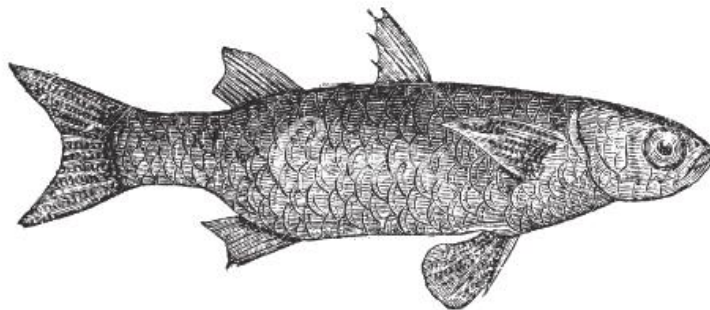
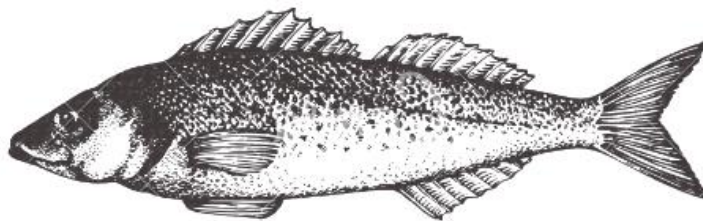




Insight into the dietary preferences of adult European seabass (*Dicentrarchus labrax*) and thicklip grey mullet (*Chelon labrosus*) in the Dutch Wadden Sea through bulk stable isotope analysis



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Utrecht University – Master of Bioinspired Innovation
Master thesis, Utrecht University 2022-2023

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1. Layman summary

The Wadden Sea is located in the southeastern part of the North Sea. This area of great ecological importance is currently facing major threats, such as anthropogenic climate change-induced temperature and sea level rise. From the 1980s onwards, the number of large fish has been in decline in the Wadden Sea. The research in this paper pertains to the Swimway project, whose aim is to investigate how fish use the Wadden Sea, what pressures they face there, and what management measures would be effective at improving this habitat for both fish species. The Swimway project is an initiative by the Royal Netherlands Institute for Sea Research (NIOZ), the University of Groningen (RUG), Wageningen Marine Research (WMR), and the Wadden Association, although others such as Rijkswaterstraat and Sportvisserij Nederland are involved too.

The goal of this study is to provide a greater understanding of the diet of adult European sea bass and thicklip grey mullet caught in the Wadden Sea. Although these two large fish species have largely differing diets and life history strategies, they are both considered to be partially migratory species, meaning that their population is composed of both migratory and resident individuals. The thicklip mullet is one of many fish species whose numbers have decreased in the Dutch Wadden Sea since the 1980s. On the other hand, the European sea bass has shown an opposing, increasing trend in abundance during the same time period. In this study, their diet is explored using bulk carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) stable isotope analysis (SIA) of their liver, muscle, and fin tissue. Stable isotopes are different forms of an element that have the same number of protons but a different number of neutrons and do not show radioactive decay. The heavy isotope form has a greater number of neutrons; whilst the form with fewer neutrons is isotopically light (Hobson and Wassenaar, 2008). The greater abundance of one of the isotope forms provides us with the opportunity to use the rarer form as a geochemical marker (MacNeil, 2006; Peterson and Fry, 1987). In summary, this approach is based on the tissue stable isotope composition performing as a natural chemical tag by revealing the necessary information to reconstruct the dietary history of the fish (MacNeil, 2006; Post, 2002; McMahon et al. 2013). Our results revealed that sea bass preferred prey item was flatfish, whilst for thicklip mullet it was the macroalgae *Ceramium rubrum*. When comparing our results to data from the stomach contents of adult sea bass and thicklip mullet from the same sampling group, the prey preferences for both fish differed between the two techniques. Although many factors come into play, the reason for this difference could be partially as a result of temporal differences in the data retrieved between the techniques. Prey data retrieved from stomach content analysis only reflects the most recent food items ingested.

A secondary aim of this study was to investigate the tissue turnover rate of the fin tissue in comparison with muscle and liver tissue. The tissue turnover rate is the rate for the isotopic composition of the consumer tissue from the past diet to be replaced with the ratio from the more recent diet. Muscle tissue has a longer turnover rate when compared to liver tissue, meaning that it reflects a longer, less recent period of feeding. Increased knowledge on the turnover rate of fin tissue would be useful as it would permit us to analyze diet at a greater temporal resolution using SIA in a non-lethal manner, which is applicable for tagged fish. This is suitable for this project, as parallel to this study and as part of the Swimway project, *D. labrax* and *C. labrosus* movements in the Dutch Wadden Sea were being tracked using acoustic tags (personal communications with Jena Edwards).

2. Abstract

Here, we aim to provide new insights into the diet of adult *C. labrosus* and *D. labrax* caught from summer to autumn in the Dutch Wadden Sea through bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ tissue stable isotope analysis. Our stable isotope

mixing model reveals that for *D. labrax*, flatfish sp. are the largest contributors to their diet, followed by *Clupea harengus*, *Carcinus maenas*, and *Crangon crangon*. For *C. labrosus*, their main prey items appear to be the macroalgae *Ceramium rubrum*, followed by *Corophium* sp., and green microphytobenthos. For both fish species, the prey preferences derived from this study's stable isotope mixing models differ from those obtained from stomach content prey frequency analysis from the same sampling group. This could be a result of the temporal differences in the data retrieved between the techniques and the partially migratory nature of both fish species, amongst other reasons discussed. A comparison in the stable isotope values between *D. labrax* caught in three regions in the Dutch Wadden Sea - Terschelling, Balgzand, and Eierlandse Gat - reveals their diet is more similar between Eierlandse Gat and Terschelling in comparison to that in Balgzand. Our results also show *D. labrax* and *C. labrosus* length and sex had a significant influence on tissue $\delta^{13}\text{C}$ values, but not on $\delta^{15}\text{N}$ values. This suggests similar trophic level feeding but differing spatial foraging habitats between fish of differing lengths and sexes. A secondary aim of this study was to determine whether the dorsal fin stable isotope signature was more correlated to that of the species' liver or muscle SIA. Fin tissue is preferable for SIA over liver or muscle tissue because the non-lethal nature of fin tissue collection means it can be applied for the diet analysis of tagged fish. For both fish species, fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were more correlated to that of muscle than liver tissue.

3. Introduction

The Wadden Sea is a temperate shallow intertidal area located in the southeastern part of the North Sea extending along the Dutch, German and Danish coasts. This dynamic landscape is the largest unbroken intertidal sand and mudflat system in the world and was appointed as a World Heritage Site by UNESCO due to its unique ecological and geological value (UNESCO World Heritage, n.d.). The inner eastern side of the Wadden Sea is connected to various estuaries and thus has a strong freshwater discharge influence. On the other hand, the outer western Wadden Sea, which is delimited by the Frisian islands, is greatly influenced by the North Sea's higher marine salinity (see Figure 2). The Wadden Sea provides various ecosystem services, including its role as an important migration stop for many bird species (Essink et al., 2005; Reise et al., 2010), serving as a rich aquatic nursery for commercially important fish (van der Veer et al., 2001), and functioning as a migration stop for marine fish seasonal visitors when conditions are suitable (Essink et al., 2005).

The Wadden Sea is made up of a diverse fish community and is an important area for many fish species, including both permanent resident species and fish that depend on this area solely for part of their lifecycle (Tulp et al., 2017). In the past decades, there has been a large decline in fish fauna in the Wadden Sea (Rijke Waddenzee, 2004; Tulp et al., 2017, 2008; van der Veer et al., 2015). This was largely made evident in Van de Veer et al. (2015)'s study where an analysis of the daily spring and autumn fish abundance and diversity between 1960 and 2011 from an ongoing fyke net program in the Dutch Wadden Sea showed an increase in abundance from 1960 peaking in 1980, which was followed by a dramatic ten-fold decrease in total fish biomass from 1980 to 2010 (van der Veer et al., 2015). In the case of the European sea bass (*Dicentrarchus labrax*), despite some small short-term declines, their numbers in the Dutch Wadden Sea have shown an overall increase since 1980 (Cardoso et al., 2015; van der Veer et al., 2015). The thicklip grey mullet (*Chelon labrosus*) has shown an increasing trend in species abundance from around 1960 – 1975, with peak catches in the 1970s, followed by a continuous decrease (van der Veer et al., 2015). Currently, there is a scarcity of knowledge on not only the drivers that cause these declines, but on the lifecycles and the role played by the Wadden sea for different fish species (Rijke Waddenzee, 2004). Improving our understanding of both is advisable to create more effective conservation and management measures to restore their numbers.

The main aim of this study is to obtain an insight into the diet preference of adult *D. labrax* and *C. labrosus* in the Dutch Wadden Sea using bulk stable isotope analysis (SIA). Since the heavy, rarer nitrogen ($\delta^{15}\text{N}$) isotope is consistently and gradually enriched at each successive trophic step within a food chain, consumer tissue $\delta^{15}\text{N}$ is indicative of prey trophic position (MacNeil, 2006; Perkins et al., 2014). Conversely, in the case of carbon, the heavier and rarer $\delta^{13}\text{C}$ isotope is highly conserved from diet to consumers, and therefore $\delta^{13}\text{C}$ can be used to detect habitat-use variation by identifying the source of primary energy (Caut et al., 2009; MacNeil, 2006; Perkins et al., 2014; Poiesz et al., 2021; Post, 2002, 2002; Shipley and Matich, 2020).

A secondary aim of this study is to determine whether *D. labrax* and *C. labrosus* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dorsal fin stable isotope signature is more correlated to that of the species' liver or muscle SIA, and thus could possibly be used as a reliable ex-situ non-lethal proxy for either in future studies. Contradicting knowledge exists on the relationship of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between fin and non-lethally sampled tissue, with some studies showing fin to have a similar and closely correlated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to that of muscle (Busst et al., 2015; Busst and Britton, 2018; Suzuki et al., 2005), while others showing it to be more similar to that of liver (McCloskey et al. 2018). Thus, the development of a species-specific model is necessary.

Our study is of value by providing new insights into the diet of the *C. labrosus* and *D. labrax* in the Wadden Sea using SIA, which may have important spatial and temporal management implications for these fish and for the Wadden Sea, an area of high natural value that currently faces major threats by human activities. The study also aims to provide an insight into the use of dorsal fin SIA as a possible tool for detecting the diet of *D. labrax* and *C. labrosus*, which would permit us to analyze consumer diets in a non-destructive and non-lethal manner and thus could be applied to tagged individuals (Busst and Britton, 2018).

4. Materials and methods

4.1. Sample collection

D. labrax and *C. labrosus* adult individuals were captured in different locations of the Dutch Wadden Sea between spring and summer from June 2021 to September 2022. Fish were captured from a small-scale commercial gillnet fishing boat. Adult fish were frozen, their serial number, site of capture, total length, and sex were recorded; and liver, muscle, and dorsal fin samples were collected in plastic Eppendorf tubes. The muscle sample comprises a skinless and boneless white or red muscle sample excised from just in front of the caudal fin. All samples collected were stored frozen until processing. Figure 1 shows a photographic depiction of the sampling locations on the fish.

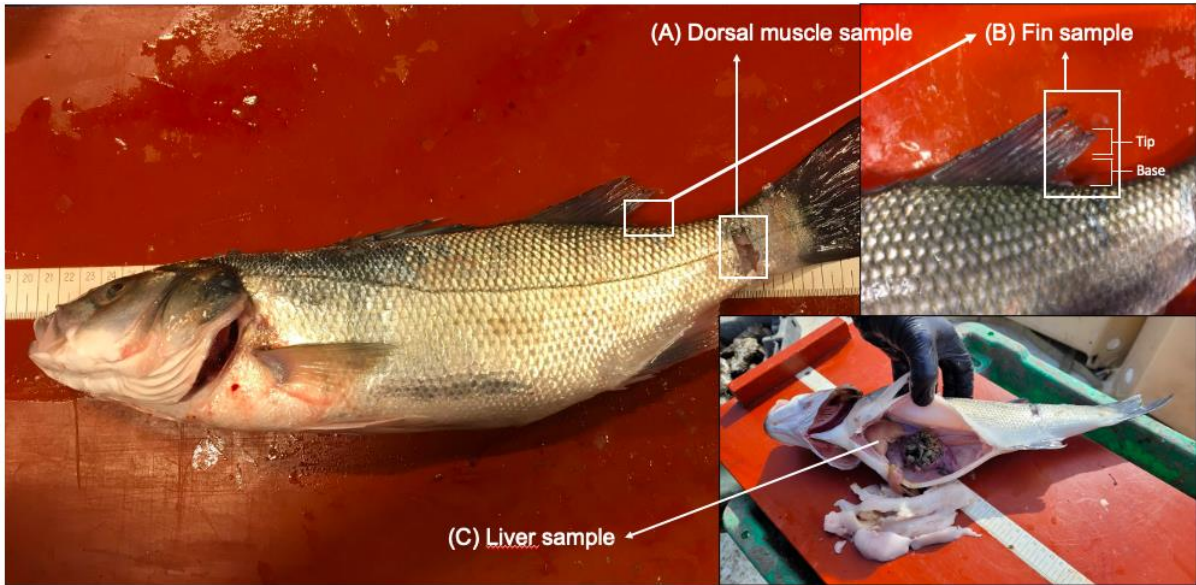


Figure 1: Photograph highlighting the locations of the (a) dorsal muscle, (b) fin, and (c) liver samples collected from each fish. Images provided courtesy of S. Popma and J. Edwards.

4.2. Sample selection

D. labrax individuals in this study were selected from three different locations within the Dutch Wadden Sea, named Balgzand, Eierlandse gat, and Terschelling. On the other hand, *C. labrosus* individuals selected were caught in Terschelling. Figure 2 depicts the sampling locations of the *D. labrax* and *C. labrosus* chosen for this study. 31 *D. labrax* (13 M, 18 F) and 18 *C. labrosus* (13 F, 5 M) were selected whose total length (TL) ranged between 40,8 – 50,4 cm and 46 – 59.5 cm respectively, corresponding well with adult fish.

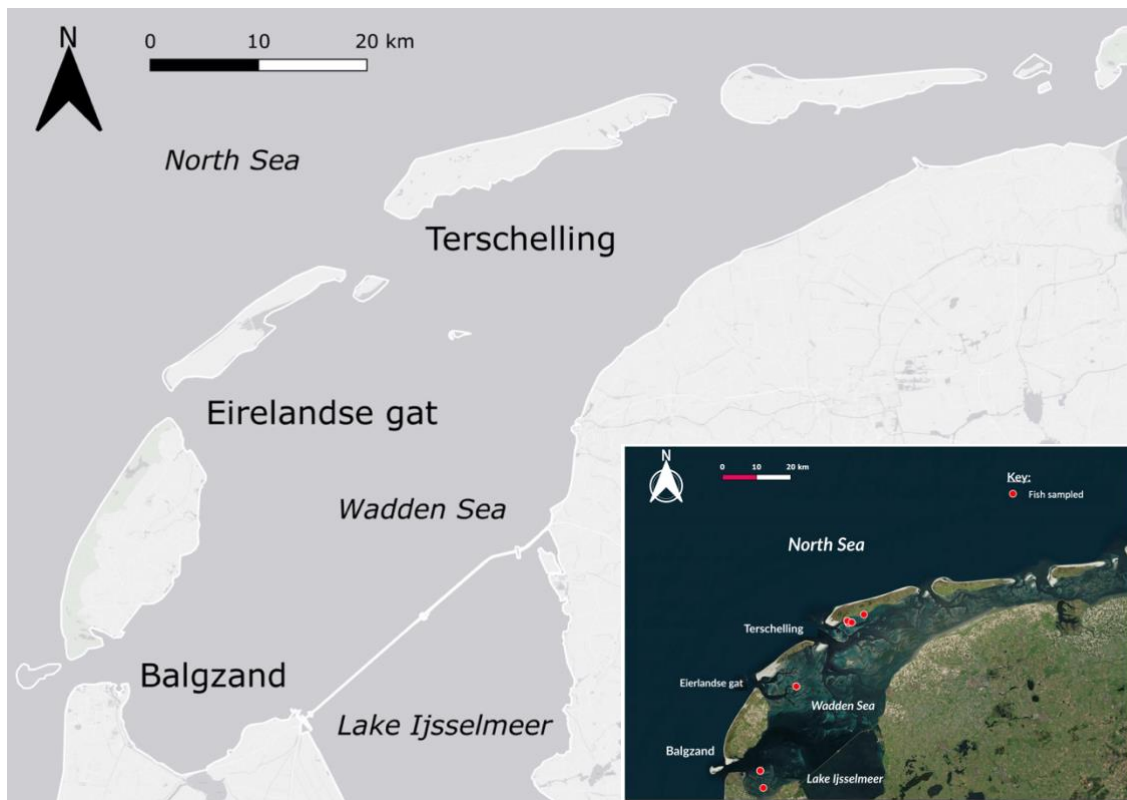


Figure 2: Map of the Dutch Wadden sea illustrating the capture locations used in this study.

4.3. Sample preparation

All samples were freeze-dried to constant weight for 24 h.

Fin samples: The dried fin sample was cut with scissors until the target mass was attained (2 ± 0.01 mg [mean \pm SD], range 1.7 to 2.3 mg). The tip or base section of the fin were used indiscriminately, as studies have shown only a slight difference in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the base and tip due to differences in composition between bone and membrane ratios driving variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of ~ 1 ‰ (Hayden et al., 2015).

Decalcification was not conducted on the fin samples, as experiments have found no benefit of decalcification on this tissue (Graham et al., 2013; Hayden et al., 2015). To remove any uncertainties we also conducted a practical and quick test for the detection of carbonates in the sample by applying HCl drops on the fin to examine whether bubbles of CO_2 formed implying the presence of carbonates (Schlacher and Connolly, 2014). No bubbles formed. Fish tissue varies in lipid content, and if lipid content is too high it must be removed prior to analysis to minimize $\delta^{13}\text{C}$ variance. Lipid correction is not deemed necessary when C/N ratios are below 4 (Hanisch et al. 2010; Graham et al 2013). In the case of fin tissue, preliminary results for fin samples ($n=30$) revealed fin tissue C/N ratio was below 4 (C/N mean = 3.52 ± 0.01 , range 3.15 to 3.98), so lipid correction was not undertaken. The weighed-out fin samples were loaded into tin capsules for analysis.

Muscle samples: The dried muscle samples were homogenized into a powder with a mortar and pestle. Samples weighing 1 ± 0.01 mg (range 0.7-1.3 mg) were loaded in tin capsules and run for SIA. Although the mean C:N ratio was below 4 (mean C/N: 3.87 ± 0.72 , range 3.20 to 6.0 for *D. labrax*, and 3.9 ± 1.0 range 3.3 to 6.7 for *C. labrosus*), some individual values were greater than 4, so precautionary lipid extraction prior to analysis should have ideally taken place to prevent these individual's high lipid content influencing the results for $\delta^{13}\text{C}$ (Graham et al., 2013).

Liver samples: The dried liver samples were homogenized after freeze-drying into a powder. Due to this tissue's known high-lipid content, lipids were extracted to remove their influence on the $\delta^{13}\text{C}$ value. Lipids were removed by treating the samples with three repeated immersions in a 3/2 hexane/DCM solution until the supernatant liquids were clear, indicating that lipids have been removed. Following the final immersion, each sample was heated and dried again using nitrogen gas. Then, 1 ± 0.01 mg (range 0.7 to 1.3 mg) was loaded onto tin capsules for analysis.

4.4. Consumer stable isotope analysis

Bulk carbon and nitrogen SIA analysis was performed at the Royal Netherlands Institute for Sea Research laboratory (NIOZ) laboratory in Texel, the Netherlands. Most samples were analyzed using a Delta V Advantage Isotope ratio mass spectrometer with a Flash 2000 Organic Element Analyser (Thermo Fisher Scientific). 29 of the fin samples were combusted in a different machine, an elemental analyzer (vario ISOTOPE cube, Elementar, Germany) coupled via continuous flow to an isotope ratio mass spectrometer (Isoprene visiON, Elementar, UK). The differences in the machine used should not interfere with the results as in both cases, the same standard (acetanilide) was included in each run for system calibration and to compensate for drift over time.

Isotope ratios were expressed in delta (δ) and defined as parts of per thousand (‰) relative to the standard as follows

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where δX is the delta value of the sample for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, R is the ratio of heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$, $\delta^{15}\text{N}/\delta^{14}\text{N}$), and the R_{standard} is Vienna Pee Dee Belemnite (VPDB) for C and atmospheric N for N.

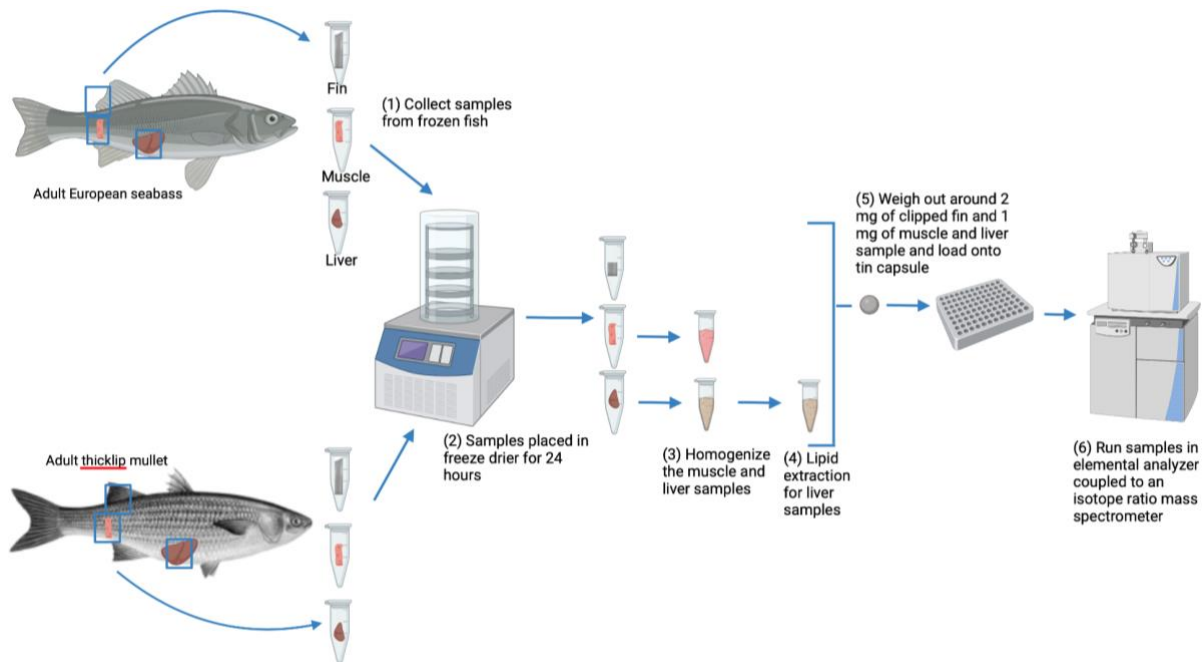


Figure 3: Illustration highlighting the method for sample analysis

5. Data analysis and modeling

All statistical analysis and graphics were carried out with the R software.

5.1. Assessing the effect of sex and length on the tissue stable isotope composition of *D. labrax* and *C. labrosus*

The significance of the interaction between sex and length on stable isotope values was first tested using an ANOVA test to compare two linear regression models (*lm* function from the *lm4* package in R), one with the interaction (Sex*Length) and the other one without the interaction (Sex + Length). An ANOVA test showed that the model with interaction did not explain significantly more variation ($p > 0.05$) than the model without the interaction for both ^{13}C and $\delta^{15}\text{N}$, suggesting there is no significant interaction between sex and length and thus the linear regression model without the interaction was used in order to determine the association between adult *D. labrax* and *C. labrosus* fish length and sex and tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values. We undertook a linear mixed-effects model with length and sex as the explanatory variables, isotope value as the response variable, and tissue as the nested random effect to accommodate for repeated measurements of the same individuals for each tissue. We ran separate models for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and for each species. The linear mixed effect model was constructed using the *lmer* function from the *lme4* package in R. As *lmer* does not give a p-value as the correct degrees of freedom for the model are not obvious, we then used the R package *lmerTest*

to calculate the degrees of freedom from Satterthwaite's approximation. Assumptions of normality of residuals and homogeneity of variances were checked by inspecting residual plots.

5.2. Assessing the effect of location on stable isotope composition of *D. labrax* tissues

We conducted an ANOVA test in R to determine whether there was a significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes between the three locations (Balgzand, Terschelling and Eierlandse Gat) for each tissue. If a significant difference between the groups was observed, a Tukey's posthoc analysis was conducted to estimate all pairwise differences and keep Type I error rate at 5%.

5.3. Assessing the use of fin tissue as an alternative to liver and/or muscle tissue

Paired t-tests were conducted to determine whether there was a significant difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between (a) muscle and fin and (b) liver and fin tissues. A linear regression analysis was then conducted to estimate dorsal fin $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (response variable) from liver and muscle $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (explanatory variable) (lm function from the *lme4* package in R). We ran separate models for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes, and for each species. The same individuals were compared between tissues. The normality of the residuals and homoscedasticity were checked by inspecting residual plots.

5.4. Determining *D. labrax* and *C. labrosus* isotopic niche using SIBER

We used the package SIBER in R to fit bivariate (x and y) ellipses containing 95% of observations around the isotopic data of each group in isotopic space to compare the breadth of each isotopic niche. Separate SIBER models were run to determine isotopic niche differences between fish species. Analyses on the overlap between ellipse areas were performed on Bayesian estimates of the standard ellipse areas (SEAb) to observe the degree of uncertainty around each SEA. Bayesian estimates were calculated using Markov chain Monte Carlo simulation for each group with the default uninformative priors of the ellipses.

5.5. Dietary proportions using the stable isotope mixing model SIMMR

To determine the relative contribution of each prey type to the diet of *D. labrax* and *C. labrosus* in the Wadden Sea, we performed two separate Bayesian isotopic mixing models calculated using Markov chain Monte Carlo simulation for each fish species using the R package SIMMR. By utilizing a Bayesian approach, this model can account for uncertainty by factoring mean and variance parameters in both the prey and consumer data. We checked for convergence diagnostics. The model included: (1) the consumer $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from the muscle tissue of each fish individual (*D. labrax* n=31; *C. labrosus* n=18), (2) the mean and standard deviation for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from each prey species making up $\geq 2.5\%$ of the diet of the fish species extracted from stomach content data, and (3) an estimated trophic enrichment factor (TEF) for each fish species. No species-specific TEF reference values were available in the literature for either *D. labrax* or *C. labrosus*. We solved this shortcoming for *D. labrax* by estimating the TEF from values provided by McCutchan et al. (2003), where they estimated TEFs from a combination of data from the published literature and original data. The $\Delta^{13}\text{C}$ used for the mixing model was $1.3 \pm 0.30\text{‰}$, which is the value from McCutchan et al. (2003) extracted for consumers analyzed as muscle. For $\Delta^{15}\text{N}$ we used the value $2.35 \pm 0.24 \text{‰}$, which is the intermediate value for consumers raised on a high protein diet ($3.3 \pm 0.26 \text{‰}$) and consumers raised on an invertebrate diet ($1.4 \pm 0.21\text{‰}$), seeming appropriate as both non-invertebrate high-protein prey and invertebrate prey make up the Wadden Sea *D. labrax* diet (McCutchan et al., 2003). In the case of *C. labrosus*, we chose the TDF value obtained from an experimental feeding study on the isotope fractionation in the muscle tissue of the mullet *Mugil liza*, a fish species from the

same Mugilidae family which shares the same detritivorous feeding habits as the *C. labrosus* (Oliveira et al., 2017). Values were 2.8 ± 0.5 ‰ for $\Delta^{13}\text{C}$ and 4.3 ± 0.4 ‰ for $\Delta^{15}\text{N}$ (Oliveira et al., 2017). We estimated both models with non-informative priors.

5.5.1. *D. labrax* and *C. labrosus* prey data used for the SIMMR mixing model

Prey item frequency was recorded from stomach content analysis from 75 *D. labrax* and 16 *C. labrosus* caught in the Dutch Wadden Sea between spring and summer from June 2021 to September 2022. *D. labrax* analyzed were caught in Terschelling (n=6), Balgzand (n=27) and Eierlandse Gat (n=42). *C. labrosus* were only caught in Terschelling. Only prey categories that comprised $\geq 2.5\%$ of prey frequency total in at least one site were selected to input in the mixing model, as the exclusion of uncommon items tends to improve mixing model accuracy. Too many prey species in the isospace plot makes it impossible to distinguish between prey items (Phillips and Gregg, 2003). For the stomach content prey frequency for both fish species including prey items contributing to $<2.5\%$ of total prey frequency, see the Appendix. As 100% of the *C. labrosus* ate sand, presumably as a source of microphytobenthos (i.e. the microscopic photosynthetic organisms living on the sediment surface dominated by diatoms and cyanobacteria), microphytobenthos were also included in the mixing model for *C. labrosus*. We excluded pieces of plastic, unknown items, and *D. labrax* and *C. labrosus* scales, which probably came in the tank whilst they were being held.

Benthic prey item $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ means and standard deviations were extracted from a subsample from the long-term monitoring SIBES sampling campaign from June to October of 2011-2014 in the Dutch Wadden Sea (Bijleveld et al., 2012; Christianen et al., 2017; Riekenberg et al., 2022). On the other hand, fish prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ means and standard deviations used in the SIMMR models were lipid-corrected values extracted from fish collected from the long-term fyke catches from NIOZ in the Dutch Wadden Sea, which took place from spring to autumn from 2012-2016 (Poiesz et al., 2021).

Various assumptions were necessary to obtain $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *D. labrax* prey species from the available data in the literature. For example, flatfish species recorded in stomach contents were assumed to be European flounder (*Platichthys flesus*) and European plaice (*Pleuronectes platessa*); Nereididae sp. were assumed to be the polychaete *Hediste diversicolor*, a scavenging benthic ragworm found at high abundance and frequency in the Wadden sea (Christianen et al., 2017), and the *Ensis* sp. from stomach content analysis was assumed to be the bivalve *Ensis directus*, an invasive species introduced in the late 1970s whose numbers rapidly increased and is has now become a commonly occurring species in the Dutch Wadden Sea (Dekker and Beukema, 2012).

Table 1: Mean bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *D. labrax* food sources considered for diet reconstruction in the Bayesian isotopic mixing model. Values were extracted from Christianen et al. (2017); Riekenberg et al. (2022); and Poiesz et al. (2021).

Name	Species name	n	$\delta^{13}\text{C}$ (‰)	Std. Dev	$\delta^{15}\text{N}$ (‰)	Std. Dev	Tissue analyzed
Shrimp	<i>Cragnon cragnon</i>	17	-14,90	1,24	13,60	1,24	Whole
Herring	<i>Clupea harengus</i>	174	-18,86	1,58	14,45	1,32	Muscle

Green Crab	<i>Carcinus maenas</i>	18	-15,80	0,85	14,00	1,27	Whole
Ragworm	<i>Hediste diversicolor</i>	17	-16,50	1,24	12,30	1,65	Whole
Razor clam	<i>Ensis directus</i>	9	-17,80	0,90	9,90	1,20	Foot/meat/whole
European Flounder	<i>Platichthys flesus</i>	177	-17,80	3,72	16,23	1,20	Muscle
European Plaice	<i>Pleuronectes platessa</i>	102	-16,10	1,62	14,67	1,11	Muscle
	<i>Corophium sp.</i>	19	-17,97	2,99	10,88	2,32	Whole

Assumptions were also made to obtain $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *C. labrosus* prey species from the literature. The gastropod species observed in the stomachs were assumed to be *Peringia ulvae*, a commonly found aquatic snail living in the intertidal which feeds mainly on microphytobenthos (Christianen et al., 2017); and the bivalves were assumed to be *Abra tenuis*, which lives in the high intertidal parts of the tidal flats and is also one of the most common benthic species in the Dutch Wadden Sea (Christianen et al., 2017; Holmes et al., 2004). These broad assumptions were made as a result of the taxonomical identification of one gastropod and one bivalve, which apparently closely resembled the other unidentified gastropods and bivalves in the *D. labrax* stomachs (personal communications with Gala van de Meer, 2022). The red and green macroalgae observed were presumed to consist of a mix of three macroalgal species: red horn weed, bladderwrack, and sea lettuce, which are found throughout the Wadden Sea sporadically attached to rocks and other hard surfaces (Albrecht, 1998); personal communications with Philip Riekenberg). The microphytobenthos consumed by *C. labrosus* were assumed to consist of a mixture of green and brown microphytobenthos (Riekenberg et al., 2022).

Table 2: Mean bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *C. labrosus* food sources considered for diet reconstruction in the Bayesian isotopic mixing model.

Common Name	Species name	<i>n</i>	$\delta^{13}\text{C}$ (‰)	Std. Dev	$\delta^{15}\text{N}$ (‰)	Std. Dev	Tissue analyzed
Cone mudsnail	<i>Corophium sp.</i>	19	-17,97	2,99	10,88	2,32	Whole
	<i>Peringia ulvae</i>	10	-15,1	2,52	9,2	0,95	Whole
	<i>Abra tenuis</i>	18	-13,21	1,40	8,45	2,47	All meat
Red Horn Weed	<i>Ceramium rubrum</i>	8	-18,4	2,55	11,6	0,85	Fresh leaf material
Bladderwrack	<i>Fucus vesiculosus</i>	8	-15,3	1,70	8	2,55	Fresh leaf material
Sea lettuce	<i>Ulva sp.</i>	8	-13,5	1,70	11,1	1,98	Fresh leaf material

Microphybenthos green	20	-14,2	1,34	7,3	0,89
Microphybenthos brown	25	-13,4	1,00	11,3	1,5

6. Results

6.1. Isotopic niche of *D. labrax* and *C. labrosus* in the Dutch Wadden Sea

The mean $\delta^{13}\text{C}$ value for *D. labrax* (n=31) muscle tissue was -16.27 ± 1.16 ‰ [mean \pm SE]. For *C. labrosus* (n=18) the mean muscle $\delta^{13}\text{C}$ was -14.10 ± 1.46 ‰, (see Table 3). The $\delta^{13}\text{C}$ range of benthic primary producers in the Wadden Sea is from -19.2 to -11.5 ‰, whilst the $\delta^{13}\text{C}$ range for pelagic producers is from -23.3 to 17.4 ‰, suggesting that benthic marine producers are a more important energy source for both species than pelagic producers (Christianen et al. 2017). The more enriched $\delta^{13}\text{C}$ value for *C. labrosus* suggests that another potentially important food source for this species are macrophytes ($\delta^{13}\text{C}$ values ranging between -15.2 ‰ and -10.9 ‰) (Christianen et al. 2017), a known contributor to their diet from stomach content analysis. Terrestrial, riverine, and estuarine carbon sources ($\delta^{13}\text{C}$ values ranging from -26 ‰ to -30 ‰) have little contribution to either species (Christianen et al. 2017). It is important to note that carbon is enriched per trophic transfer, so the importance of the contribution of food sources in the Dutch Wadden Sea from Christianen et al. (2017)'s analysis on primary carbon sources for consumers may be a slightly inaccurate estimate for our species. Finally, *D. labrax* muscle tissue was enriched in $\delta^{15}\text{N}$ compared to *C. labrosus* (mean *D. labrax* $\delta^{15}\text{N}$ is 16.81 ± 0.43 ‰; mean $\delta^{15}\text{N}$ for *C. labrosus* is 13.69 ± 0.75 ‰).

Table 3: Table showing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (\pm SD) of *D. labrax* and *C. labrosus* muscle data in the Dutch Wadden Sea. This data was used for the SIBER and SIMMR models.

	N	Mean $\delta^{13}\text{C} \pm$ SD (‰)	Mean $\delta^{15}\text{N} \pm$ SD (‰)	C/N
<i>D. labrax</i>	31	$-16,267 \pm 1,162$	$16,811 \pm 0,429$	$3,87 \pm 0,72$
<i>C. labrosus</i>	18	$-14,10 \pm 1,46$	$13,69 \pm 0,75$	$3,9 \pm 1,0$

Analysis of *D. labrax* and *C. labrosus* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the SIBER isospace showed no ellipse overlap between the two fish species, which was expected due to their greatly differing feeding habits (see Figure 4). The Bayesian Standard Ellipse Area (SEAb) was 1.26 ‰² for *D. labrax* (95% confidence interval= $0.92 - 1.78$) and 3.30 ‰² for *C. labrosus* (95% confidence interval= $2.00 - 5.44$). If isotopic niche breadth is a function of trophic niche, this means that *D. labrax* has a lower diet variety than *C. labrosus*.

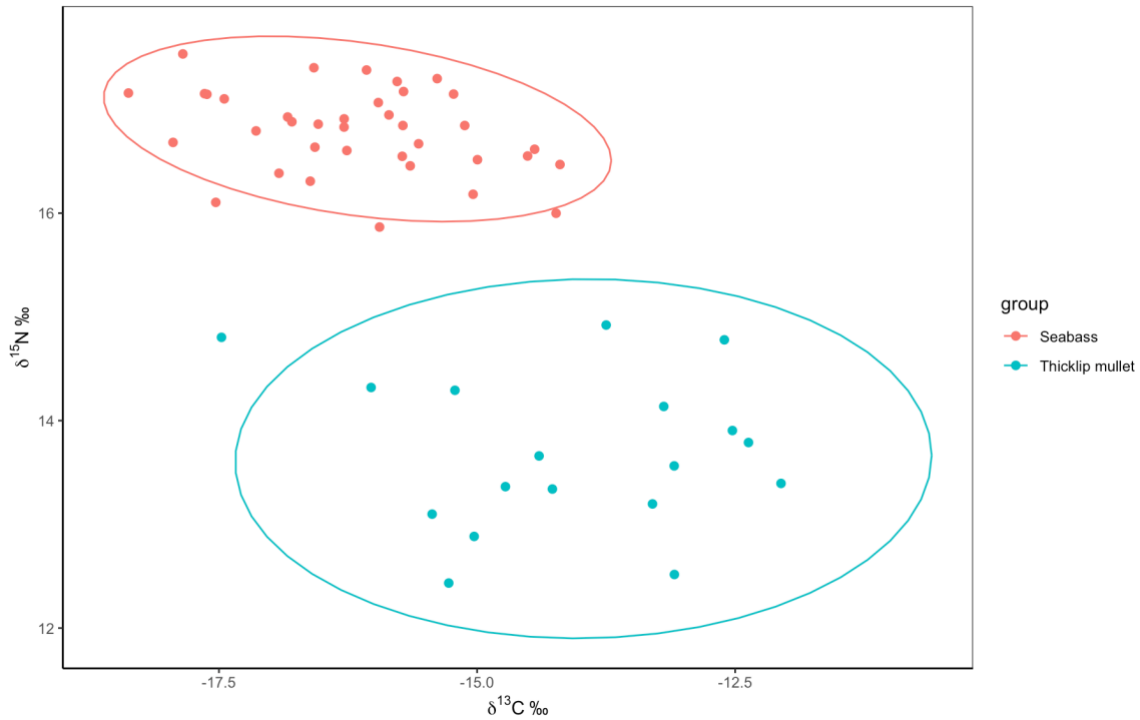


Figure 4: Ellipse area representing the core isotopic niche utilized by *D. labrax* (red) and *C. labrosus* (blue) in the Dutch Wadden Sea during summer and autumn. Isotopic niches were scaled to contain 95% of observations.

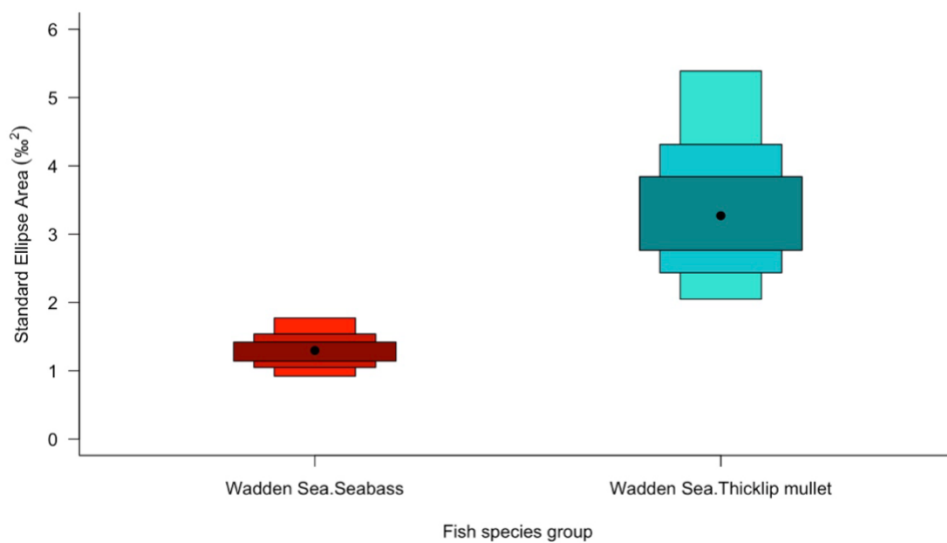


Figure 5: Density plots showing the credible intervals of the Bayesian Standard Ellipse areas (SEAb) for *D. labrax* (red) and *C. labrosus* (blue) in the Wadden Sea during summer and autumn. The black dot represents the mode, the credible intervals (50%, 75%, and 95%) are represented by the colors of the boxes from dark to light.

Table 4: Table containing summary of data for SIBER comparing the isotopic niche of *D. labrax* and *C. labrosus*

	<i>D. labrax</i>	<i>C. labrosus</i>
<i>n</i>	37	18
Mean $\delta^{13}\text{C}$	-16,17	-14,10

$\delta^{13}\text{C}$ standard deviation	1,08	1,46
Mean $\delta^{15}\text{N}$	16,79	13,69
$\delta^{15}\text{N}$ standard deviation	0,41	0,75
SEAb mode	1.26	3,30
SEAb 99% confidence interval	0.82 – 2.00	1.74-6.41
SEAb 95% confidence interval	0.92 – 1.78	2.00-5.44
SEAb 50% confidence interval	1.126-1.42	2.78– 3.90

6.2. Assessing *D. labrax* and *C. labrosus* diet in the Dutch Wadden Sea using SIMMR isotopic mixing model

The output of the SIMMR model provides the proportional contribution of each food source to the diet of *D. labrax* and *C. labrosus* during autumn and summer in the Wadden Sea. For both fish species, the consumer isotope values generally lay within the polygon, indicating the data was acceptable for running the mixing model.

Unfortunately, the large number of prey groups and the fact that some of our prey groups were isotopically similar to each other might have limited the performance of our model.

6.2.1. Assessing *D. labrax* diet in the Dutch Wadden Sea using SIMMR

For *D. labrax*, the isotope values for *P. flesus* and *P. platessa* were merged into one group named "Flatfish sp." due to their proximity in the isospace plot and their taxonomic similarity (see Appendix for SIMMR plot with the two flatfish species separated in isospace).

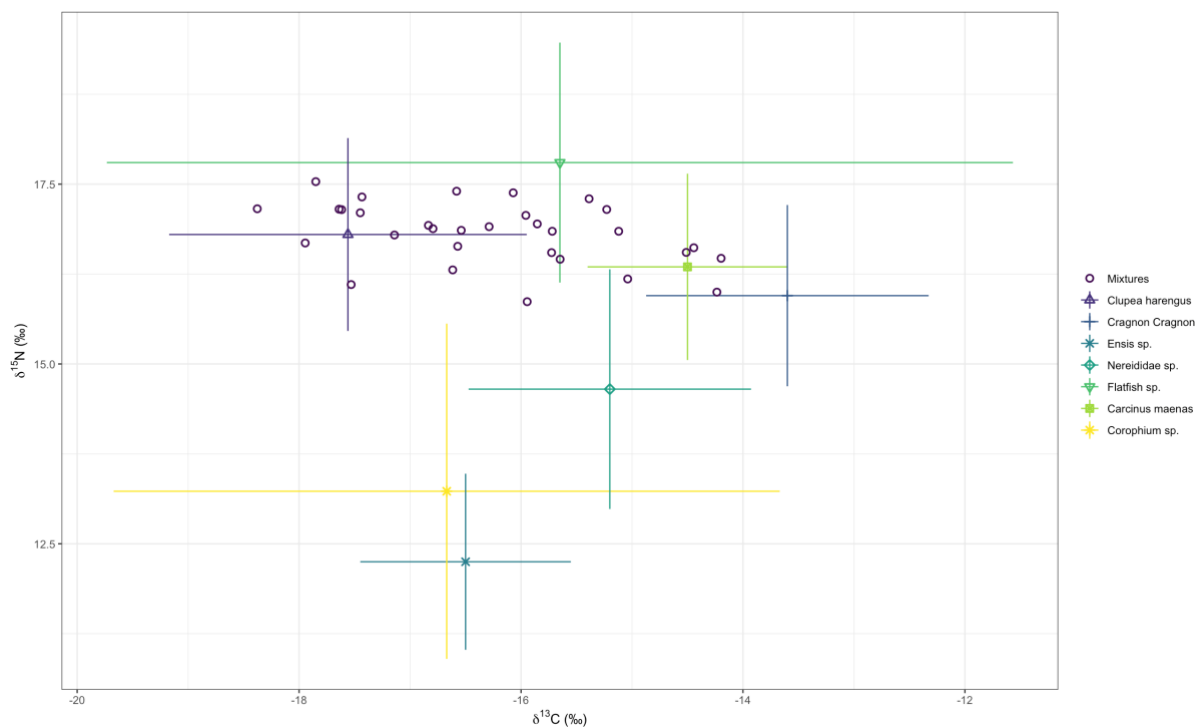


Figure 6: SIMMR isospace plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual *D. labrax* and their main food sources in the Dutch Wadden Sea. Vertical and horizontal bars represent the standard deviation of the food sources, which can be differentiated by differing colours and symbols

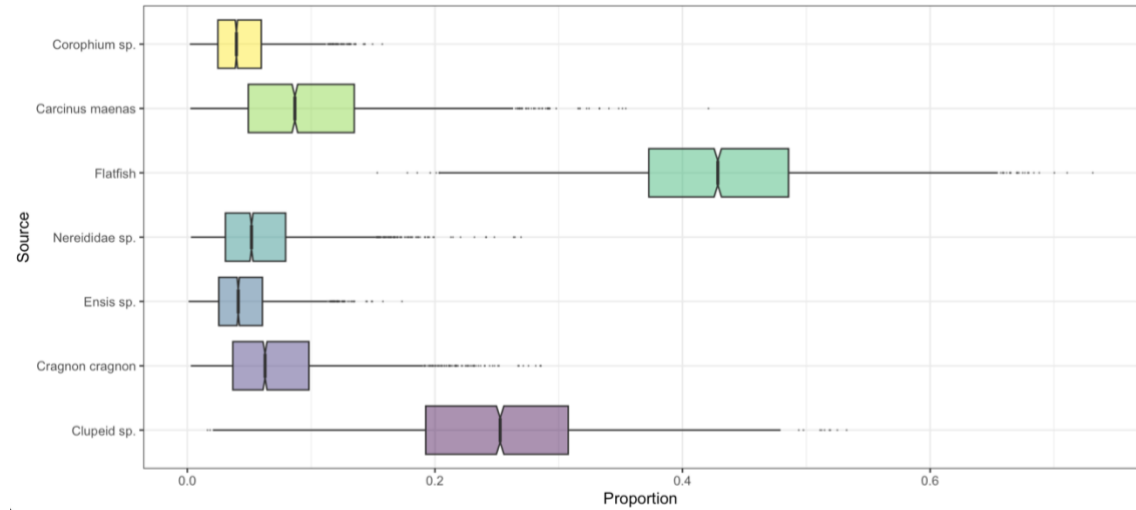


Figure 7: Proportion of the contribution of prey items in the diet of *D. labrax* in the Wadden Sea estimated based on the Bayesian model output with SIMMR. Boxes represent the credible interval of 50% and error bars the credible interval of 95% obtained with SIMMR.

Table 5: Estimates of diet proportions (means and standard deviations) for adult *D. labrax* captured in the Dutch Wadden Sea based on SIMMR stable isotope mixing model output. Prey are ordered from highest to lowest abundance.

	Mean proportion	St. dev.
Flatfish sp.	0.431	0.084
<i>Clupeid harengus</i>	0.249	0.082
<i>Carcinus maenas</i>	0.098	0.061
<i>Cragnon cragnon</i>	0.072	0.047
Nereididae sp.	0.061	0.039
Corophium sp.	0.045	0.026
Ensis sp.	0.044	0.025

Table 5 shows the proportional contribution of each food source according to the model. For *D. labrax*, flatfish sp. provided by far the largest contribution to their diet (43.1%). The next most common prey species were *Clupea harengus* (24.9%), *Carcinus maenas* (9.8%), and *Cragnon cragnon* (7.2%). Minor contributions were given by Nereididae sp. (6.1%), Corophium sp. (4.5%), and Ensis sp. (4.4%) (see Figures 6 and 7).

6.2.2. Assessing the diet of *C. labrosus* in the Dutch Wadden Sea using SIMMR

In our study, the SIMMR mixing model estimated that the dietary source with the largest contribution in their diet was the macroalgae *Ceramium rubrum* (21.2%), followed by Corophium sp. (18.1%), green microphytobenthos (17.0%), *Fucus vesiculosus* (14.8%), and *Peringia ulvae* (13.4%) (see Figures 8 and 9). Minor contributions were

given by *Abra tenuis* (6.7%), *Ulva* sp. (4.4%), and brown microphytobenthos (4.3%). Some agreement was found with stomach content prey frequency analysis in *C. labrosus* (n=20) caught during the same sampling period in Terschelling, which showed that relative to anything other than sand, *Corophium* sp. had the highest prey frequency (46%), followed by gastropod sp. (38.5%), bivalve sp. (6.6%), and seaweed sp. (4.9%).

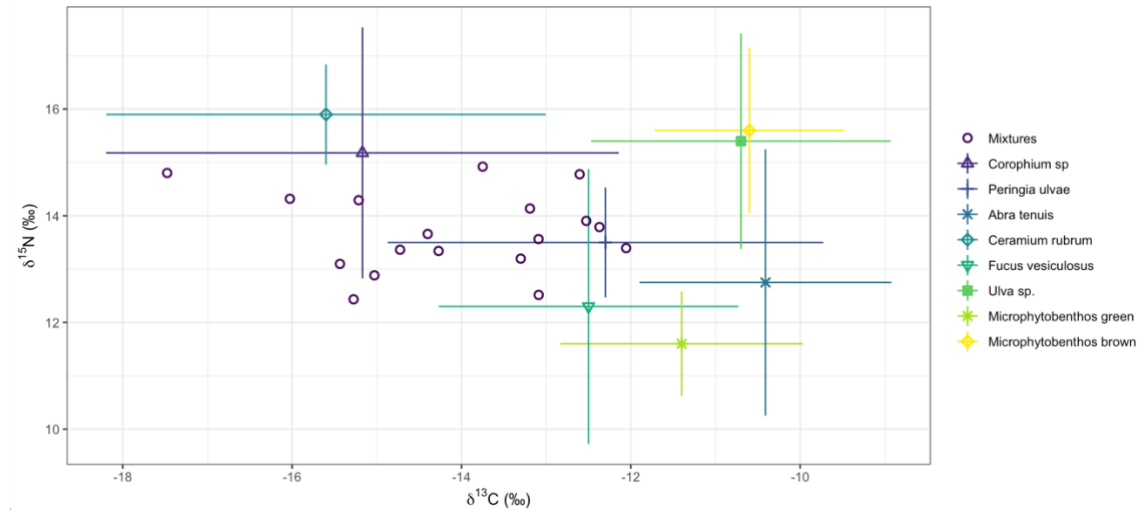


Figure 8: SIMMR isospace plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual *C. labrosus* adults and their main food sources in the Dutch Wadden Sea. Vertical and horizontal bars represent the standard deviation of the food sources. The food sources can be differentiated by differing colours and symbols.

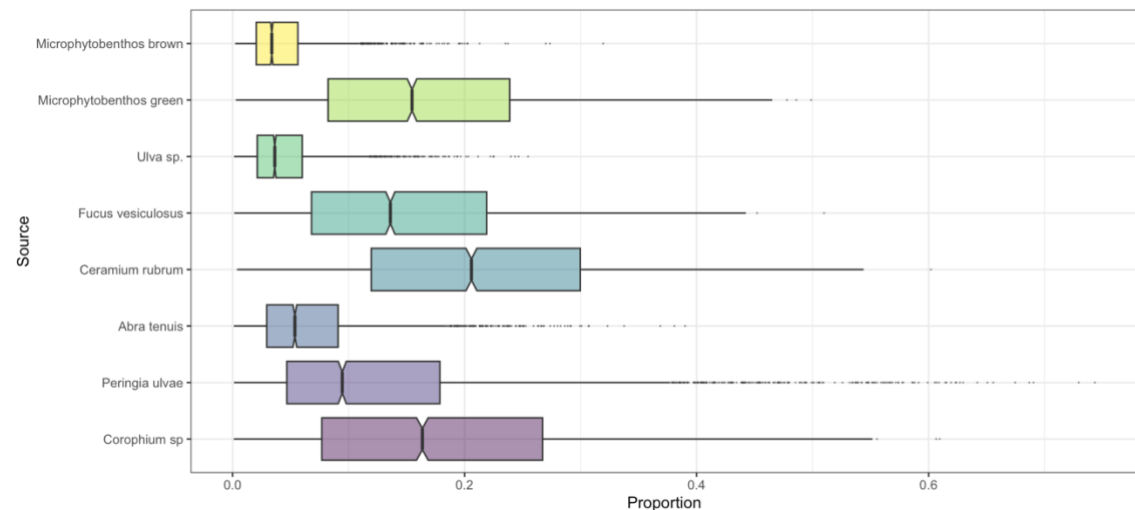


Figure 9: Proportion of the contribution of prey items in the diet of *C. labrosus* adults in the Dutch Wadden Sea estimated based on the Bayesian model output with SIMMR. Boxes represent the credible interval of 50% and error bars the credible interval of 95% obtained with SIMMR.

Table 6: Estimates of diet proportions (means and standard deviations) for adult *C. labrosus* captured in the Dutch Wadden Sea based on SIMMR stable isotope mixing model output. Prey are ordered from highest to lowest abundance.

	Mean proportion	St. dev.
<i>Ceramium rubrum</i>	0.212	0.115

Corophium sp.	0.181	0.119
Microphytobenthos green	0.170	0.101
<i>Fucus vesiculosus</i>	0.148	0.095
<i>Peringia ulvae</i>	0.134	0.124
<i>Abra tenuis</i>	0.067	0.052
Ulva sp.	0.044	0.032
Microphytobenthos brown	0.043	0.032

6.3. What is the influence of location on the stable isotope values of *D. labrax* in the Dutch Wadden Sea?

Table 7: Mean \pm SD of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C:N values of muscle, fin and liver tissue from *D. labrax* caught in Balgzand, Terschelling and Eierlandse Gat

Location	Muscle n	Muscle $\delta^{13}\text{C}$	Muscle $\delta^{15}\text{N}$	Muscle C/N	Liver n	Liver $\delta^{13}\text{C}$	Liver $\delta^{15}\text{N}$	Liver C/N	Fin n	Fin $\delta^{13}\text{C}$	Fin $\delta^{15}\text{N}$	Fin C/N
Balgzand	23	-16,73 \pm 0,86	16,90 \pm 0,35	3,9	19	-15,48 \pm 0,91	16,48 \pm 0,29	5,6	20	-15,21 \pm 0,51	18,00 \pm 0,47	3,6
Terschelling	8	-15,20 \pm 0,60	16,83 \pm 0,35	3,4	9	-15,31 \pm 0,81	15,98 \pm 0,49	5,5	11	-13,84 \pm 1,0	17,94 \pm 0,61	3,4
Eierlandse gat	6	-15,33 \pm 0,92	16,30 \pm 0,37	3,8	6	-14,14 \pm 1,10	15,88 \pm 0,42	5,5	8	-13,622 \pm 1,01	17,73 \pm 0,70	3,4

An ANOVA test was performed to determine whether there was a significant difference in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in *D. labrax* tissue between Balgzand, Terschelling and Eierlandse Gat (see Table 7). A significant difference between the locations was observed for muscle $\delta^{15}\text{N}$ (ANOVA, $F_{2, 34}=7.055$, $p=0.00274$) and $\delta^{13}\text{C}$ (ANOVA, $F_{2, 34}=13.99$, $p=3.7\text{e-}05$), and so a Tukey's Test was performed to estimate all pairwise differences (see Figure 10). In the case of muscle $\delta^{13}\text{C}$, a highly significant depletion of $\delta^{13}\text{C}$ in BG compared to TS ($p=0.000202$) and EG ($p=0.002104$) was observed, but there was no significant difference between TS and EG ($p=0.952$). For muscle $\delta^{15}\text{N}$, there was a significant enrichment of $\delta^{15}\text{N}$ in BG ($p=0.0193$) and TS ($p=0.0228$) compared to EG ($p=0.0193$), but there was no significance difference between TS and BG ($p=0.870$). After an ANOVA test was conducted on the liver isotopic signature between locations for $\delta^{13}\text{C}$ (ANOVA, $F_{2, 31}=4.929$, $p=0.0138$) and $\delta^{15}\text{N}$ (ANOVA, $F_{2, 31}=9.224$, $p=0.000719$), a Tukey's test showed unlike muscle $\delta^{13}\text{C}$, liver $\delta^{13}\text{C}$ was not significantly different between BG and TS ($p=0.888$). The $\delta^{15}\text{N}$ of liver was also different to the pattern observed in muscle. Liver $\delta^{15}\text{N}$ in BG was significantly enriched compared to both TS ($p=0.00557$) and EG ($p=0.00406$), and no significant difference existed between TS and EG liver $\delta^{15}\text{N}$ ($p=0.865$) (see Figure 10).

A SIMMR mixing model was used to compare the most common food items between the three areas from *D. labrax* muscle isotope signature (see Figure 11). An important limitation of the model is that the underlying shifts in the baseline $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values across the three locations were not accounted for in the mixing models. Despite the close proximity between the three locations, we must be cautious before making any

conclusions as Christianen et al. (2017)'s study provided evidence that benthic primary producers show a heterogeneous pattern across the Dutch Wadden Sea, with $\delta^{13}\text{C}$ values ranging from ($\delta^{13}\text{C}$ -19.2 to - 11.5‰). Another important limitation of the interlocation comparison in the diet of *D. labrax* is the small sample size of fish caught in Eierlandse Gat (n=6) and Terschelling (n=8) relative to Balgzand (n=23) (see Table 7). Their sample size may be too small to provide a reliable representation of *D. labrax*'s isotopic signature in those areas. Despite these limitations, in all three areas, flatfish made up the largest proportional contribution to the diet, making up between 33.6 and 40.7% (Balgzand: 37.9%; Terschelling: 40.7%; Eierlandse Gat 33.6%) (see Figure 11). The proportional contribution of *Clupeid harengus* was much greater in Balgzand (34.9%) than in the other two areas (Terschelling 11.4%; Eierlandse Gat 12.2%), whilst the proportional contribution of *C. cragnon* and *C. maenas* were much greater in Terschelling (*C. cragnon*= 13.3%, *C. maenas*= 19.4%) and Eierlandse Gat (*C. cragnon*= 14.2%, *C. maenas*= 16.6%) than in Balgzand (*C. cragnon*= 5.6%; *C. maenas*= 8.2%) (see Figure 11).

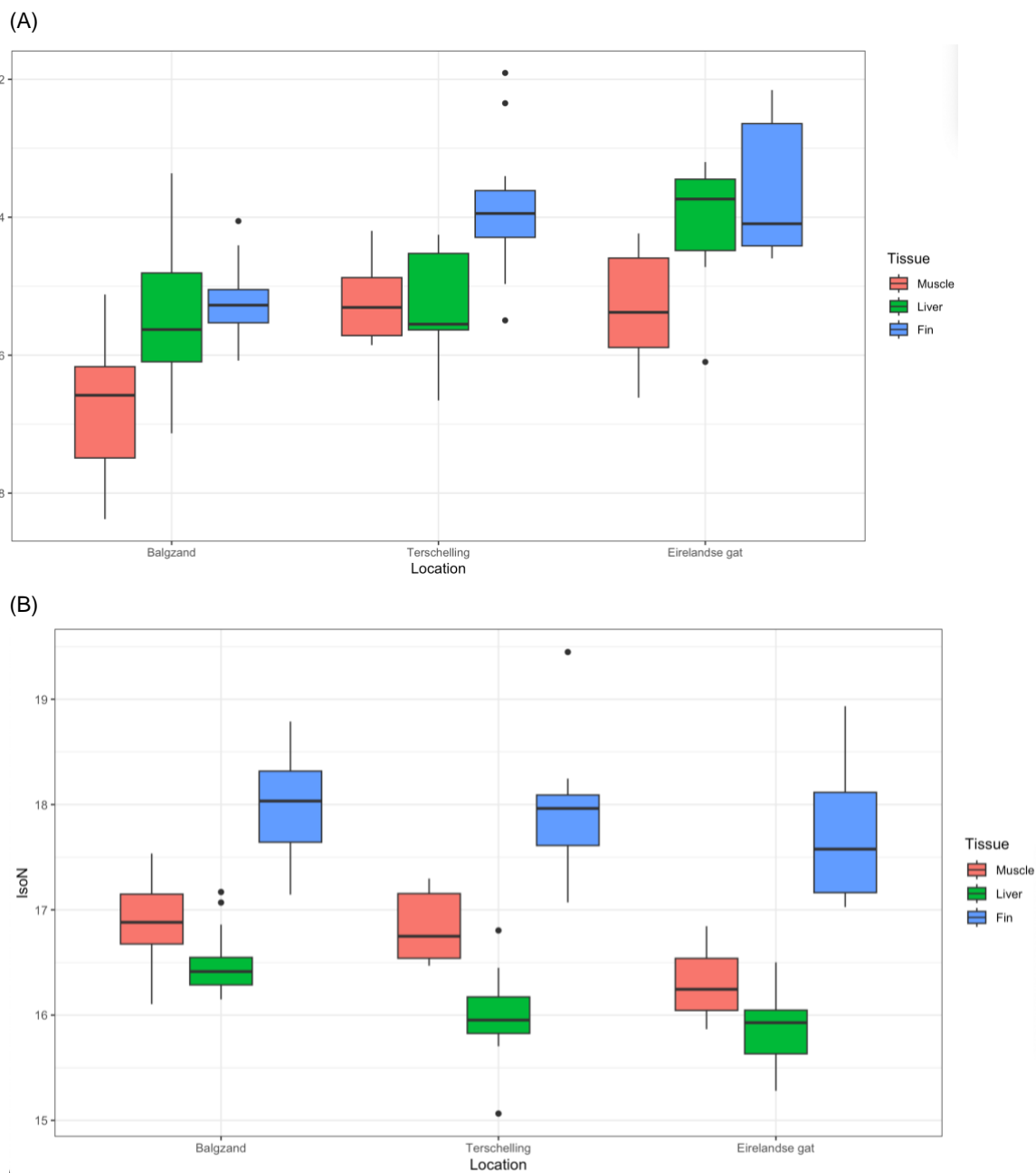
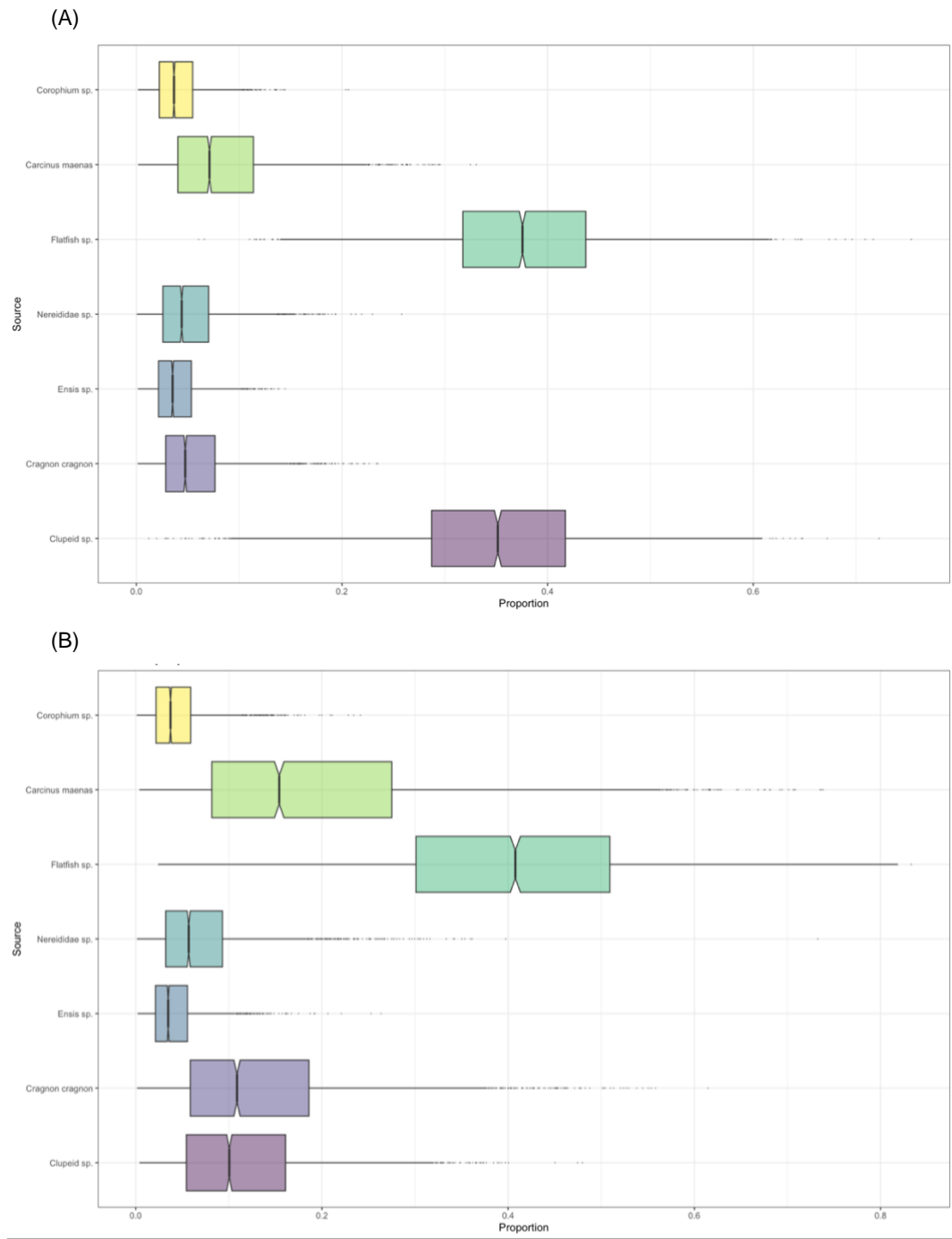


Figure 10: Boxplots visualizing the (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ values of *D. labrax* for muscle, liver and fin tissue in Balgzand (n=23), Terschelling (n=8) and Eierlandse Gat (n=6) in the Dutch Wadden Sea.



(C)

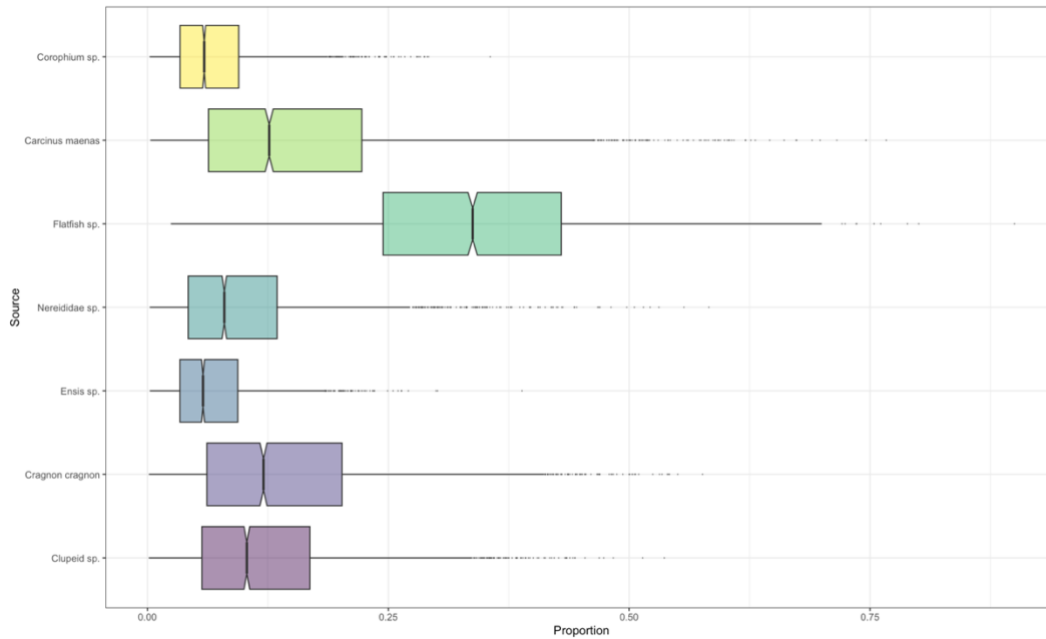


Figure 11: Posterior probabilities for the proportional contribution of each food source to the diet of *D. labrax* captured from spring to summer 2021-2022 in the Dutch Wadden Sea in (a) Balgzand, (b) Terschelling, and (c) Eierlandse Gat, obtained from the SIMMR mixing model.

Table 8: Estimates of diet proportions (means and standard deviations) based on SIMMR stable isotope mixing model captured in three different locations: Balgzand (BG), Terschelling (TS) and Eierlandse Gat (EG).

	BG Mean	BG St Dev.	TS Mean	TS St. Dev	EG Mean	EG St Dev
Flatfish sp.	0.379	0.088	0.407	0.144	0.336	0.133
<i>Clupea harengus</i>	0.349	0.099	0.114	0.074	0.122	0.086
<i>Carcinus maenas</i>	0.082	0.054	0.194	0.142	0.166	0.128
<i>Cragnon cragnon</i>	0.056	0.038	0.133	0.097	0.142	0.103
Nereididae sp.	0.052	0.034	0.068	0.053	0.098	0.076
Corophium sp.	0.042	0.024	0.044	0.030	0.068	0.047
Ensis sp.	0.039	0.023	0.041	0.028	0.067	0.045

6.4 Influence of sex and length on the stable isotope values of *C. labrosus* and *D. labrax*

6.4.1 What is the influence of *D. labrax* and *C. labrosus* length on tissue isotope signature?

There was a significant enrichment in tissue $\delta^{13}\text{C}$ with increasing fish length for both fish species ($p < 0.05$), (see Tables 9 and 10). On the other hand, there was no significant relationship between length and tissue $\delta^{15}\text{N}$ value ($p = 0.7930$ for *D. labrax*, $p = 0.5539$ for *C. labrosus*).

6.4.2 What is the influence of sex on tissue isotope signature?

There is a significant relationship between sex and tissue $\delta^{13}\text{C}$ value for both *D. labrax* and *C. labrosus* ($p < 0.05$) (see Tables 9 and 10). For *D. labrax*, males have a more enriched $\delta^{13}\text{C}$ value than females; whilst for *C. labrosus*, females have a more enriched $\delta^{13}\text{C}$ value than males. On the other hand, there is no significant relationship between sex and tissue $\delta^{15}\text{N}$ value (*D. labrax*: $p = 0.0753$; *C. labrosus*: $p = 0.1410$). We must note that although female *D. labrax* and *C. labrosus* are typically larger than males, as males reach maturation when they are a smaller size than females (Hickling, 1970; Kennedy and Fitzmaurice, 1972, 1969), prior to the analysis we ensured there was no significant interaction between sex and length for either species (see methods section).

Table 9: Results from LMMs investigating how the stable isotope values of *D. labrax* depending on the sex and length of the fish.

Explanatory variable	Estimate	Std. Error	df	T value	P value
Sex effect on $\delta^{13}\text{C}$	0.514	0.231	88	2.231	<0.05
Sex effect on $\delta^{15}\text{N}$	-0.168	0.094	88	-1,800	0.075
Length effect on $\delta^{13}\text{C}$	0.085	0.030	88	2.809	<0.05
Length effect on $\delta^{15}\text{N}$	0.003	0.0123	88	0.263	0.793

In LMMs for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, sex and length are included as fixed effects. Tissue is included as the nested random effect.

Table 10: Results from LMMs investigating how stable isotope values of *C. labrosus* vary depending on the sex and length of the fish.

Explanatory variable	Estimate	Std. Error	df	T value	P value
Sex effect on $\delta^{13}\text{C}$	-1.159	0.355	49	-3.269	<0.05
Sex effect on $\delta^{15}\text{N}$	0.368	0.245	49	1.496	0.141
Length effect on $\delta^{13}\text{C}$	0.198	0.0490	49	4.036	<0.05
Length effect on $\delta^{15}\text{N}$	-0.020	0.034	49	-0.596	0.554

In LMMs for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, sex and length are included as fixed effects. Tissue is included as the nested random effect.

6.5 Assessing whether fin tissue can be used instead of liver and/or muscle tissue

Tables 11 and 12 below provide a summary of the data used for *D. labrax* (Table x) and *C. labrosus* (Table y) for Section 6.5.

Table 11: Mean \pm SD of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N values of muscle, fin and liver tissue from *D. labrax* caught in the Dutch Wadden Sea.

Tissue type	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
Dorsal fin	31	-14,506 \pm 1,168	17,821 \pm 0,482	3,5 \pm 0,2
Muscle	31	-16,267 \pm 1,162	16,811 \pm 0,429	3,87 \pm 0,72
Liver	31	-15,258 \pm 1,091	16,296 \pm 0,419	5,6 \pm 0,8

Table 12: Mean \pm SD of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N values of muscle, fin and liver tissue from *C. labrosus* caught in Terschelling, in the Dutch Wadden Sea.

Tissue type	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
Dorsal fin	18	-12,36 \pm 1,27	14,18 \pm 0,94	3,4 \pm 0,19
Muscle	18	-14,10 \pm 1,46	13,69 \pm 0,75	3,9 \pm 1,0
Liver	18	-12,46 \pm 1,26	13,67 \pm 0,64	5,4 \pm 1,12

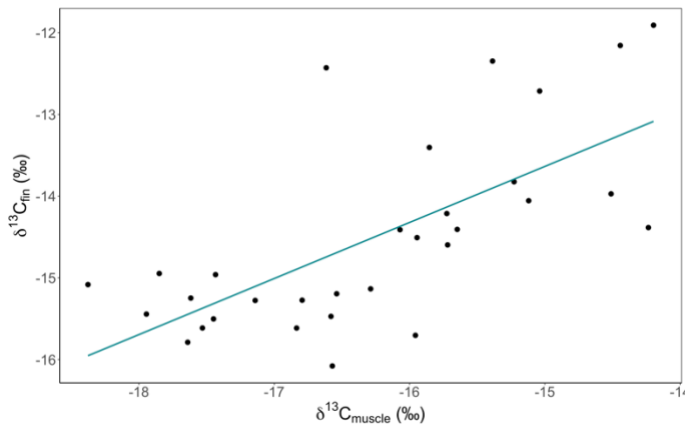
Results from the paired t-test showed there was a significant difference (<0.05) in fin tissue $\delta^{13}\text{C}$ compared to muscle and liver tissue $\delta^{13}\text{C}$ value in both *D. labrax* and *C. labrosus*. There was also a significant difference (<0.05) in all but one of the $\delta^{15}\text{N}$ isotopic signatures between fin tissues and both muscle and liver tissue in *D. labrax* and *C. labrosus*. Only fin and liver tissue $\delta^{15}\text{N}$ value for *C. labrosus* did not show a significant difference ($p=0.701$) (see Table 13).

Table 13: Results of paired t-test comparing muscle and liver $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values with fin values examined in *D. labrax* ($n=33$) and *C. labrosus* ($n=18$). Significant *p* values are in bold.

	$\delta^{13}\text{C}$ <i>p</i> value	$\delta^{15}\text{N}$ <i>p</i> value
<i>D. labrax</i> muscle vs. fin	<0.05	<0.05
<i>D. labrax</i> liver vs. fin	<0.05	<0.05
<i>C. labrosus</i> muscle vs. fin	<0.05	<0.05
<i>C. labrosus</i> liver vs. fin	0.7017	<0.05

A linear regression model was then conducted to determine whether there was a significant relationship between the stable isotope signature of fin tissue and the stable isotope signature of liver and muscle tissue for *D. labrax* and *C. labrosus* (see Figure 12). Predictive power was estimated from significance (*p* value) and R^2 value.

(A) *D. labrax* $\delta^{13}\text{C}$ muscle vs fin

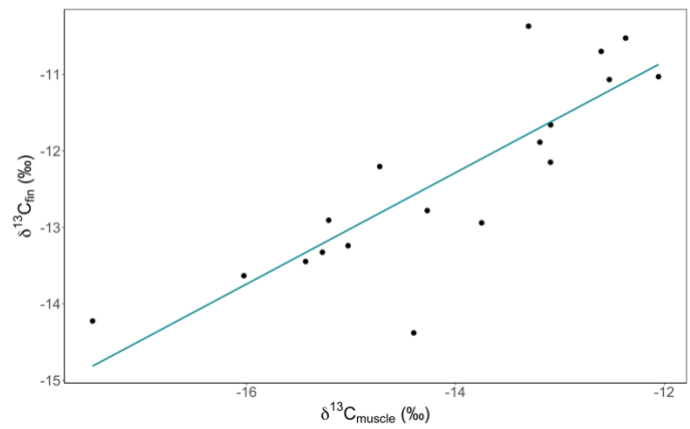


$R^2 = 0.47$, $p = < 0.05$

$Y = 0.70x - 3.34$

$F_{29} = 25.29$

(B) *C. labrosus* $\delta^{13}\text{C}$ muscle vs fin

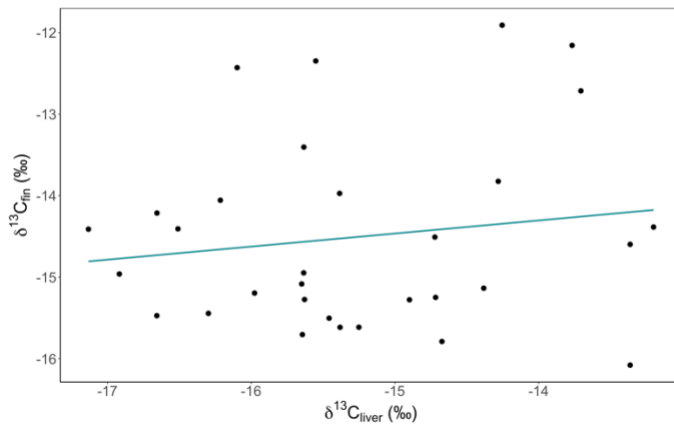


$R^2 = 0.71$, $p < 0.05$

$Y = 0.73x - 2.10$

$F_{16} = 38.98$

(C) *D. labrax* $\delta^{13}\text{C}$ liver vs fin

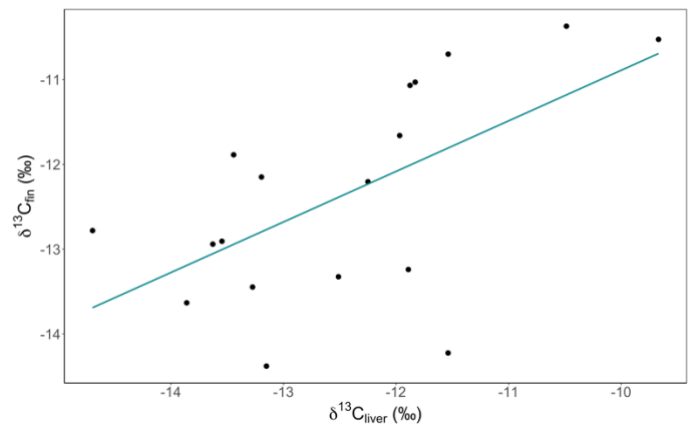


$R^2 = 0.02$, $p = 0.42$

$Y = 0.16x - 12.06$

$F_{29} = 0.66$

(D) *C. labrosus* $\delta^{13}\text{C}$ liver vs fin



$R^2 = 0.35$, $p < 0.05$

$Y = 0.60x - 4.93$

$F_{16} = 8.69$

(E) *D. labrax* $\delta^{15}\text{N}$ muscle vs fin

(F) *C. labrosus* $\delta^{15}\text{N}$ muscle vs fin

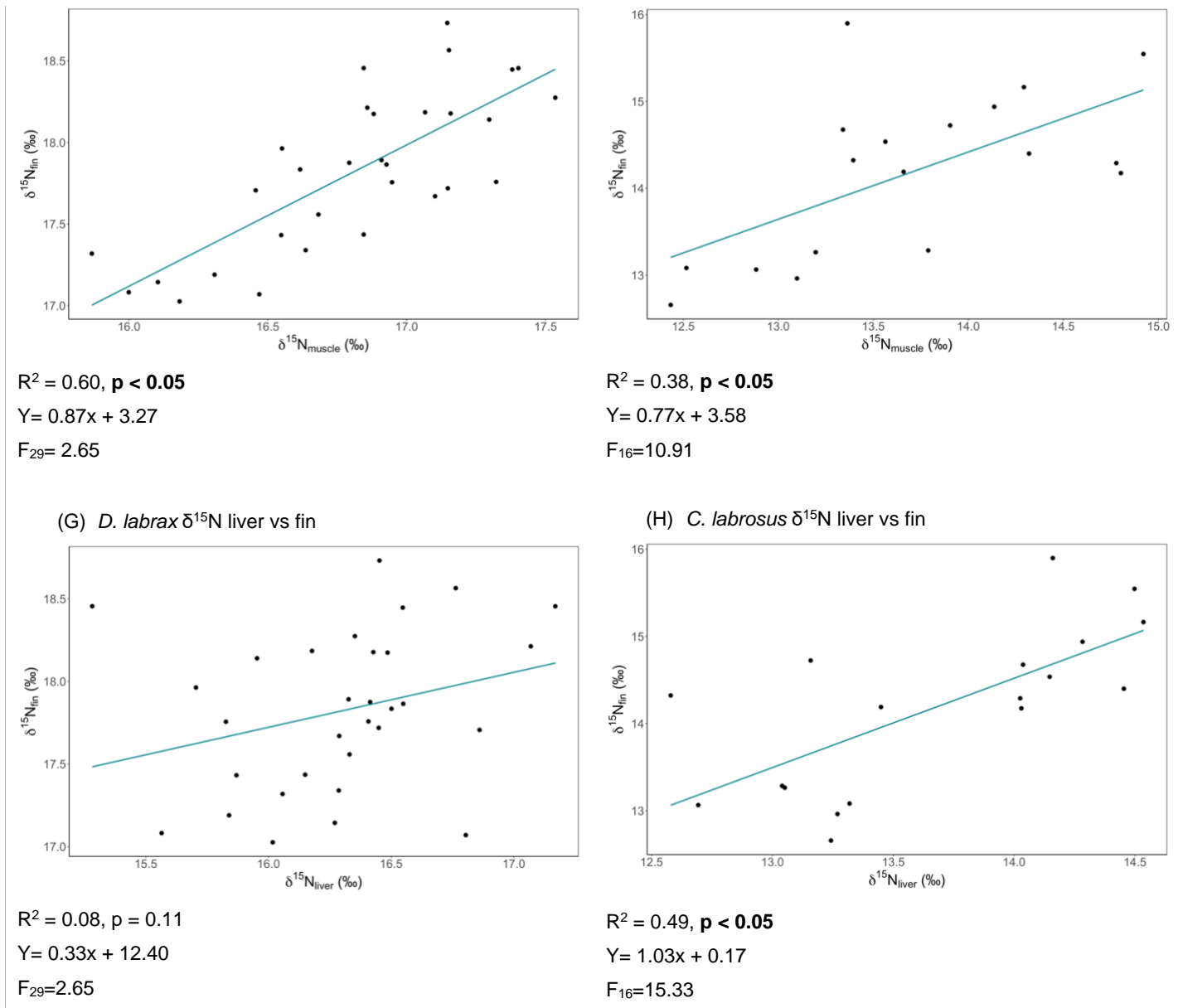


Figure 12: Linear regression of the stable isotope signature of fin tissue ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fin on the y-axis) and stable isotope signature of liver and muscle tissue ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ muscle and liver, on the x-axis) in *D. labrax* (left column) and *C. labrosus* (right column). Model statistics (Equation, R^2 , p , and F) for regressions are below each graph.

7. Discussion

7.1 The isotopic niche of *D. labrax* and *C. labrosus* in the Dutch Wadden Sea

The finding that benthic marine producers are a more important energy source for both *D. labrax* and *C. labrosus* than pelagic producers is unsurprising, as benthic primary producers are the most important energy source for the majority of consumers at higher trophic levels in the Dutch Wadden Sea (Christianen et al., 2017) (see Table 3). The more enriched $\delta^{13}\text{C}$ value for *C. labrosus* suggesting that macrophytes are another important food source for this species (see Table 3) is also as expected, as stomach content analysis has revealed macrophytes are a known contributor to their diet (van de Meer 2022, unpublished) (see Figure 14). However, the little contribution

of terrestrial riverine and estuarine carbon sources to the diet if *C. labrosus* is very surprising, as we would have expected a greater terrestrial influence in the diet of *C. labrosus* because of its nature as a euryhaline marine species that spends a lot of time in the vicinity of the coast and in freshwater and brackish waters, frequently entering brackish lagoons, river mouths, and estuaries (Poiesz et al., 2021).

The higher *D. labrax* $\delta^{15}\text{N}$ compared to *C. labrosus* is to be expected, as $\delta^{15}\text{N}$ is used to estimate the trophic positions of consumers because of $\delta^{15}\text{N}$ enrichment per trophic transfer. *D. labrax* displays one of the highest trophic positions (TP) in the western Dutch Wadden Sea, with an estimated TP of 3.70 based on stomach content analysis, and a slightly lower 3.42 derived from SIA (Poiesz et al., 2021, 2020) (see Table 3). On the other hand, *C. labrosus* has one of the lowest TPs in the coastal fish community in the western Wadden sea, with a TP varying between 2.36 (stomach content based) to 2.33 (SIA based) (Poiesz et al., 2021). Interestingly, Poiesz et al. (2021)'s study on the stable isotope signature of fish caught from the Texel fyke from spring to autumn showed a similar range of isotope values for *D. labrax* ($\delta^{13}\text{C} = -16.36 \pm 0.11$, $\delta^{15}\text{N} = 17.09 \pm 0.09$ [mean +/- SE]), but for *C. labrosus* our muscle $\delta^{13}\text{C}$ value was much more enriched than Poiesz et al. (2021)'s value ($\delta^{13}\text{C} = -18.45 \pm 0.19$, $\delta^{15}\text{N} = 15.19 \pm 0.18$) (see: Supplementary data from Poiesz et al. 2021). This suggests *C. labrosus* caught in the Marsdiep (which contains the Texel fyke) have a greater terrestrial, riverine, and estuarine influence, which could be as a result of currents from lake IJsselmeer or movement of these fish further inland.

The finding that the isotopic niche breadth is smaller for *D. labrax* than for *C. labrosus* was unexpected, as *D. labrax* are known to be opportunistic feeders which use a variety of techniques to forage on a variety of prey types, switching between chasing fish in open water to feeding on crustaceans among rocks and seaweed (Pickett and Pawson, 1994). On the other hand, the diet of *C. labrosus* entails a mainly herbivorous diet, grazing on the seafloor for diatoms, benthic small benthic invertebrates, detritus, and suspended organic matter (Carlier et al., 2007; Harrison, 2003; Koussoroplis et al., 2011; Leijzer, 2008).

7.2 *D. labrax* diet in the Dutch Wadden Sea using SIMMR

Our SIMMR model shows that *D. labrax* has consumed a variety of prey types, which is consistent with its known diet as a largely piscivorous feeder that opportunistically forages on a wide range of prey (Cardoso et al., 2015; Laffaille et al., 2000; Pickett and Pawson, 1994) (see Figure 7). It is surprising, however, that flatfish is the most consumed food item, considering that long-term trends in the western Wadden Sea have shown a decrease in juvenile flatfish abundance, especially for *P. platessa* (Veer et al., 2022). The adverse environmental conditions and the decline of *C. harengus* in the Dutch Wadden Sea since 2005 signify these factors are also unlikely to be responsible for the overall increase in *D. labrax* numbers since the 1980s (Supplementary data from Van de Veer et al. 2015; Cardoso et al., 2015; Tulp et al., 2022). In contrast, despite interannual population fluctuations, the population densities of *Crangon Crangon* and *Carcinus Maenas* have not shown any evidence of decrease and have instead shown an overall increase from the 1990s up to 2010 in the Dutch Wadden Sea (Campos et al., 2010; Tulp et al., 2017, 2012). Therefore, given *D. labrax*'s opportunistic feeding strategies, they may have played an important role in the species' growth in numbers over time.

The finding that flatfish sp. are the main prey species of *D. labrax* contrasts with data on *D. labrax* feeding behavior in the Dutch Wadden Sea from SCA (Cardoso et al., 2015; Poiesz et al., 2020). SCA from adult *D. labrax* caught in the Dutch Wadden Sea in the same sampling period, show *Crangon crangon* to have the greatest contribution to their diet in terms of prey frequency (64%), followed by *Clupea harengus*. (11%), and

Carcinus maenas (8%) (Van de Meer et al. 2022, unpublished) (see Figure 13). Flatfish sp. made up a mere 2.9% of the diet (van de Meer et al. 2022, unpublished). *Crangon crangon* as *D. labrax* preferred food item is corroborated by Cardoso et al. (2015)'s SCA of *D. labrax* in the Dutch Wadden Sea. The dominance of *Crangon crangon* in their diet has been attributed to the continual presence and high abundance of this shrimp species in Wadden Sea waters (Beukema, 1992; Kuipers and Dapper, 1984).

However, it's important to note that although SCA provides important insights into this fish's feeding preferences, it is limited by only providing a snapshot of diet composition whilst neglecting dietary items that quickly break down (Pinnegar and Polunin, 2000; Poiesz et al., 2020). Preference for flatfish rather than shrimp is supported by *D. labrax*'s high trophic position in the coastal fish community in the Wadden Sea, although its high trophic position could also be a result of the avid consumption of *Clupea harengus*, rather than a high intake of flatfish (Poiesz et al., 2021, 2020). SCA and the isotopic mixing model agree that *Clupea harengus*, a pelagic fish that typically travel in schools, is the second most abundant prey item in the diet of the *D. labrax*. Their abundance in *D. labrax*'s diet is unsurprising due to the following factors: (1) pelagic fish considerably outnumber demersal fish biomass in the Dutch Wadden Sea, and (2) *Clupea harengus* is one of the most common pelagic fish in this area (Couperus et al., 2016).

The reason for the disagreement in prey item choice between SCA and SIA may be that whilst SCA can only reveal the most recent food items ingested by *D. labrax*, diet is only isotopically reflected in muscle tissue a few months after ingestion. *D. labrax* from the northern stock comprises a population made of migratory and non-migratory individuals (Pawson et al., 2007), with part of the adolescent or mature fish population found around England and Wales migrating to the Wadden Sea during the warmer months (Pawson et al., 1987). Recent tagging data from the Swimway project has shown that all *D. labrax* leave the Wadden Sea during winter and come back in early spring (personal communications with Jena Edwards). Thus, the muscle tissue isotope signature of *D. labrax* in this study, which were caught in spring and summer, most likely also reflects their diet during the spring migration into the Wadden Sea. Their diet during their migration may be dominated by flatfish, or by a dietary item that occupies the same isotopic niche as flatfish but isn't found or consumed in the Wadden Sea. The consumption of small fish during their migration is likely if they traverse offshore areas, which may be revealed from movement data from *D. labrax* tagged in the Dutch Wadden Sea as part of the SWIMWAY project. Multiple studies have shown that *D. labrax* typically has a more fish-dominated diet offshore than in-shore (Kelley, 1987; Kennedy and Fitzmaurice, 1972; Spitz et al., 2013), made evident by Picket and Pawson (1995), who showed that *D. labrax* caught offshore England and Wales had a fish-dominated diet; whilst *D. labrax* caught close to the shore of these countries had crabs as their dominant feed (Kelley, 1987)

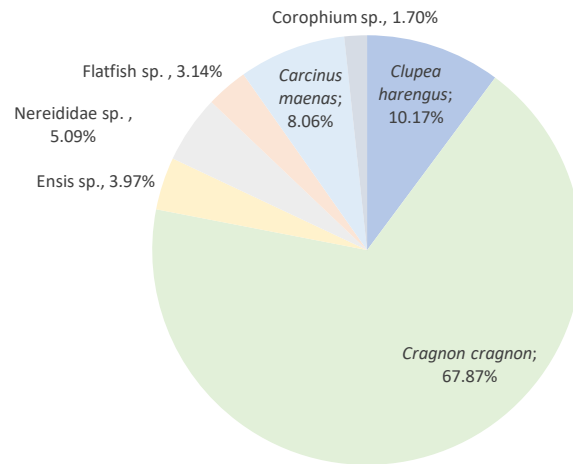


Figure 13: The frequency of prey consumed by *D. labrax* determined by stomach content analysis of individuals from the same sample group. Pie chart excludes prey species making up <2.5% of the total prey frequency (van de Meer 2022, unpublished).

7.3 *C. labrosus* diet in the Dutch Wadden Sea using SIMMR

C. labrosus is known as a demersal species and benthic feeder (Koussoroplis et al., 2011; Poiesz et al., 2020), grazing from the seafloor consuming algae, detritus, meiofauna, and suspended organic matter (Carlier et al., 2007; Harrison, 2003; Koussoroplis et al., 2011; Leijzer, 2008).

The relative significance of *Corophium* species as an important dietary source compared to other food items for *C. labrosus* is agreed upon by the stable isotope mixing model (18.10%) and stomach content data (48.3%) (personal communications with Gala van de Meer) (see Figures 8 & 14). *Corophium* sp. are amphipod crustaceans that are found at high densities in the Wadden Sea (Farrell et al., 2022). Two *Corophium* species which occupy different geographical distributions are found in this system: *Corophium voluntator*, which are found in silty areas in the upper coastal parts of the intertidal flats, and *Corophium arenarium*, abundant in locations with sandy sediment (Beukema and Flach, 1995). *C. labrosus* can exploit this important dietary source by grazing on the seafloor, which is their main foraging strategy (Carlier et al., 2007; Harrison, 2003; Koussoroplis et al., 2011; Leijzer, 2008).

There is a large disagreement in the importance of macroalgae between SIA and SCA to the diet of *C. labrosus* (see Figures 8 & 14). Whilst the mixing model estimates that *Ceramium rubrum* macroalgae make up 21.2% of their diet, SCA reveals that all seaweed species make up less than 5%. This small proportion in the macroalgae stomachs could possibly be explained because the macroalgae in the fish stomachs were so macerated or digested they could not be distinguished from the rest of the stomach contents. Research from Cardona et al. (2001) shows that *C. labrosus* in the Mediterranean expands its niche breadth during summer and spring by feeding on more micro and macroalgae to expand its dietary niche during these warmer months to prevent food limitation. During summer and spring, the detritus is poorer due to having a lower organic matter content, and thus competition for detritus between grey mullets and meiofauna is high due to the scarcity of nutritious detritus (Cardona, 2001; Lasserre et al., 1976). It is possible that the same phenomenon happens in the Wadden Sea. This possibility is supported by the SIBER plot (see Figure 4), which shows a surprisingly large niche breadth for *C. labrosus* and an enriched $\delta^{13}\text{C}$ value in *C. labrosus* muscle tissue, which suggests macroalgae may potentially

be an important food source for this species (*C. labrosus* $\delta^{13}\text{C}$ value: -14.10 ± 1.46 ‰; macroalgae $\delta^{13}\text{C}$ values ranging between -15.2 ‰ and -10.9 ‰, [mean \pm SE]) (Christianen et al., 2017). The SIMMR model also revealed that *C. labrosus* predominantly consumed green microphytobenthos (17%) over brown microphytobenthos (4.3%), implying that this fish has a greater reliance on energy obtained from newly fixed organic matter from primary producers from the overlying water column than microbially-reworked re-use of detrital organic matter (Riekenberg et al., 2022). This again provides support for *C. labrosus* sampled in spring and summer being more reliant on energy obtained from plants or algae (i.e. by herbivory through grazing and/or the consumption of herbivores), rather than decaying biomass from detritus (Cordone et al., 2020).

However, despite their dietary contribution possibly being greater than the one put forward by SCA, macroalgae species are unlikely to be *C. labrosus* main dietary source. This is because of macroalgae's spatial limitation and scarcity in the Wadden Sea due to their need to attach to hard substrate to exist (personal communications with Riekenberg et al. 2022; Albrecht, 1998). A large limitation of our mixing model is that the three macroalgal species fed into the mixing model were not taxonomically identified from stomach contents analysis. Instead, we used the broad assumption that the green and red macroalgae observed in their stomach consisted of a mix of these three macroalgal species as they are all found in the Wadden Sea and we had access to prior data on their isotopic signature. Many other macroalgal species are found in this system (personal communications with Philip Riekenberg, Lang and Buschbaum, 2010). Although taxonomic identification of the macroalgae from the stomach contents could have proven useful, this was not possible using visual identification only due to the small size of the particles present in the fish stomachs, and genetic analysis would have had to be conducted.

C. labrosus has been observed to be a migratory species, with schools of adults of *C. labrosus* migrating to warmer coastal waters in spring and disappearing with the arrival of the cold season (Leijzer, 2008; Muus et al., 1999; Schaber et al., 2011). Although their migration pattern and the proportion of the population that migrates are not well understood yet, preliminary tracking data from thicklip mullet caught in the Dutch Wadden Sea has shown evidence that they all migrate out of the Wadden Sea during winter, and only return with the arrival of spring (Leijzer, 2008; Muus et al., 1999; Schaber et al., 2011, personal communications with Jena Edwards). Thus, as in the case of *D. labrax*, we must be cautious with interpretations made from the SIMMR model as due to the migratory nature of *C. labrosus*, the differences in diet and environmental baseline stable isotope values experienced during their migration will have been reflected in the stable isotope signature.

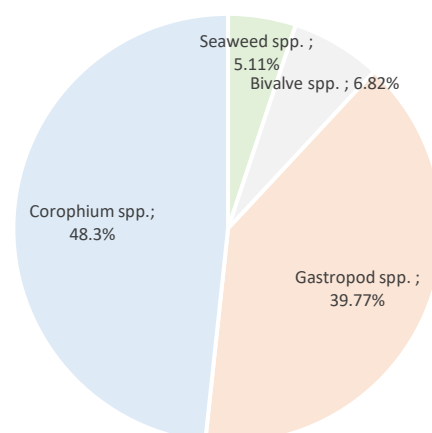


Figure 14: The frequency of prey consumed by *C. labrosus* determined by stomach content analysis of individuals from the same sample group. Pie chart excludes prey species making up < 2.5% of the total prey frequency (van de Meer 2022, unpublished).

7.4 The influence of location on the stable isotope values of *D. labrax* in the Dutch Wadden Sea

The SIMMR mixing model reveals that the diet of *D. labrax* in Terschelling and Eierlandse Gat was very similar, and differed from that in Balgzand (see Figure 11). The reason for the similarity between EG and TS may be because of a similarity in prey species due to the spatial proximity of the two areas to each other. Possible reasons for the difference between EG and TS from BG include a higher proportion of *Clupea harengus* in BG than in the two other areas, and/or a greater richness of *C. cragnon* and *C. maenas* in TS and EG than in BG.

We found little agreement between the SIMMR model prey abundances and that from stomach contents of *D. labrax* caught from these three locations during the same sampling period. Stomach content analysis showed BG to have the greatest abundance of shrimp (Balgzand's *D. labrax* had the lowest *C. cragnon* abundance in the SIMMR model), and *D. labrax* from EG had the highest proportion of fish in their diet, which was not the case in our mixing model. The reason for this mismatch may be because of the difference in temporal resolution between SCA and SIA. As the stable isotope signature most likely includes *D. labrax* migration into the Wadden Sea, differences in SIA may be a reflection of differences in diet or geographic baseline isotopic signatures during migration. Data from electronic tagging may reveal if differences in migration patterns between individuals caught in the three different locations exist. Moreover, the stable isotope signature of liver, the tissue with the shortest turnover rate of all three, does not show exactly the same pattern in stable isotope signature as muscle for the three locations, suggesting temporal diet changes (see Figure 10).

Thus, the reasons for the interlocation isotopic differences can be one or a combination of (1) differences in diet and geographical baselines experienced during their migration, (2) differences in diet between the three locations, (3) sample size limitation, and/or (4) the lack of inclusion of interlocation isotopic baseline differences. BG had the most depleted $\delta^{13}\text{C}$ signature between locations in all three tissues (Figure 10). This could be due to this area's proximity to the Texel inlet, from which most of the terrestrial freshwater discharged at the sluice at Den Oever enters the Wadden Sea (Christianen et al., 2017; Duran-Matute et al., 2014).

7.5 The influence of *D. labrax* and *C. labrosus* length on tissue isotope signature

The significant enrichment in tissue $\delta^{13}\text{C}$ with increasing fish length for both fish species suggests size-based differences in diet and/or spatial foraging patterns (see Tables 9 and 10). This may be the result of the need for larger fish to forage in more offshore locations and have a larger range of food sources to maintain a larger body size. Larger fish may be (a) feeding in areas with a lower terrestrial input (i.e. more offshore environments), resulting in them ingesting prey that is more enriched in $\delta^{13}\text{C}$, (b) consuming a greater proportion of benthic (as opposed to pelagic) prey, and/or (c) consuming a greater proportion of macroalgae (Christianen et al. 2017). Data from ongoing telemetry studies for *D. labrax* and *C. labrosus* populations in the Wadden Sea may offer some evidence for length-related spatial differences in foraging habitat use.

No significant relationship between length and tissue $\delta^{15}\text{N}$ value for both fish species indicates there is no evidence of $\delta^{15}\text{N}$ enrichment as consumer size increases due to the consumption of prey with higher trophic value to sustain their larger body size (Tables 9 & 10). For *D. labrax*, this contrasts with evidence from Cardoso

et al (2015), which showed $\delta^{15}\text{N}$ enrichment with increasing fish body length for individuals from 3.6 to 70 cm TL, reflecting an increase in piscivory as they age (Spitz et al., 2013). SCA from adult *D. labrax* from the same sample group as this study showed the proportion of shrimp consumed decreased from 79% to 35% and the proportion of herring increased from 4% to 31% with an increase in *D. labrax* size (van de Meer et al. 2022, unpublished). It is possible that the lack of trophic gradient observed in this study is a result of the narrow length range of adult fish sampled (for *D. labrax*: 40.80 to 54.4 cm; for *C. labrosus*: 46 to 59.5 cm).

7.6 The influence of sex on *D. labrax* and *C. labrosus* tissue isotope signature

The significant relationship between sex and tissue $\delta^{13}\text{C}$ value for both *D. labrax* and *C. labrosus* may be a result of differences in spatial foraging patterns and/or differences in the location from where the consumed prey derived their $\delta^{13}\text{C}$ (see Tables 9 and 10). This may be due to resource partitioning as a result of intra-species competition and/or different geographical movements due to distinct sex-related lifecycle patterns. The number of male *C. labrosus* sampled was much smaller than females (M=5; F=13) because much fewer males than females were caught in that area. This inequality between the number of *C. labrosus* females and males caught in Terschelling may provide evidence for sex-related differences in foraging location. Males may be found in more nearshore locations with higher terrestrial input, reflected by the significantly more depleted $\delta^{13}\text{C}$ values in males (see Table 10).

The lack of a significant relationship between sex and tissue $\delta^{15}\text{N}$ value for both species indicates that both sexes feed from the same trophic level. These results support observations from *D. labrax* stomach content analysis from the same sampling period and location, which showed that the mean sizes of prey items and the frequency of each prey item did not significantly differ between sexes (Van de Meer et al. 2022, unpublished).

7.7 Can fin tissue be used instead of liver and/or muscle tissue?

For *D. labrax*, fin had a significant regression line with muscle for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($\delta^{13}\text{C}$: $p < 0.05$, $R^2 = 0.47$; $\delta^{15}\text{N}$: $p < 0.05$, $R^2 = 0.60$); whilst the fin and liver $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not have a significant relationship ($\delta^{13}\text{C}$: $p = 0.42$; $\delta^{15}\text{N}$: $p = 0.11$) (see Figure 12). Thus, for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, muscle has a higher predictive power for fin stable isotope values than liver. However, the relationship between muscle and fin $\delta^{13}\text{C}$ ($R^2 = 0.47$) is less robust compared with the muscle-fin $\delta^{15}\text{N}$ relationship ($R^2 = 0.60$). In any case, muscle seems to be a better predictor and thus a more suitable proxy of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for fin than liver. The regression of *D. labrax* muscle on fin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ allows for a simple arithmetic correction to estimate fin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from muscle tissues (see Figure 12, Equations A and E).

For *C. labrosus*, both muscle and liver have a significant positive regression line with fin $\delta^{13}\text{C}$ ($p < 0.05$). However, muscle shows a higher predictive power for fin ($R^2 = 0.71$) than liver ($R^2 = 0.35$) (see Figure 12). For $\delta^{15}\text{N}$, muscle and liver have a significant regression line with fin ($p < 0.05$). In this case, liver shows a slightly higher predictive power for fin ($R^2 = 0.49$) than muscle ($R^2 = 0.38$). All relationships had a similar strength (R^2 between 0.35 and 0.49), apart from the muscle-fin relationship with a greater R^2 value of 0.71, suggesting that the regression of *C. labrosus* fin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be better estimated from muscle tissue than from liver tissue. A simple equation (see Figure 12, Equations B and F) can predict fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to muscle.

A key consideration in SIA is tissue isotopic turnover, which determines the rate for the isotopic composition of the consumer tissues from the past diet to be replaced with ratios from more recent diets. Tissue turnover rate

depends on the tissue type, fish species, growth rate, and the degree of metabolic turnover (Busst et al., 2015; Herzka, 2005). Differences in turnover rate between the tissues can provide temporal data about diet (MacNeil, 2006). Tissues with a higher metabolic rate (e.g. liver) have a higher turnover rate and thus reflect a shorter, more recent period of dietary feeding than less metabolically active tissue (e.g. muscle) (Busst and Britton, 2018; Tieszen et al., 1983). In our study, muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were more correlated with that of fin tissue than liver for both fish species, suggesting that the isotopic signature of fin tissue may reflect an isotopic turnover rate more similar to muscle, and thus a relatively slower isotopic turnover rate than that of liver. However, it is important to note that there is considerable scatter around the regression line, resulting in a relatively low R^2 value for all linear regressions. Controlled diet-shift experiments must be conducted to determine with certainty the tissue turnover rates of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes for each tissue. If these laboratory-controlled experiments take place, it would help better understand this study's data in much greater geographical and temporal resolution to determine these fish's diet in the Wadden sea and diet changes in relation to migration patterns.

7.8 Caveats of the SIMMR mixing models

One issue to be considered is that the SIMMR mixing models were created using prey stable isotope values collected opportunistically from other projects undertaken in the Dutch Wadden Sea. As such, their isotopic values temporally and spatially differ from our consumer data, and include some SI values that have been lipid corrected and that have not. Another issue is that fish species have a wide dietary range, and in the SIMMR mixing models various prey groups are so isotopic similar it is hard to determine prey preferences with certainty. The incorporation of informative priors into the mixing model based on prey biomass from SCA would have narrowed the range of feasible solutions for each source in the SIMMR model. However, we only had stomach contents prey frequency, not biomass. Incorporating priors based on prey frequency would have been misleading and unrecommended. In any case, incorporating informative priors from SCA should be done with caution, as they can transfer biases from dietary estimates derived from stomach contents into the mixing model ((Franco-Trecu et al., 2013; Swan et al., 2020).

The high uncertainty of TEF values for both consumer species is another limitation of our study. The reason for this uncertainty is that the TEFs were estimated from literature values of proxies of our species due to the absence of muscle tissue TEFs for both species in the literature. Although our SIMMR plots generally showed consumer individuals inside the prey polygon, there were a small number of mixtures that were slightly outside of the prey isospace, which could be an indication that: (1) the TEFs are wrongly estimated, or (2) that potential prey items are missing. Although it is unlikely we missed a prey item for *D. labrax* and *C. labrosus* in the Wadden Sea, both fish species are partially migratory species, so it is possible that their tissue stable isotope values also reflect prey consumed during their migration, but are not included in the mixing model. Moreover, the fish most likely move between isotopically distinct oceanographic regions during the course of their migrations. Unfortunately, determining whether consumer stable isotope values originated from shifts in diet and/or the migratory movement of the fish through habitats with different baseline stable isotope values was not possible (Post, 2002).

Next, the lack of proxies for liver and fin tissue made it impossible to undertake a mixing model to determine diet preference on the timescale of these tissues in this study. Undertaking controlled diet-shift experiments to determine diet-tissue discrimination factors of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for muscle, liver, and fin tissue would have been of

great use in this study to remove much of the uncertainty from our muscle tissue SIMMR model, and to additionally conduct mixing models for fish liver and fin tissue.

Another consideration is that the SIMMR plots presented in this report do not factor in the differences in prey trophic level. To address this, an alternative improved method could have been employed to generate SIMMR plots that correct for these trophic level differences. This alternative method would still use the same TDFs as before, but with the inclusion of adjustments to account for trophic levels by shifting both prey and consumers to trophic level 2 using the equation provided below.

$$\text{New } \delta^{13}\text{C}/\delta^{15}\text{N} \text{ value of prey/consumer} = \text{Actual } \delta^{13}\text{C}/\delta^{15}\text{N} \text{ value} - (\text{Trophic level of prey/consumer} - \text{trophic level } 2) * \text{TDF} (\delta^{13}\text{C} \text{ or } \delta^{15}\text{N})$$

The Appendix contains the trophic levels for prey and consumers for both fish species. Most of these values were extracted from Riekenberg (2022), who used literature sources to determine the invertebrate values (Borst et al., 2018; Christianen et al., 2015; 2017), and interpolated the remaining species relative to ecosystem trends. Although trophic level values were not available for the prey items *Corophium* sp. and *Abra tenuis*, due to their deposit-feeding diet based on bacteria, diatoms, and particulate organic matter, we assigned them trophic level 2. No value was found for green and brown microphytobenthos. However, as primary producers, they were placed on trophic level 1. For *D. labrax* and *C. labrosus*, we determined the values 2.36 and 3.50, respectively, based on literature values based on stomach contents (Poiesz et al., 2021; Riekenberg et al., 2022). Interestingly, this alternative model showed significant differences in prey proportions. In the case of *D. labrax*, the greatest contribution was from *Clupea harengus*, followed by *Ensis* sp. Flatfish sp. was only in third place, and *Cragnon cragnon* continued to have a minimal contribution of less than 10% (see Figure 15). For *C. labrosus*, *Ceramium rubrum* macroalgae and *Corophium* sp. remain the dominant prey species, although their proportional contribution in this new model is much greater (see Figure 16). These findings highlight the need for future studies to build on the work from this research to provide more accurate information regarding the diet of these two fish species.

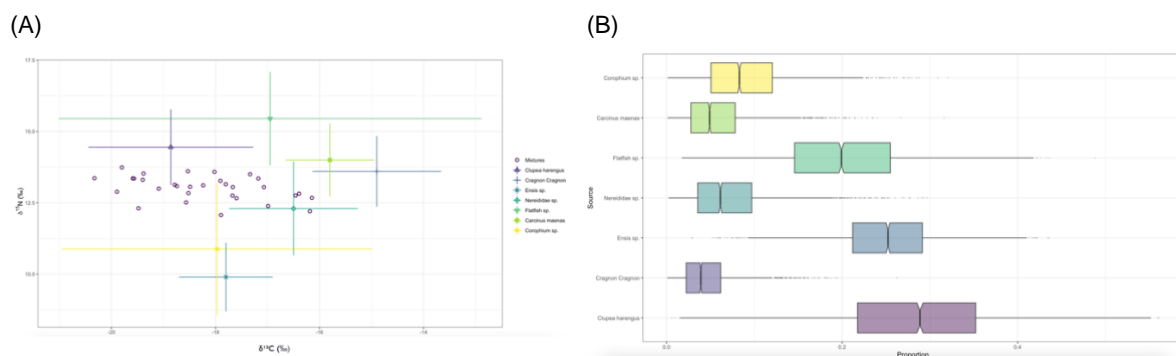


Figure 15: (A) Alternative SIMMR isospace plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual *D. labrax* adults and their main food sources in the Dutch Wadden Sea. Vertical and horizontal bars represent the standard deviation of the food sources. (B) Boxplot showing the proportional contribution of *D. labrax* prey items based on the Bayesian model output from SIMMR.

(A)

(B)

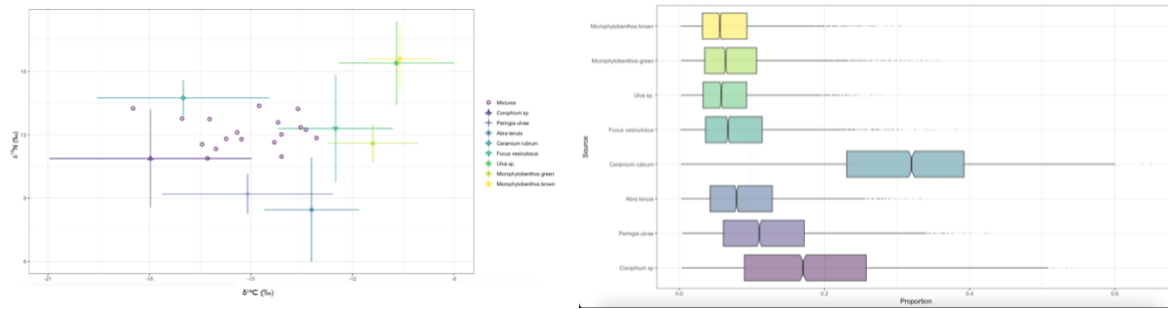


Figure 16: (A) Alternative SIMMR isospace plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual *C. labrosus* adults and their main food sources in the Dutch Wadden Sea. Vertical and horizontal bars represent the standard deviation of the food sources. (B) Boxplot showing the proportional contribution of *C. labrosus* prey items based on the Bayesian model output from SIMMR.

8. Conclusion

Our stable isotope mixing model reveals that for *D. labrax*, flatfish sp. are the largest contributors to their diet, followed by *Clupea harengus*, *Carcinus maenas*, and *Crangon crangon*. For *C. labrosus*, their main prey items appear to be the macroalgae *Ceramium rubrum*, followed by *Corophium* sp., and green microphytobenthos. Data on *D. labrax* and *C. labrosus* feeding behavior in the Dutch Wadden Sea had been previously researched using stomach content analysis, granting us the possibility to taxonomically identify prey items, unobtainable using SIA (Cardoso et al., 2015; Poiesz et al., 2020). In this study, we combined data extracted from both techniques, as they can each overcome problems the other cannot resolve (Pinnegar and Polunin, 2000; Poiesz et al., 2021; Post, 2002; Vander Zanden et al., 2010). Disagreements between the two methods were found, which could be a result of temporal differences in the data retrieved between the techniques. Although this research has significant caveats that need to be addressed in the future, this study provides new insights and raises new questions on the diet of *D. labrax* and *C. labrosus* in the Dutch Wadden Sea. Studies such as this one can have important management implications at both local and global scales to mitigate the impacts of anthropogenic threats on migrating fish stocks.

9. Appendix

Table 14: Prey item abundance extracted from stomach content analysis of *D. labrax* in Terschelling, Balgzand, and Eirelangse gat. The table includes species whose frequency was below 2.5% in at all sites and thus excluded from the SIMMR model. This resulted in the exclusion of *Gammarus* sp., Seaweed sp., Bivalve spp, *Liocarcinus holsatus*, unknown crab sp., Nematode sp., *Ammodytes tobianus*, Gastropod sp., and *Sygnathus acus*. Values below 2.5% are highlighted in red.

Prey species	Terschelling (n= 6)		Balgzand (n=27)		Eierlandse gat (n= 42)		In all three areas (n=75)	
	Frequency of prey item	Proportion of prey frequency contribution (%)	Frequency of prey item	Proportion of prey frequency contribution (%)	Frequency of prey item	Proportion of prey frequency contribution (%)	Frequency of prey item	Proportion of prey frequency contribution (%)
Clupeid sp.	2	0,49	7	0,59	237	28,01	246	10,07
<i>Cragnon cragnon</i>	261	63,97	1054	88,57	326	38,53	1641	67,14
Ensis sp.	27	6,62	10	0,84	59	6,97	96	3,93
Nereididae sp.	25	6,13	1	0,084	97	11,47	123	5,03
Flatfish sp.	46	11,27	3	0,25	27	3,19	76	3,11
<i>Carcinus maenas</i>	25	6,13	87	7,31	83	9,81	195	7,98
Gammarus spp.	4	0,98	24	2,02	13	1,54	41	1,68
Seaweed spp.	5	1,23	2	0,17	1	0,12	8	0,32
Bivalve spp	6	1,47	0	0	0	0	6	0,24
<i>Liocarcinus holsatus</i>	3	0,73	2	0,25	0	0	5	0,20
Unknown crab sp.	4	0,98	0	0	1	0,12	5	0,20
Nematode sp.	0	0	0	0	1	0,12	1	0,04
<i>Ammodytes tobianus</i>	0	0	1	0,084	0	0	1	0,04
Gastropod spp.	0	0	0	0	1	0,12	1	0,04
<i>Corophium</i> sp.	0	0	41	3,45	0	0	41	0,29
<i>Sygnathus acus</i>	0	0	7	0,59	0	0	7	0,29
TOTAL	408	100	1190	100	846	100	2444	100

Table 15: Prey species frequency in stomach contents of *C labrosus* (n=16) caught in Terschelling assessed from stomach content analysis. The table includes prey species occurring at < 2.5% and thus excluded from SIMMR mixing model. This resulted in the exclusion of Mollusca sp. and *Gammarus* sp.

Prey species	Frequency of prey item	Proportion of prey frequency contribution (%)
Seaweed spp.	9	5,03
Mollusca spp.	1	0,56

Bivalve spp.	12	6,70
Gastropod spp.	70	39,11
Gammarus spp.	2	1,12
Corophium sp.	85	47,49
Total	179	100

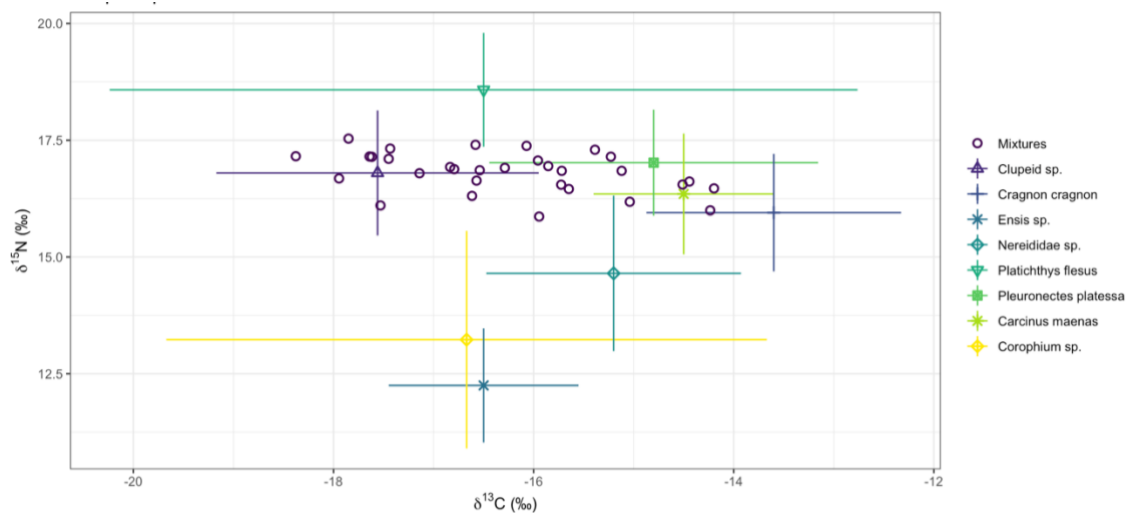


Figure 17: SIMMR isospace plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *D. labrax* individuals and their main food sources in the Dutch Wadden Sea. The isotope signatures for the two flatfish species (*P. flesus* and *P. platessa*) are separated. Vertical and horizontal bars represent the standard deviation of the food sources, which can be differentiated by differing colours and symbols.

Table 16: Trophic level *D. labrax* and *D. labrax* prey items considered in the SIMMR model (Sources: Borst et al., 2018; Christianen et al., 2015, 2017; Poesz et al., 2020; Riekenberg et al., 2022).

Name	Species name	Trophic level
European sea bass	<i>Dicentrarchus labrax</i>	3,5
Shrimp	<i>Cragnon cragnon</i>	3,2
Herring	<i>Clupea harengus</i>	3,4
Green Crab	<i>Carcinus maenas</i>	3
Ragworm	<i>Hediste diversicolor</i>	2,6
Razor clam	<i>Ensis directus</i>	2
European Flounder	<i>Platichthys flesus</i>	3,5
European Plaice	<i>Pleuronectes platessa</i>	3,2
	Corophium sp	2

Table 167 Trophic level *C. labrosus* and *C. labrosus* prey items considered in the SIMMR model (Sources: Borst et al., 2018; Christianen et al., 2015, 2017; Poesz et al., 2020; Riekenberg et al., 2022).

Species Name	Common name	Trophic level
Thicklip mullet	<i>Chelon labrosus</i>	2,36
	<i>Corophium sp.</i>	2
Cone mudsnail	<i>Peringia ulvae</i>	2
	<i>Abra tenuis</i>	2
Red Horn Weed	<i>Ceramium rubrum</i>	1,5
Bladderwrack	<i>Fucus vesiculosus</i>	1
Sea lettuce	<i>Ulva sp.</i>	1
Green microphytobenthos		1
Brown microphytobenthos		1

10. Bibliography

- Albrecht, A.S., 1998. Soft bottom versus hard rock:: Community ecology of macroalgae on intertidal mussel beds in the Wadden Sea. *Journal of Experimental Marine Biology and Ecology* 229, 85–109. [https://doi.org/10.1016/S0022-0981\(98\)00044-6](https://doi.org/10.1016/S0022-0981(98)00044-6)
- Beukema, J., 1992. Dynamics of juvenile shrimp *Crangon crangon* in a tidal-flat nursery of the Wadden Sea after mild and cold winters. *Marine Ecology-progress Series - MAR ECOL-PROGR SER* 83, 157–165. <https://doi.org/10.3354/meps083157>
- Beukema, J., Flach, E., 1995. Factors controlling the upper and lower limits of the intertidal distribution of two *Corophium* species in the Wadden Sea. *Marine Ecology-progress Series - MAR ECOL-PROGR SER* 125, 117–126. <https://doi.org/10.3354/meps125117>
- Bijleveld, A.I., van Gils, J.A., van der Meer, J., Dekinga, A., Kraan, C., Van der Veer, H.W., Piersma, T., 2012. Designing a benthic monitoring programme with multiple conflicting objectives. *Methods in Ecology and Evolution* 3, 526–536. <https://doi.org/10.1111/j.2041-210X.2012.00192.x>
- Borst, A.C.W., Verberk, W.C.E.P., Angelini, C., Schotanus, J., Wolters, J.-W., Christianen, M.J.A., van der Zee, E.M., Derksen-Hooijberg, M., van der Heide, T., 2018. Foundation species enhance food web complexity through non-trophic facilitation. *PLoS One* 13, e0199152. <https://doi.org/10.1371/journal.pone.0199152>
- Busst, G.M.A., Bašić, T., Britton, J.R., 2015. Stable isotope signatures and trophic-step fractionation factors of fish tissues collected as non-lethal surrogates of dorsal muscle. *Rapid Commun Mass Spectrom* 29, 1535–1544. <https://doi.org/10.1002/rcm.7247>
- Busst, G.M.A., Britton, J.R., 2018. Tissue-specific turnover rates of the nitrogen stable isotope as functions of time and growth in a cyprinid fish. *Hydrobiologia* 805, 49–60. <https://doi.org/10.1007/s10750-017-3276-2>
- Campos, J., Bio, A., Cardoso, J., Dapper, R., Witte, J., Veer, H.W., 2010. Fluctuations of brown shrimp *Crangon crangon* abundance in the western Dutch Wadden Sea. <https://doi.org/10.3354/MEPS08493>
- Cardona, L., 2001. Non-competitive coexistence between Mediterranean grey mullet: evidence from seasonal changes in food availability, niche breadth and trophic overlap. *Journal of Fish Biology* 59, 729–744. <https://doi.org/10.1111/j.1095-8649.2001.tb02376.x>
- Cardoso, J.F.M.F., Freitas, V., Quilez, I., Jouta, J., Witte, J.Ij., Van Der Veer, H.W., 2015. The European sea bass *Dicentrarchus labrax* in the Dutch Wadden Sea: from visitor to resident species. *J. Mar. Biol. Ass.* 95, 839–850. <https://doi.org/10.1017/S0025315414001714>
- Carlier, A., Riera, P., Amouroux, J.-M., Bodiou, J.-Y., Escoubeyrou, K., Desmalades, M., Caparros, J., Grémare, A., 2007. A seasonal survey of the food web in the Lapalme Lagoon (northwestern Mediterranean) assessed by carbon and nitrogen stable isotope analysis. *Estuarine, Coastal and Shelf Science* 73, 299–315. <https://doi.org/10.1016/j.ecss.2007.01.012>
- Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* 46, 443–453. <https://doi.org/10.1111/j.1365-2664.2009.01620.x>
- Christianen, M. J. A., Holthuijsen, S. J., van der Zee, E. M., van der Eijk, A., Govers, L. L., van der Heide, T., et al. 2015. Ecotopen- en Kansrijkdomkaart van de Nederlandse Waddenzee. Rapportnummer 2015.04.01, Waddenfondsproject Waddensleutels.
- Christianen, M.J.A., Middelburg, J.J., Holthuijsen, S.J., Jouta, J., Compton, T.J., van der Heide, T., Piersma, T., Sinninghe Damsté, J.S., van der Veer, H.W., Schouten, S., Olf, H., 2017. Benthic primary producers are key to sustain the Wadden Sea food web: stable carbon isotope analysis at landscape scale. *Ecology* 98, 1498–1512. <https://doi.org/10.1002/ecy.1837>
- Cordone, G., Salinas, V., Marina, T.I., Doyle, S.R., Pasotti, F., Saravia, L.A., Momo, F.R., 2020. Green vs brown food web: Effects of habitat type on multidimensional stability proxies for a highly-resolved Antarctic food web. *Food Webs* 25, e00166. <https://doi.org/10.1016/j.fooweb.2020.e00166>
- Couperus, B., Gastauer, S., Fässler, S.M.M., Tulp, I., van der Veer, H.W., Poos, J.J., 2016. Abundance and tidal behaviour of pelagic fish in the gateway to the Wadden Sea. *Journal of Sea Research* 109, 42–51. <https://doi.org/10.1016/j.seares.2016.01.007>
- Dekker, R., Beukema, J.J., 2012. Long-term dynamics and productivity of a successful invader: The first three decades of the bivalve *Ensis directus* in the western Wadden Sea. *Journal of Sea Research* 71, 31–40. <https://doi.org/10.1016/j.seares.2012.04.004>
- Duran-Matute, M., Gerkema, T., de Boer, G., Nauw, J., Gräwe, U., 2014. Residual circulation and freshwater transport in the Dutch Wadden Sea: A numerical modelling study. *Ocean Science* 10, 611–632. <https://doi.org/10.5194/os-10-611-2014>
- Essink, K., Dettmann, C., Frake, H., Laursen, K., Lüerssen, G., Wiersinga, W.A., 2005. Wadden Sea Quality Status report 2004 (No. No. 19). Common Wadden Sea Secretariat, Wilhelmshaven, Germany.
- Farrell, E.M., Beermann, J., Neumann, A., Wrede, A., 2022. The interplay of temperature and algal enrichment intensifies bioturbation of the intertidal amphipod *Corophium volutator*. *Journal of Experimental Marine Biology and Ecology* 151837. <https://doi.org/10.1016/j.jembe.2022.151837>
- Franco-Trecu, V., Drago, M., Riet-Sapriza, F., Parnell, A., Frau, R., Inchausti, P., 2013. Bias in Diet Determination: Incorporating Traditional Methods in Bayesian Mixing Models. *PLoS ONE* 8, e80019. <https://doi.org/10.1371/journal.pone.0080019>

- Graham, C.T., Harrison, S.S.C., Harrod, C., 2013. Development of non-lethal sampling of carbon and nitrogen stable isotope ratios in salmonids: effects of lipid and inorganic components of fins. *Isotopes in Environmental and Health Studies* 49, 555–566. <https://doi.org/10.1080/10256016.2013.808635>
- Harrison, I.J., 2003. *The Freshwater Fishes of Europe*, Volume 8, Part I: Mugilidae, Atherinidae, Atherinopsidae, Blenniidae, Odonotbutidae, Gobiidae 1.
- Hayden, B., Soto, D.X., Jardine, T.D., Graham, B.S., Cunjak, R.A., Romakkaniemi, A., Linnansaari, T., 2015. Small Tails Tell Tall Tales – Intra-Individual Variation in the Stable Isotope Values of Fish Fin. *PLoS ONE* 10, e0145154. <https://doi.org/10.1371/journal.pone.0145154>
- Herzka, S.Z., 2005. Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. *Estuarine, Coastal and Shelf Science, Connectivity in the life cycles of fishes and invertebrates that use estuaries* 64, 58–69. <https://doi.org/10.1016/j.ecss.2005.02.006>
- Hickling, C.F., 1970. A Contribution to the Natural History of the English Grey Mulletts [Pisces, Mugilidae]. *Journal of the Marine Biological Association of the United Kingdom* 50, 609–633. <https://doi.org/10.1017/S0025315400004914>
- Holmes, S., Dekker, R., Williams, I., 2004. Population dynamics and genetic differentiation in the bivalve mollusc *Abra tenuis*: Aplanic dispersal. *Marine Ecology-progress Series - MAR ECOL-PROGR SER* 268, 131–140. <https://doi.org/10.3354/meps268131>
- Kelley, D.F., 1987. Food of bass in U.K. waters. *Journal of the Marine Biological Association of the United Kingdom* 67, 275–286. <https://doi.org/10.1017/S002531540002659X>
- Kennedy, M., Fitzmaurice, P., 1972. The Biology of the Bass, *Dicentrarchus Labrax*, in Irish Waters. *Journal of the Marine Biological Association of the United Kingdom* 52, 557–597. <https://doi.org/10.1017/S0025315400021597>
- Kennedy, M., Fitzmaurice, P., 1969. Age and growth of thick-lipped grey mullet *Crenimugil labrosus* in Irish waters. *Journal of the Marine Biological Association of the United Kingdom* 49, 683–699. <https://doi.org/10.1017/S002531540003722X>
- Koussoroplis, A.-M., Bec, A., Perga, M.-E., Koutrakis, E., Bourdier, G., Desvillettes, C., 2011. Fatty acid transfer in the food web of a coastal Mediterranean lagoon: Evidence for high arachidonic acid retention in fish. *Estuarine, Coastal and Shelf Science* 91, 450–461. <https://doi.org/10.1016/j.ecss.2010.11.010>
- Kuipers, B.R., Dapper, R., 1984. Nursery function of Wadden Sea tidal flats for the brown shrimp *Crangon crangon*. *Marine Ecology Progress Series* 17, 171–181.
- Laffaille, P., Lefeuvre, J.-C., Feunteun, E., 2000. Impact of sheep grazing on juvenile sea bass, *Dicentrarchus labrax* L., in tidal salt marshes. *Biological Conservation* 96, 271–277. [https://doi.org/10.1016/S0006-3207\(00\)00081-1](https://doi.org/10.1016/S0006-3207(00)00081-1)
- Lang, A.C., Buschbaum, C., 2010. Facilitative effects of introduced Pacific oysters on native macroalgae are limited by a secondary invader, the seaweed *Sargassum muticum*. *Journal of Sea Research* 63, 119–128. <https://doi.org/10.1016/j.seares.2009.11.002>
- Lasserre, P., Renaud-Mornant, J., Castel, J., 1976. Metabolic activities of meiofaunal communities in a semi-enclosed lagoon: possibilities of trophic competition between meiofauna and mugilid fish.
- Leijzer, T.B., 2008. Kennisdocument diklipharder, *Chelon labrosus* (Risso, 1827) | Hydrotheek. Kennisdocument 17.
- MacNeil, A.M., 2006. Scientific basis for ecosystem-based management in the Lesser antilles including interactions with marine mammals and other top predators. Cruise report for the LAPE ecosystem survey on RV Celtic Explorer (CE0607).
- McCutchan, J., Lewis Jr, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390. <https://doi.org/10.1034/j.1600-0706.2003.12098.x>
- Muus, B.J., Nielsen, J.G., Muus, B.J., 1999. Sea fish. *Scandinavian Fishing Year Book*, Hedehusene [Denmark].
- Oliveira, M.C.L.M., Mont'alverne, R., Sampaio, L.A., Tesser, M.B., Ramos, L.R.V., Garcia, A.M., 2017. Elemental turnover rates and trophic discrimination in juvenile Lebranche mullet *Mugil liza* under experimental conditions. *J Fish Biol* 91, 1241–1249. <https://doi.org/10.1111/jfb.13408>
- Pawson, M.G., Pickett, G.D., Kelley, D.F., 1987. The distribution and migrations of bass, *Dicentrarchus labrax* L., in waters around England and Wales as shown by tagging. *Journal of the Marine Biological Association of the United Kingdom* 67, 183–217. <https://doi.org/10.1017/S0025315400026448>
- Pawson, M.G., Pickett, G.D., Leballeur, J., Brown, M., Fritsch, M., 2007. Migrations, fishery interactions, and management units of sea bass (*Dicentrarchus labrax*) in Northwest Europe. *ICES Journal of Marine Science* 64, 332–345. <https://doi.org/10.1093/icesjms/fsl035>
- Perkins, M.J., McDonald, R.A., Veen, F.J.F. van, Kelly, S.D., Rees, G., Bearhop, S., 2014. Application of Nitrogen and Carbon Stable Isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) to Quantify Food Chain Length and Trophic Structure. *PLOS ONE* 9, e93281. <https://doi.org/10.1371/journal.pone.0093281>
- Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136, 261–269. <https://doi.org/10.1007/s00442-003-1218-3>
- Pickett, G.D., Pawson, M.G., 1994. *Sea Bass: Biology*. Springer Science & Business Media.
- Pinnegar, J.K., Polunin, N.V.C., 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia* 122, 399–409. <https://doi.org/10.1007/s004420050046>
- Poiesz, S., Witte, J., van der Meer, M., van der Veer, H., Soetaert, K., 2021. Trophic structure and resource utilization of the coastal fish community in the western Wadden Sea: evidence from stable isotope data analysis. *Mar. Ecol. Prog. Ser.* 677, 115–128. <https://doi.org/10.3354/meps13855>

- Poiesz, S.S.H., Witte, J.I., Veer, H.W. van der, 2020. Only a few key prey species fuel a temperate coastal fish food web. *Marine Ecology Progress Series* 653, 153–166. <https://doi.org/10.3354/meps13472>
- Post, D.M., 2002. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology* 83, 703–718. [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
- Reise, K., Baptist, M., Burbridge, P., Dankers, N., Fischer, L., Flemming, B., Oost, A., Smit, C., 2010. The Wadden Sea - a universally outstanding tidal wetland, *Wadden Sea Ecosystem No. 29*. Wilhelmshaven, Germany.
- Riekenberg, P.M., van der Heide, T., Holthuijsen, S.J., van der Veer, H.W., van der Meer, M.T.J., 2022. Compound-specific stable isotope analysis of amino acid nitrogen reveals detrital support of microphytobenthos in the Dutch Wadden Sea benthic food web. *Frontiers in Ecology and Evolution* 10.
- Rijke Waddenzee, 2004. Species fact sheets for the Wadden Sea fish strategy. *Journal of Sea Research* 51, 157–166. <https://doi.org/10.1016/j.seares.2003.09.001>
- Schaber, M., Marohn, L., Petereit, C., Schroeder, J.P., Zumholz, K., Hanel, R., 2011. Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet *Chelon labrosus*. *Fish Sci* 77, 757–764. <https://doi.org/10.1007/s12562-011-0384-1>
- Schlacher, T.A., Connolly, R.M., 2014. Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. *Methods in Ecology and Evolution* 5, 541–550. <https://doi.org/10.1111/2041-210X.12183>
- Shipley, O.N., Matich, P., 2020. Studying animal niches using bulk stable isotope ratios: an updated synthesis. *Oecologia* 193, 27–51. <https://doi.org/10.1007/s00442-020-04654-4>
- Spitz, J., Chauvelon, T., Cardinaud, M., Kostecki, C., Lorange, P., 2013. Prey preferences of adult sea bass *Dicentrarchus labrax* in the northeastern Atlantic: implications for bycatch of common dolphin *Delphinus delphis*. *ICES Journal of Marine Science* 70, 452–461. <https://doi.org/10.1093/icesjms/fss200>
- Suzuki, K.W., Kasai, A., Nakayama, K., Tanaka, M., 2005. Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. *Can. J. Fish. Aquat. Sci.* 62, 671–678. <https://doi.org/10.1139/f04-231>
- Swan, G.J.F., Bearhop, S., Redpath, S.M., Silk, M.J., Goodwin, C.E.D., Inger, R., McDonald, R.A., 2020. Evaluating Bayesian stable isotope mixing models of wild animal diet and the effects of trophic discrimination factors and informative priors. *Methods in Ecology and Evolution* 11, 139–149. <https://doi.org/10.1111/2041-210X.13311>
- Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57, 32–37. <https://doi.org/10.1007/BF00379558>
- Tulp, I., Bolle, L.J., Chen, C., Dänhardt, A., Haslob, H., Jepsen, N., van Leeuwen, A., Poiesz, S.S.H., Scholle, J., Vrooman, J., Vorberg, R., Walker, P., 2022. Fish | Wadden Sea Quality Status Report. Common Wadden Sea Secretariat, Germany.
- Tulp, I., Bolle, L.J., Dänhardt, A., de Vries, P., Haslob, H., Jepsen, N., Scholle, J., 2017. Wadden Sea Quality Status Report Fish 25.
- Tulp, I., Bolle, L.J., Meesters, E., Vries, P. de, 2012. Brown shrimp abundance in northwest European coastal waters from 1970 to 2010 and potential causes for contrasting trends. *Marine Ecology Progress Series* 458, 141–154. <https://doi.org/10.3354/meps09743>
- Tulp, I., Bolle, L.J., Rijnsdorp, A.D., 2008. Signals from the shallows: In search of common patterns in long-term trends in Dutch estuarine and coastal fish. *Journal of Sea Research, Dynamics of Fish and Fishers* 60, 54–73. <https://doi.org/10.1016/j.seares.2008.04.004>
- UNESCO World Heritage, n.d. Wadden Sea [WWW Document]. UNESCO World Heritage Centre. URL <https://whc.unesco.org/en/list/1314/> (accessed 7.1.22).
- van Aken, H.M., 2010. Meteorological forcing of long-term temperature variations of the Dutch coastal waters. *Journal of Sea Research* 63, 143–151. <https://doi.org/10.1016/j.seares.2009.11.005>
- van der Veer, H.W., Dapper, R., Henderson, P.A., Jung, A.S., Philippart, C.J.M., Witte, J.I.J., Zuur, A.F., 2015. Changes over 50 years in fish fauna of a temperate coastal sea: Degradation of trophic structure and nursery function. *Estuarine, Coastal and Shelf Science* 155, 156–166. <https://doi.org/10.1016/j.ecss.2014.12.041>
- van der Veer, H.W., Dapper, R., Witte, J.I., 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). *Journal of Sea Research* 45, 271–279. [https://doi.org/10.1016/S1385-1101\(01\)00062-4](https://doi.org/10.1016/S1385-1101(01)00062-4)
- Vander Zanden, H.B., Bjorndal, K.A., Reich, K.J., Bolten, A.B., 2010. Individual specialists in a generalist population: results from a long-term stable isotope series. *Biology Letters* 6, 711–714. <https://doi.org/10.1098/rsbl.2010.0124>
- Veer, H.W.V.D., Tulp, I., Witte, J.I.J., Poiesz, S.S.H., Bolle, L.J., 2022. Changes in functioning of the largest coastal North Sea flatfish nursery, the Wadden Sea, over the past half century. *Marine Ecology Progress Series* 693, 183–201. <https://doi.org/10.3354/meps14082>