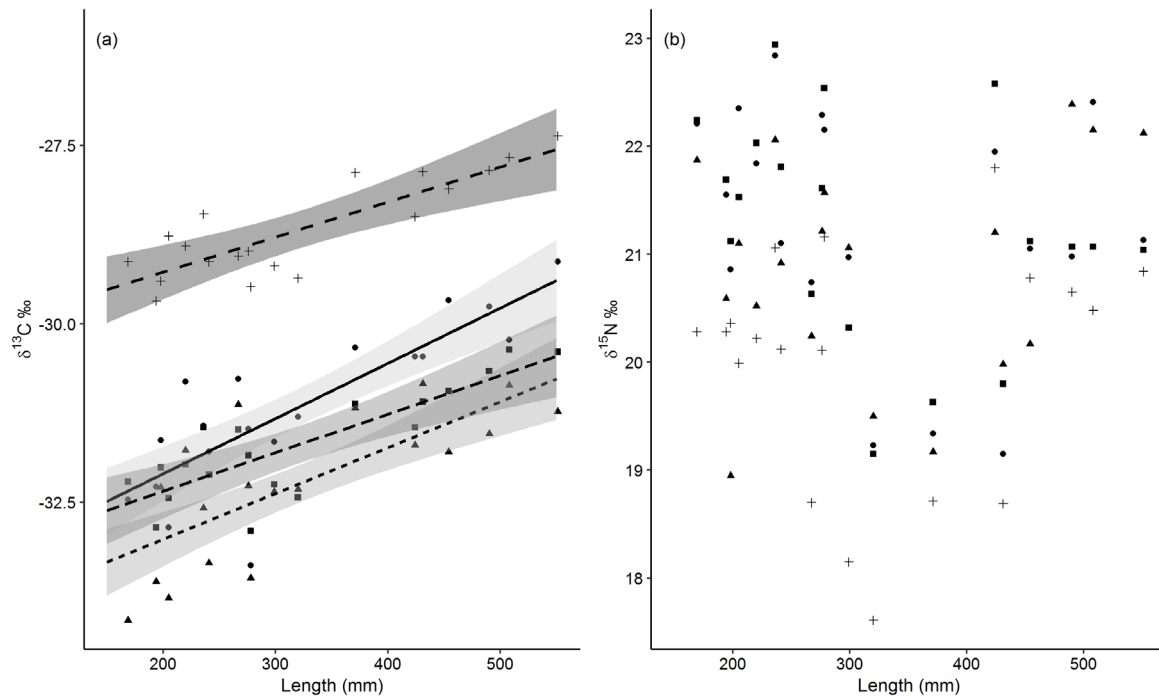
**Fig. 3.** Linear relationships of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ for muscle (■), mucus (▲), fin (●) and scale (+). Significant relationships are fitted with 95% confidence intervals around the line for muscle (long dashed line, light grey), mucus (short dashed line), fin (solid line) and scale (long dashed line, dark grey).

**Table 3.** Mean predicted dietary contributions from Bayesian mixing models of 'Cyprinidae', 'Invertebrates' and 'Percidae' to the diet of *S. lucioperca* by size class (<350 mm and >350 mm), showing standard deviation and 95% confidence limits. Mixing models were (1) consumer as muscle values, (2) consumer as mucus values, (3) consumer as scale values, (4) consumer as muscle values based on conversion using the linear models for $\delta^{13}C$ and $\delta^{15}N$ mucus to muscle, and (5) consumer as muscle values based on conversion using the linear models for $\delta^{13}C$ and $\delta^{15}N$ scale to muscle.

| Resource | Model | I < 350 mm | | | | II > 350 mm | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | 2.5% CI | 95.7% CI | Mean | SD | 2.5% CI | 95.7% CI |
| Cyprinidae | 1 | 0.32 | 0.13 | 0.07 | 0.552 | 0.47 | 0.22 | 0.04 | 0.83 |
| | 2 | 0.23 | 0.11 | 0.03 | 0.459 | 0.45 | 0.21 | 0.02 | 0.79 |
| | 3 | 0.28 | 0.11 | 0.07 | 0.499 | 0.40 | 0.18 | 0.05 | 0.73 |
| | 4 | 0.27 | 0.11 | 0.07 | 0.498 | 0.40 | 0.20 | 0.04 | 0.76 |
| | 5 | 0.31 | 0.12 | 0.07 | 0.535 | 0.46 | 0.21 | 0.04 | 0.81 |
| Invertebrates | 1 | 0.46 | 0.1 | 0.26 | 0.646 | 0.27 | 0.14 | 0.06 | 0.60 |
| | 2 | 0.63 | 0.11 | 0.4 | 0.811 | 0.33 | 0.13 | 0.11 | 0.59 |
| | 3 | 0.50 | 0.09 | 0.33 | 0.668 | 0.31 | 0.09 | 0.13 | 0.50 |
| | 4 | 0.49 | 0.09 | 0.31 | 0.648 | 0.38 | 0.17 | 0.10 | 0.76 |
| | 5 | 0.47 | 0.09 | 0.27 | 0.65 | 0.28 | 0.13 | 0.06 | 0.58 |
| Percidae | 1 | 0.23 | 0.12 | 0.03 | 0.475 | 0.26 | 0.19 | 0.01 | 0.68 |
| | 2 | 0.15 | 0.10 | 0.01 | 0.375 | 0.22 | 0.18 | 0.004 | 0.64 |
| | 3 | 0.22 | 0.10 | 0.04 | 0.439 | 0.29 | 0.16 | 0.02 | 0.62 |
| | 4 | 0.24 | 0.1 | 0.05 | 0.443 | 0.23 | 0.16 | 0.02 | 0.59 |
| | 5 | 0.23 | 0.11 | 0.04 | 0.454 | 0.27 | 0.18 | 0.02 | 0.68 |

$R^2 = 0.09$, $F_{(1,6)} = 0.58$, $P = 0.47$) (Fig. 5b). The maximum prey length to predator length ratio was 0.38, whilst the minimum was 0.08 (mean ± SD; 0.22 ± 0.06), with the majority of *S. lucioperca* consuming small prey sizes relative to their body size (85% of prey <0.3 prey length/predator length; Fig. 5c).

The prey-specific abundance (% $P_i$) was highest for Cyprinidae at 79.8%, followed by Percidae (13.1%) and then invertebrates (7.11%) (Tab. 4). Invertebrates were only represented in the diet of individuals up to 396 mm, whilst Cyprinidae were present in individuals from 204 to 532 mm
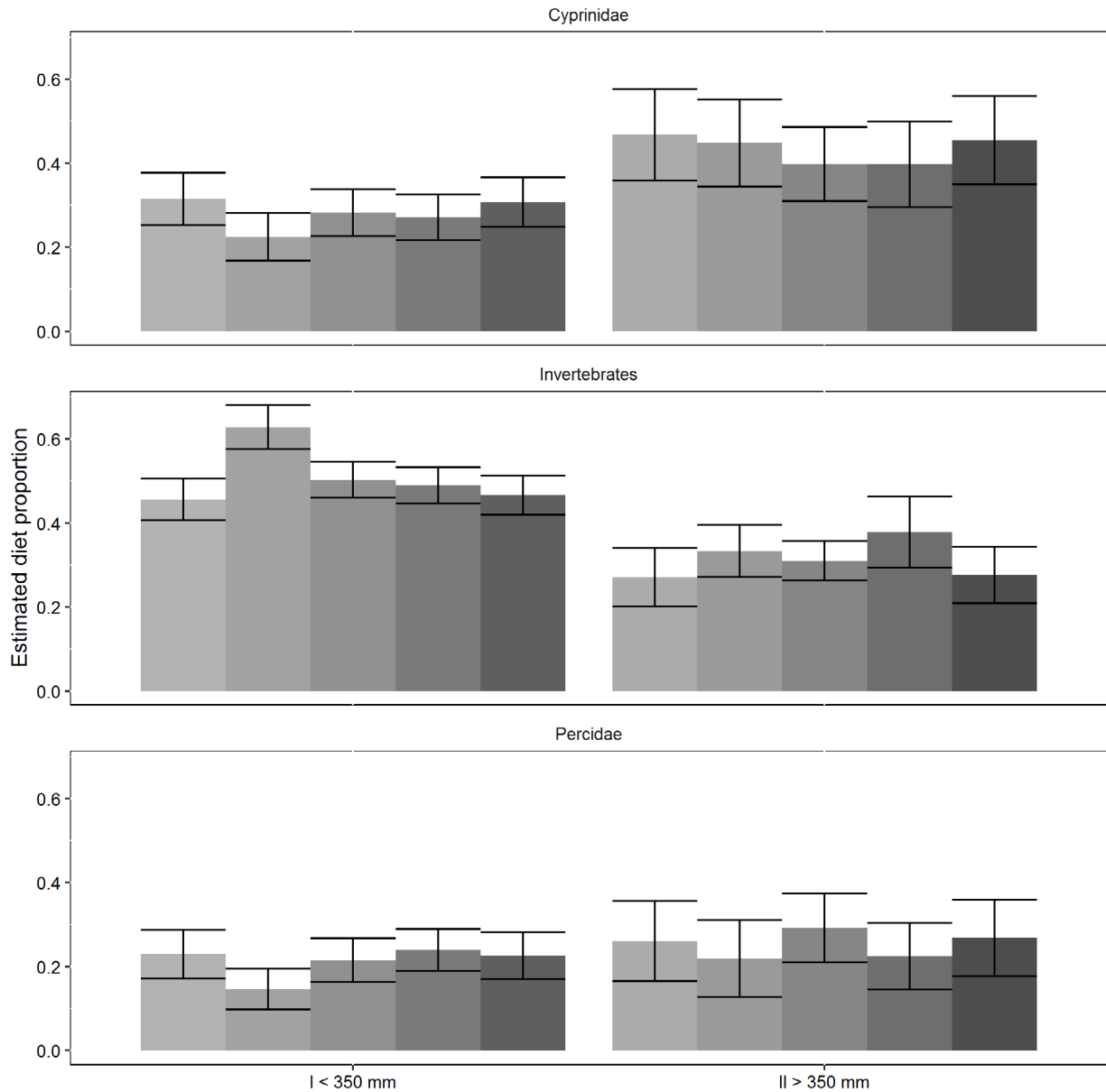
**Fig. 4.** Mean predicted dietary contributions (0–1) of 'Cyprinidae', 'Invertebrates' and 'Percidae' to the diet of *S. lucioperca* by size class (<350 and >350 mm) for each Bayesian mixing model. Models are represented by colour in sequence from light to dark, where model 1 is represented by light grey and model 5 by dark grey, and error bars represent the standard deviation. Mixing models were (1) consumer as muscle values, (2) consumer as mucus values, (3) consumer as scale values, (4) consumer as muscle values based on conversion using the linear models for $\delta^{13}C$ and $\delta^{15}N$ mucus to muscle, and (5) consumer as muscle values based on conversion using the linear models for $\delta^{13}C$ and $\delta^{15}N$ scale to muscle.

and Percidae from 221 to 464 mm. Grouping *S. lucioperca* into the two size classes of <350 mm (194–340 mm, $n=41$) and >351 mm (352–532 mm, $n=41$) revealed the percentage prey abundance was higher for invertebrates in the smaller size category (<350 mm = 15.8%) than in the larger size class (>350 mm = 0.5%). For Percidae, the opposite pattern was evident, with higher % $P_i$ for Percidae in the larger size class (>350 mm = 20.3%) than in the smaller size class (<350 mm = 3.7%). Percentage prey abundance remained similar for Cyprinidae in both size classes (<350 mm = 80.5%, >350 mm = 79.3%; Tab. 4).

## 4 Discussion

The predictable relationships between the SI data of dorsal muscle and from fins, scales and epidermal mucus revealed that the tissues that can be collected by non-destructive methods can be used reliably within trophic studies on *S. lucioperca*, negating the collection of dorsal muscle samples. Mucus and fin showed no significant differences in isotope values compared to muscle, while scale was significantly depleted in $\delta^{15}N$ and enriched in $\delta^{13}C$. Moreover,
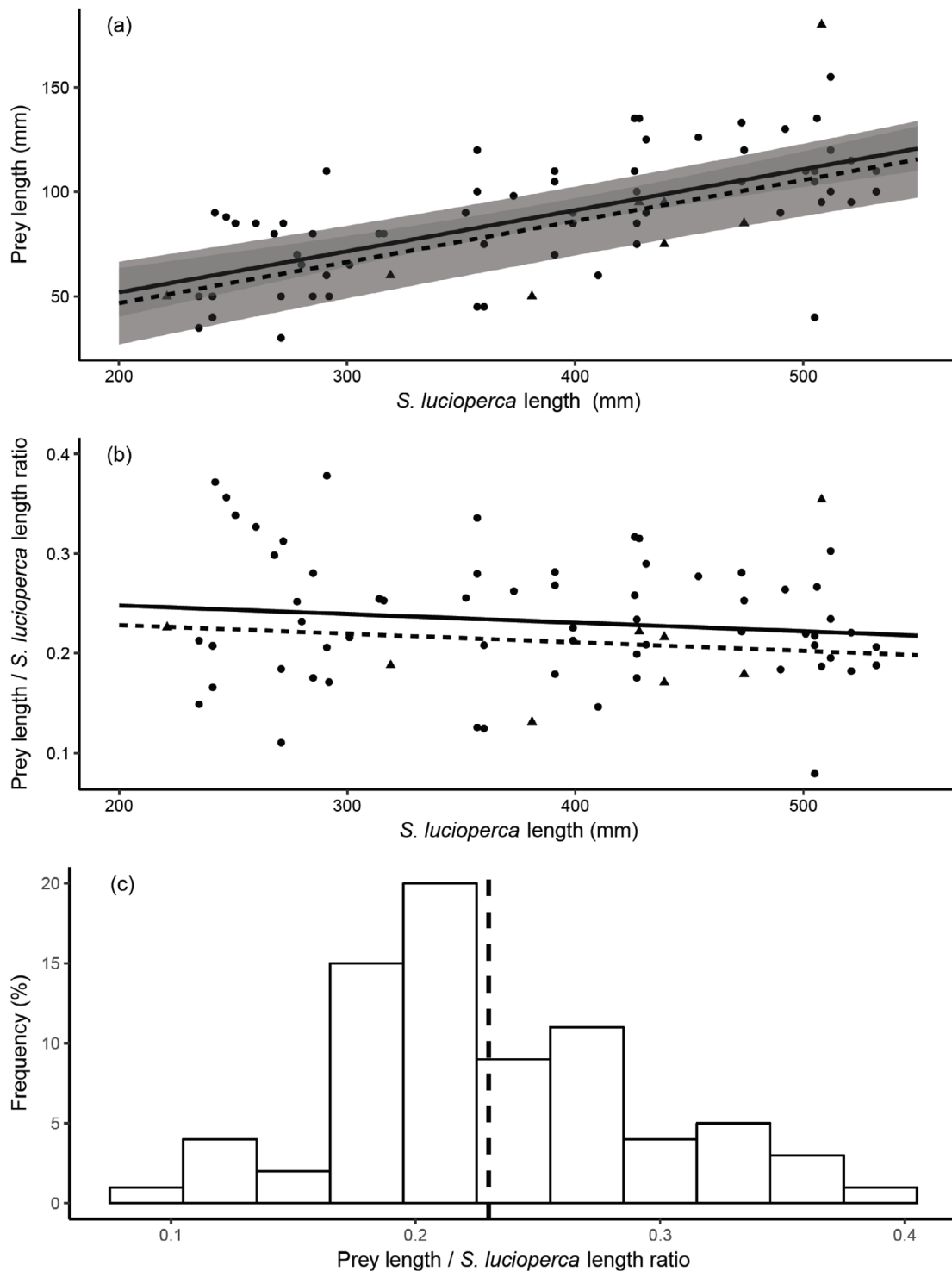
**Fig. 5.** *S. lucioperca* predator–prey relationships in the Grand Union Canal. (a) Predator size to prey size linear relationships (with 95% confidence intervals) for 'Cyprinidae' (● solid line) and 'Percidae' (▲ dashed line). (b) Prey: predator length ratios versus *S. lucioperca* body length, where lines represent relationships according to linear regression for 'Cyprinidae' (● solid line) and 'Percidae' (▲ dashed line). (c) Relative frequency distributions of prey: predator length ratios, where the mean prey size to predator size ratio is shown at 0.22.

the data provided here enables the application of the SI data from these tissues to Bayesian mixing models for predicting diet composition from putative prey SI data (Parnell *et al.*, 2013; Phillips *et al.*, 2014; Stock *et al.*, 2018). In this study, the

diet composition predictions from Bayesian mixing model results were broadly similar to those from stomach content analyses. The addition of stomach contents analysis, however, also provided data on the structured feeding relationships of

**Table 4.** Frequency occurrence (% $F_i$) and prey-specific abundance (% $P_i$) of diet by prey types 'Cyprinidae', 'Percidae' and 'Invertebrate' for *S. lucioperca* from the Grand Union Canal.

| Prey type | Size class | (% $F_i$) | (% $P_i$) |
| --- | --- | --- | --- |
| Cyprinidae | 194–532 mm | 70.2 | 79.8 |
| | 194–340 mm | 73.2 | 80.5 |
| | 352–532 mm | 67.9 | 79.3 |
| Percidae | 194–532 mm | 21.3 | 13.1 |
| | 194–340 mm | 9.8 | 3.7 |
| | 352–532 mm | 30.2 | 20.3 |
| Invertebrates | 194–532 mm | 8.5 | 7.1 |
| | 194–340 mm | 17.1 | 15.8 |
| | 352–532 mm | 1.9 | 0.5 |

these non-native piscivorous fish and their prey, revealing that these *S. lucioperca* were consuming small prey sizes relative to their body size. Finally, where diet assessments are being made in catch and release fisheries, the results suggest that tissue collection can successfully involve anglers, such as through scale collection (Kopp *et al.*, 2009; Amat Trigo *et al.*, 2017). In turn, this can help engage the public in research and build support for the conservation and management of aquatic resources (Cooke *et al.*, 2013; Elmer *et al.*, 2017; Arlinghaus *et al.*, 2017).

The stomach contents analysis of this *S. lucioperca* population emphasised an inherent problem with the method; despite 180 fish being sampled, 98 had empty stomachs. Moreover, other studies that have utilised greater numbers of *S. lucioperca* have also reported this as an issue with, for example, over 20% of 376 sampled individuals having empty stomachs in a sample from an Iberian reservoir (Pérez-Bote and Roso, 2012), an average of 57.5% of *S. lucioperca* stomachs reported to be empty across seasons and years in a German lake (Schulze *et al.*, 2012) and 42% of 591 sampled *S. lucioperca* from Lake Peipsi in Estonia with empty stomachs (Kangur and Kangur, 1998). Additionally, high proportions of empty stomachs could be due to the sampling period, as data were collected during the spawning period (Lappalainen *et al.*, 2003), which is known to be associated with reduced feeding in other piscivorous fishes (Dörner *et al.*, 2003). Where this type of sampling regime is considered problematic, such as where it removes large numbers of fish from fisheries, where *S. lucioperca* (or other piscivorous sport fishes) is an important target species for C&R (Hickley and Chare, 2004), stable isotope analysis clearly has high utility as a non-destructive dietary analysis tool.

Studies on the relationships of the SI values of fish dorsal muscle versus fin and scale tissues have shown that whilst differences in $\delta^{15}$N are usually minor and often non-significant, there tends to be predictable shifts in $\delta^{13}$C between the tissues (*e.g.* Pinnegar and Polunin, 1999; Tronquart *et al.*, 2012; Vašek *et al.*, 2017). For example, in cyprinid fishes such as chub *Squalius cephalus*, barbel *Barbus barbus* and goldfish *Carassius auratus*, there was a predictable pattern of significant $\delta^{13}$C enrichment from muscle to fin to scales (Busst *et al.*, 2015; Busst and Britton, 2016). This pattern of

$\delta^{13}$C enrichment between these tissues was also apparent here for *S. lucioperca*, although only significant from scales to muscle, mucus and fin. For epidermal mucus, however, studies have only recently started to determine how its SI values compare with other tissues, with limited differences in $\delta^{15}$N but with more variability in $\delta^{13}$C (*e.g.* Shigeta *et al.*, 2017). Here, it was revealed that differences in $\delta^{13}$C between mucus and muscle were primarily in mucus being depleted, a contrast to fin and scales. In a study of catfish (*Silurus asotus*), there was also a general trend of depleted $\delta^{13}$C values of mucus relative to muscle (Maruyama *et al.*, 2017), and depleted relative to both muscle and fin in three freshwater cyprinid species (Shigeta *et al.*, 2017). The tissues used in this study are also known to have considerable differences in their stable isotope turnover rates, with mucus generally having shorter half-lives when compared with fin and scale tissues (Church *et al.*, 2009; Maruyama *et al.*, 2017; Shigeta *et al.*, 2017). The complementary use of these tissues in SIA could therefore provide insights into diet over different timescales, although this was not able to be assessed here. The use of mucus in fish isotope studies is still relatively new compared to tissues such as muscle and fin (Church *et al.*, 2009; Maruyama *et al.*, 2015, 2017). As such, further development work is needed, both specifically for *S. lucioperca* and for fishes more generally, with increased focus required on the isotopic relationship of mucus with other tissues, their turnover rates and their fractionation factors with prey (Heady and Moore, 2013). This work should then enable the wider application of epidermal mucus to fish stable isotope studies, with this potentially highly advantageous due to its ability to be collected by non-invasive sampling techniques from live fish.

The results of both dietary assessment methods here revealed that this *S. lucioperca* population was functioning as an obligate piscivore, but only in its larger sizes. Some ontogenetic dietary shifts were evident, with smaller individuals having diets that included macroinvertebrates. Whilst *S. lucioperca* tend to switch to piscivory during the first year of life (Mittelbach and Persson, 1998), predictions from the Bayesian mixing models here suggested higher dietary contributions of invertebrates than fish for pikeperch <350 mm, where all fish were greater than 1 year old (E. Nolan, unpublished data). This pattern was also reflected in stomach content analyses. Obligate piscivory in *S. lucioperca* has been reported in a number of studies (*e.g.* Campbell, 1992; Kangur *et al.*, 2007; Pérez-Bote and Roso, 2012), with the benefits of becoming piscivorous early in life being well documented (Mehner *et al.*, 1996; van Densen *et al.*, 1996; Mittelbach and Persson, 1998). However, in the absence of suitable-sized prey fish, *S. lucioperca* will continue to consume invertebrates species (Ginter *et al.*, 2011), but are likely to grow slower than those that are completely piscivorous (Persson and Brönmark, 2008).

The stomach contents analysis of this *S. lucioperca* population also revealed that as *S. lucioperca* length increased, their prey fish size significantly increased, but that prey length to predator length ratios ranged between 0.08 and 0.38. These ratios were similar to those of Keskinen and Marjomäki (2004), who also revealed that while lengths of *S. lucioperca* and their prey were positively correlated, their prey:predator size ratio was negatively correlated. Most prey were thus

relatively small to the size of the predator, an outcome that cannot be explained by gape size limitations alone (Dörner et al., 2007). There was also no significant relationship between S. lucioperca length and $\delta^{15}N$ values, indicating that larger individuals were generally not feeding at higher trophic levels than smaller individuals (Post, 2002). Active prey choice is thought to be more important in explaining diet patterns in S. lucioperca than passive selection mechanisms (Turesson et al., 2002). This behavioural trait could explain the trends seen here, indicating that in the absence of suitable-sized fish prey, S. lucioperca will utilise the resources available (i.e. invertebrates), but when fish prey are available, prey sizes are chosen, which give the highest energy return per time spent foraging. These results on prey sizes highlight the value that SCA data can provide SIA studies, albeit with the caveat that its use is destructive or, if using non-lethal stomach evacuation techniques, is invasive to the individual fish.

Pikeperch also usually occupy higher trophic positions than other piscivorous fishes, with this apparent from across a range of habitat typologies (Campbell, 1992; Kangur and Kangur, 1998; Keskinen and Marjomäki, 2004). This has been attributable to their piscivory of omnivorous cyprinid fishes (Keskinen and Marjomäki, 2004) and, in larger S. lucioperca, on other piscivores such as perch P. fluviatilis (Kopp et al., 2009). Other studies have also highlighted that cannibalism can be feature of S. lucioperca diet that tends to increase in importance with lengths over 250 mm (Campbell, 1992; Didenko and Gurbyk, 2016; Hempel et al., 2016), and so can help explain the high trophic position of larger individuals versus other piscivores (Kopp et al., 2009). The results here are generally consistent with these findings, with both R. rutilus and P. fluviatilis being the principal prey items encountered in stomachs. However, there was minimal evidence suggesting that these S. lucioperca were cannibalistic. This might be explained by the time of sampling, as young-of-the-year (YOY) S. lucioperca would not have been present in the population due to timing of spawning (Lappalainen et al., 2003). Both inter- and intra-cohort cannibalism in pikeperch has been shown to correlate with the density of juveniles in a population (Frankiewicz et al., 1999; Lappalainen et al., 2006). Indeed, cannibalism in S. lucioperca is seen as a key regulatory force in some populations (Mehner et al., 1996; Frankiewicz et al., 1999; Lappalainen et al., 2006). This again points to the limitations of the stomach content analyses in providing accurate dietary assessments, as it was only completed at a single time of year.

In summary, this study has provided relationships on the stable isotope data of a range of tissues from S. lucioperca. The application of these data to Bayesian mixing models predicted strong ontogenetic dietary patterns, with shifts from macro-invertebrates/fish to fish only as S. lucioperca length increased. These ontogenetic patterns were similarly evident in SCA, but with these data also highlight that as S. lucioperca length increased, their prey size significantly increased, although prey items remained relatively small. In entirety, these results suggest that when non-destructive sampling is required for sport fishes such as S. lucioperca, SIA can be used to provide robust dietary assessments. However, if SCA can be completed, then it can provide dietary data that are complementary to SIA and so help provide greater insights into their piscivory and predation pressure on native prey fishes.

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