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Bass and ray ecology in Liverpool Bay

Report prepared for MMO/NWIFCA as part of an EMFF-funded project

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Marine
Management
Organisation



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Contents

Executive summary	1
List of figures	3
List of tables	6
1. Introduction.....	7
1.1 Background	7
1.2 Fisheries in the North Western IFCA district.....	7
1.3 Aims of the current project.....	9
2. Methods	11
2.1. Sample collection	11
<i>0-group bass</i>	11
<i>Bass</i>	11
<i>Thornback rays</i>	11
2.2. Processing and analysis.....	12
<i>0-group bass</i>	12
<i>Bass & thornback rays</i>	13
<i>Stable isotope analysis</i>	14
3. Results & Discussion.....	16
3.1. 0-group bass.....	16
<i>Field sampling</i>	16
<i>Otolith ageing</i>	18
3.2. Bass	20
<i>Sample composition</i>	20
<i>Sex ratio</i>	21
<i>Length-weight</i>	21
<i>Gonadosomatic index (GSI), hepatosomatic index (HSI) and fat index</i>	22
<i>Maturity</i>	25
<i>Age & growth</i>	26
<i>Diet</i>	29
<i>Stable isotope analysis</i>	30
3.2. Thornback rays.....	35
<i>Sample composition</i>	35
<i>Sex ratio</i>	36
<i>Maturity status</i>	36

<i>Size at maturity</i>	38
<i>Spawning</i>	40
<i>Minimum landing size (MLS) in relation to maturity</i>	40
<i>Diet</i>	41
<i>Stable isotope analysis</i>	41
Acknowledgements	47
References	48
Appendices	54

Executive summary

European seabass (*Dicentrarchus labrax*) and thornback ray (*Raja clavata*) support important commercial and recreational fisheries in the UK and require evidence-based sustainable management. To complement ongoing research on these species in neighbouring Welsh waters, this preliminary study aimed to provide evidence on the following aspects in Liverpool Bay (including the North Western IFCA District): i) population biology (both species) ii) stock structure and movement (both species), and iii) possible spawning locations (bass).

Samples were collected by fieldwork and from commercial fishers. Although limited by relatively small sample size (130 bass, 152 thornback ray) across a limited temporal window (June-November, mostly September-October), key findings were:

- 0-group bass recorded in the Wyre estuary suggest it is worthy of further investigation as a previously unrecognised bass nursery, one of the northernmost in the UK.
- Daily increment otolith analysis of these 0-group bass was conducted to estimate spawning dates. These will be input into retrospective oceanographic modelling, to identify possible spawning locations in an updated version of this report.
- Bass caught in fisheries had a significant bias towards females, consistent with data from North Wales, which could possibly indicate localized spawning. Gonadal development stage, gonado-somatic index, and fat index confirmed that the sampled period (July-November) is outside the spawning season. Most sampled fish were aged 5 years, ranging from 4.3 to 13.2 years. Growth curves confirmed that females grow slower and to a larger maximum size than males, with sexual dimorphism in growth rates at around 6 years of age. Other biological findings (e.g. length-weight relationship; greater gonadal investment with increasing length and in females compared to males; growth) were broadly comparable to known parameters for this species.
- Stable isotope analysis ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of bass eye lenses was successfully applied for the first time to this species. Whole eye lens diameters varied linearly with total length, indicating that lenses grow across the lifetime of the bass. Isotopes varied across the lens diameter, suggesting that shifts in spatial and trophic ecology can be detected in the eye lenses. Isotopic niches were widest in the core of sampled lenses than the outer layers, suggesting the sampled individuals came from varied juvenile habitats. Isotopes values differed among tissues (muscle, outer lens and scale), suggesting that the different tissues represent the most recent period of the individual's life, but over different time scales. There were no significant isotopic differences between sexes.
- Thornback ray length at 50% maturity (L_{50}) was 651 mm total length (TL) for males and 771 mm TL for females and corresponded well with changes in clasper length and nidamental gland width respectively, and the regional literature. Trawl catch samples were biased towards one sex, and egg cases were found in females in June. Most (53% of males, 68% of females) individuals from commercial catch samples were immature. In comparison to the minimum landing size (MLS) of 45 cm disk width applicable in the northern part of the NWIFCA District, a relatively high proportion (54.7%) of females at or above this MLS were immature, suggesting it may not be adequately protecting females.

- $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures varied across thornback ray eye lenses, suggesting shifts in spatial and trophic ecology across the lifetime of the sampled individuals. Isotopic niches were widest in the core of sampled lenses than the outer layers, suggesting these individuals or their mothers came from varied habitats. Like the bass, isotopes values differed among tissues (muscle and outer lens), suggesting that the different tissues represent different time scales. There were significant differences in carbon isotopes between sexes in the early development of these thornback rays, suggesting sexual segregation during this developmental period.
- Portunid crab and crangonid shrimp were important diet items for both bass and thornback rays, with teleost fish (notably whiting) also important for bass.

Sample collection and analysis, and oceanographic modelling, during spring 2020 was impacted by Covid-19 restrictions; however, some further samples were collected in May 2020, and results from these and the oceanographic modelling will be reported in an updated report at a later date.

List of figures

Figure 1. Annual landings of a) bass (<i>Dicentrarchus labrax</i>) and b) thornback ray (<i>Raja clavata</i>) into English ports of Liverpool Bay (Lancashire and Merseyside) by inshore vessels (≤ 10 m, UK registered). Note that 2009-2013 data is for thornback ray, and 2008 and 2014-2019 is for 'skates and rays' not identified to species, but likely to be mostly, or all, thornback ray. Source: Marine Management Organisation.	8
Figure 2. Seasonal trends in landings of a) bass (<i>Dicentrarchus labrax</i>) and b) thornback ray (<i>Raja clavata</i>) into English ports of Liverpool Bay (Lancashire and Merseyside) by inshore vessels (≤ 10 m, UK registered). Note that 2009-2013 data is for thornback ray, and 2008 and 2014-2019 is for 'skates and rays' not identified to species, but likely to be mostly, or all, thornback ray. Source: Marine Management Organisation.	9
Figure 3. Map of Liverpool Bay study area, showing approximate locations where bass (<i>Dicentrarchus labrax</i>) and thornback ray (<i>Raja clavata</i>) samples were caught by commercial fishers.	12
Figure 4. Sampling locations, and records of, 0-group bass <i>Dicentrarchus labrax</i> in the Wyre estuary (Lancashire). Circles indicate successful (yellow) and unsuccessful (blue) sampling in August 2019. Red diamonds indicate 0-group bass caught in previous small fish surveys in 2010 and 2012 (Environment Agency 2019).	16
Figure 5. Length-frequency distribution of 0-group bass <i>Dicentrarchus labrax</i> caught in the Wyre estuary (Shard Bridge), Lancashire, 9 th August 2019.	17
Figure 6. Sampling locations, and records of, 0-group bass <i>Dicentrarchus labrax</i> in the Ribble estuary (Lancashire). Blue circles indicate unsuccessful sampling in August 2019. Red diamonds indicate 0-group bass caught in previous small fish surveys from 2004-2017 (Environment Agency 2019).	17
Figure 7. Frequency of estimated birth dates of 0-group bass <i>Dicentrarchus labrax</i> caught in the Wyre estuary (Lancashire) on the 9th August 2019.	18
Figure 8. Sagittal otolith of a 91-day-old 0-group bass <i>Dicentrarchus labrax</i> caught in the Wyre estuary (Lancashire) on the 9th August 2019. Viewed at x400 magnification. The blue dots highlight the daily increments read from the core to the distal edge of the otolith.	19
Figure 9. Size and sex composition of all fished bass <i>Dicentrarchus labrax</i> sampled from Morecambe Bay in 2019. Dashed line shows minimum conservation reference size (420 mm total length). Photo shows undersized bass caught under MMO/NWIFCA dispensation.	20
Figure 10. Length-weight relationship of combined male and female bass <i>Dicentrarchus labrax</i> from Morecambe Bay and the Wyre estuary in 2019.	22
Figure 11. Gonadosomatic index (%) (mean with error bars indicating standard deviation) of bass <i>Dicentrarchus labrax</i> in Morecambe Bay across the sampling period of July-November 2019. The dashed line indicates the demarcation of higher GSI found in the spawning season of bass in Wales (Cambiè <i>et al.</i> , 2015).	23

Figure 12. Total length (mm) of bass <i>Dicentrarchus labrax</i> against gonadosomatic index for fish caught in Morecambe Bay in July-November 2019.	24
Figure 13. Total length (mm) of bass <i>Dicentrarchus labrax</i> against hepatosomatic index for fish caught in Morecambe Bay in July-November 2019.	24
Figure 14. Maturity stages in female (left) and male (right) bass <i>Dicentrarchus labrax</i> sampled from Morecambe Bay, July-November 2019.	25
Figure 15. Examples of maturity stages in female bass <i>Dicentrarchus labrax</i> from Morecambe Bay, autumn 2019. L-R: Stage III (developing, early) AB4, 425 mm TL, September; Stage IV (developing, late) AB92, 612 mm TL, October; Stage VII (spent) AB24, 605 mm TL, September.	26
Figure 16. Von Bertalanffy growth curves for fished (Morecambe Bay) and 0-group (Wyre estuary) bass <i>Dicentrarchus labrax</i> collected in 2019. Points are mean values for each age class, with standard deviation as error bars. Shaded areas indicate 95% confidence limits.	27
Figure 17. Examples of stomach contents in bass from Morecambe Bay, autumn 2019. (Left) Whiting, (right) portunid crabs.....	29
Figure 18. Left: Whole eye lens diameter (mm) and total length (mm) of individual bass <i>Dicentrarchus labrax</i> from Morecambe Bay caught in 2019. Right: Whole eye lens weight and total length of individual bass.	30
Figure 19. $\delta^{13}\text{C}$ across eye lens diameter for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019. A. sampled population trend B. Individual eye lens tracks.	31
Figure 20. $\delta^{15}\text{N}$ across eye lens diameter for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019. A: sampled population trend. B: Individual eye lens tracks.	32
Figure 21. Stable isotope across eye lens diameter by sex for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019. A: $\delta^{13}\text{C}$. B: $\delta^{15}\text{N}$	32
Figure 22. Isotopic niches of 3 phases of eye lenses (core, outer layer and the remaining central lens) for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019. Standard ellipse areas (solid lines) and convex hulls (dotted lines).	33
Figure 23. Difference in standard ellipse area among three lens phases for data presented in Figure 22.	33
Figure 24. Differences in (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ between tissue types taken from the same individuals for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019.	34
Figure 25. Length-frequency distribution by sex of combined thornback ray <i>Raja clavata</i> sample from the Liverpool Bay area, June & September 2019.	36
Figure 26. a) ICES Maturity status and b) ICES maturity stage of thornback <i>Raja clavata</i> from the Liverpool Bay area, June & September 2019, based on macroscopic examination of reproductive organs (ICES, 2013).	37

Figure 27. Maturity of male thornback ray <i>Raja clavata</i> from Liverpool Bay in 2019 a) Total length at 50% maturity ogive (L_{50}), based on macroscopic examination of gonads (ICES, 2013). L_{50} = 651 mm TL; r^2 = 0.65. Dashed blue lines indicate 95% confidence intervals (633 – 670 mm TL). b) Change in clasper length with total length. Maturity status (ICES, 2013) and L_{50} of 651 mm TL (dashed line) is indicated.....	38
Figure 28. Maturity of female thornback ray <i>Raja clavata</i> from Liverpool Bay in 2019 a) Total length at 50% maturity ogive (L_{50}), based on macroscopic examination of reproductive tract (ICES, 2013). L_{50} = 771 mm TL; r^2 = 0.70. Dashed blue lines indicate 95% confidence intervals (752 – 791 mm TL). b) Change in nidamental gland width with total length. Maturity status (ICES, 2013) and L_{50} of 771 mm TL (dashed line) is indicated.....	39
Figure 29. $\delta^{13}\text{C}$ across eye lens diameter of thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019. A: sampled population trend B: Individual eye lens tracks.	42
Figure 30. $\delta^{13}\text{C}$ across eye lens diameter by sex of thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019.	43
Figure 31. $\delta^{15}\text{N}$ across eye lens diameter for thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019. A: sampled population trend B: Individual eye lens tracks.	44
Figure 32. $\delta^{15}\text{N}$ across eye lens diameter by sex of thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019.	44
Figure 33. Isotopic niches of 3 phases of eye lenses (core, outer layer and the remaining central lens) for thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019. Standard ellipse areas (solid lines) and convex hulls (dotted lines).	45
Figure 34. Difference in standard ellipse area among three lens phases for data presented in Figure 33.	45
Figure 35. Differences in (Left) $\delta^{13}\text{C}$ and (Right) $\delta^{15}\text{N}$ between tissue types taken from the same individuals of thornback ray <i>Raja clavata</i> sampled in the Liverpool Bay area in 2019.....	46

List of tables

Table 1. Sample size for bass (<i>Dicentrarchus labrax</i>) and thornback ray (<i>Raja clavata</i>) stable isotope analysis.....	15
Table 2. Estimated ages of 0-group bass <i>Dicentrarchus labrax</i> caught in the Wyre estuary (Lancashire) on the 9th August 2019.	18
Table 3. Details of fished bass <i>Dicentrarchus labrax</i> samples obtained for this study. ‘Sized’ and ‘undersized’ means above and below, respectively, the minimum conservation reference size (MCRS) of 42 cm total length.....	20
Table 4. Length (cm)-weight (g) relationship ($W=aL^b$) for bass <i>Dicentrarchus labrax</i> from Morecambe Bay and Wales.	21
Table 5. Von Bertalanffy growth parameters L_{∞} and K , and the growth performance index (Φ , Pauly and Munro, 1984) for <i>Dicentrarchus labrax</i> data from the present study (in bold) and others from different locations (adapted from Cardoso et al., 2015). M: males, F: females, C: combined.	28
Table 6. Stomach contents of bass <i>Dicentrarchus labrax</i> ($n=94$) from Morecambe Bay, mostly sampled in October and November 2019. Indet. = indeterminate.....	29
Table 7. Paired t -test results comparing tissues from the same individuals for bass <i>Dicentrarchus labrax</i> caught in Morecambe Bay in 2019.	34
Table 8. Details of thornback ray <i>Raja clavata</i> samples obtained for this study.	35
Table 9. Length at maturity (in mm TL) of thornback ray <i>Raja clavata</i> from the present study (in bold) and others in the Celtic Seas ICES ecoregion (including the Irish Sea).....	40
Table 10. Proportion of sampled thornback ray <i>Raja clavata</i> from the Liverpool Bay area in 2019 that were immature in relation to the Cumbria Sea Fisheries Committee minimum landing size of 45 cm disk width (converted to total length of 67.1 cm using McCully et al., 2012).....	41
Table 11. Stomach contents of thornback ray ($n=13$) from Morecambe Bay in September 2019. Indet. = indeterminate.	41

1. Introduction

1.1 Background

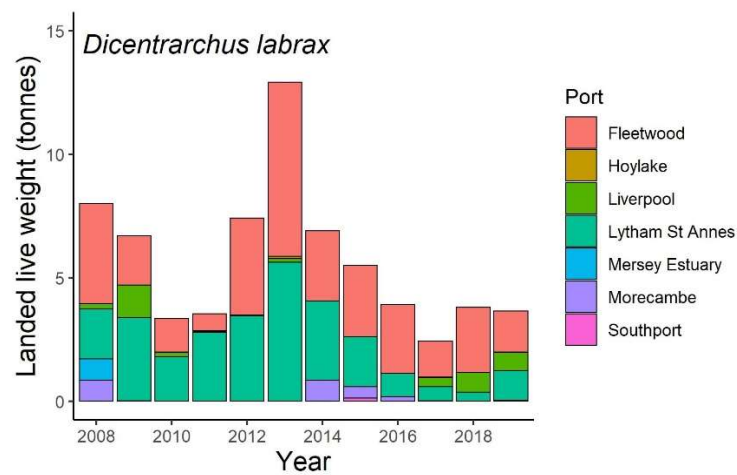
In UK waters, European sea bass (*Dicentrarchus labrax*) and thornback ray (*Raja clavata*) support important commercial and recreational fisheries. Declines in abundance and landings have occurred for both species, highlighting the need for evidence-based management. Widespread concern about bass stocks and their exploitation led to the introduction of a package of emergency measures in the UK in 2015, such as an increase in minimum landing size (MLS) ('minimum conservation reference size', MCRS) from 36 cm to 42 cm, and recreational bag limits. While thornback ray abundance in the Irish Sea is currently thought to be increasing, this is in the context of historic declines around the UK (ICES, 2018; Rogers and Ellis, 2000). There is no UK-wide MLS for thornback ray, and none in some local administrations, such as in north Wales and in the neighbouring English waters forming the southern part of the North Western Inshore Fisheries and Conservation Authority (NWIFCA) region. In contrast, there is an MLS in some local administrations, such as in South Wales (Welsh Assembly Government, 2011). In the northern part of the NWIFCA region (former Cumbria Sea Fisheries Committee), this is 45 cm disk width for all skates and rays (excluding exceptions regarding the Landing Obligation 1380/2013).

1.2 Fisheries in the North Western IFCA district

Landings data were obtained from the Marine Management Organisation (MMO) for landings of bass and rays into English ports of Liverpool Bay (Lancashire and Merseyside; i.e. the southern part of the NWIFCA district) by inshore vessels (≤ 10 m, UK registered) for 2008-2019. Figure 1 presents annual trends. For bass, these show recent landings around a third lower than a peak in 2013, with Fleetwood and Lytham St Annes the most consistently important local ports. Rays show a similar pattern (2019 landings less than half the peak in 2015), with Fleetwood the only local port of importance.

Monthly landings (Figure 2) show that the bass fishery is highly seasonal, with most landings between May and September, peaking in July and August. Rays are landed year-round and show a less distinct pattern, with August the most important month for landings in recent years (2018 and 2019).

a)



b)

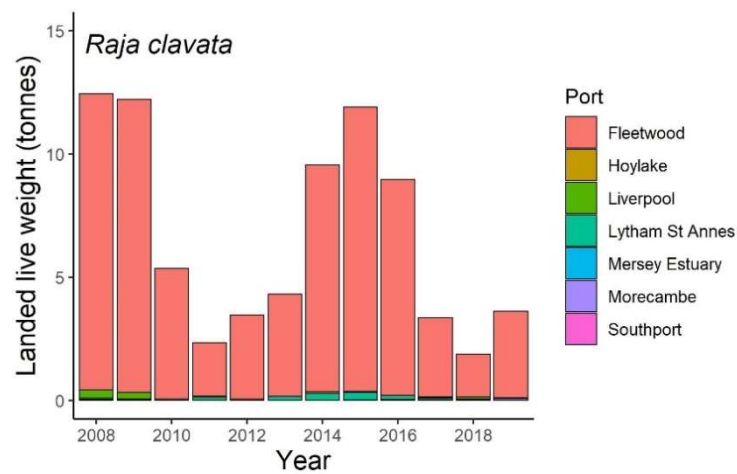
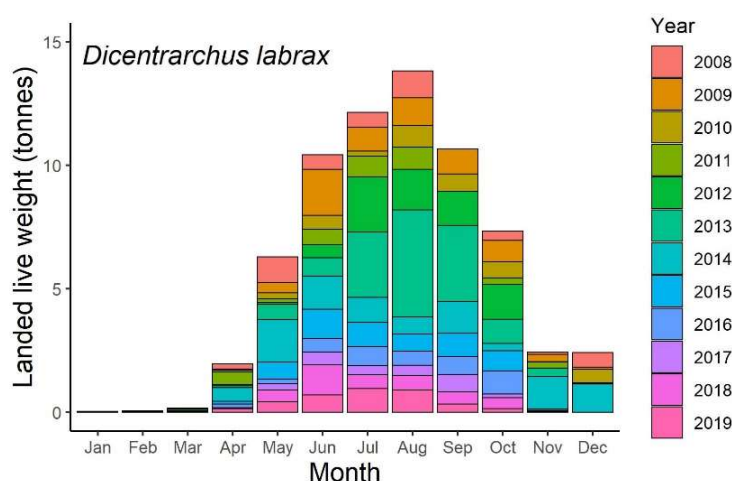


Figure 1. Annual landings of a) bass (*Dicentrarchus labrax*) and b) thornback ray (*Raja clavata*) into English ports of Liverpool Bay (Lancashire and Merseyside) by inshore vessels (≤ 10 m, UK registered). Note that 2009-2013 data is for thornback ray, and 2008 and 2014-2019 is for 'skates and rays' not identified to species, but likely to be mostly, or all, thornback ray. Source: Marine Management Organisation.

a)



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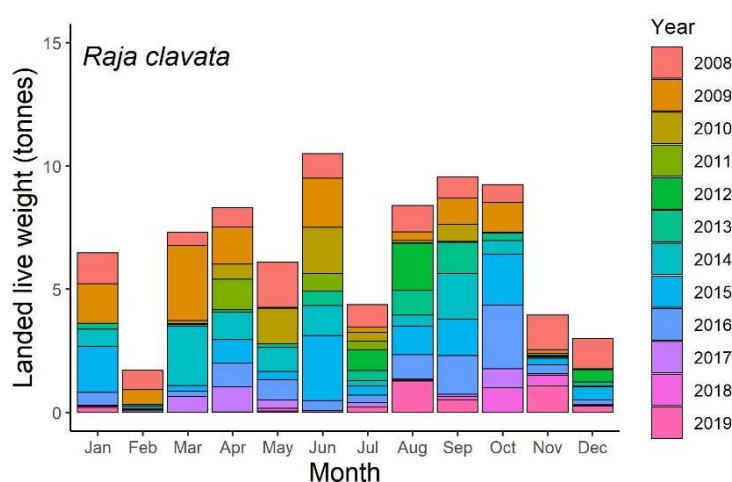


Figure 2. Seasonal trends in landings of a) bass (*Dicentrarchus labrax*) and b) thornback ray (*Raja clavata*) into English ports of Liverpool Bay (Lancashire and Merseyside) by inshore vessels (≤ 10 m, UK registered). Note that 2009-2013 data is for thornback ray, and 2008 and 2014-2019 is for 'skates and rays' not identified to species, but likely to be mostly, or all, thornback ray. Source: Marine Management Organisation.

1.3 Aims of the current project

Bangor University Sustainable Fisheries group currently has a project gathering evidence to inform the sustainable management of these species in Welsh waters (Welsh-EMFF funded – “Fisher-Science Partnership for sustainable fisheries”). Due to the lack of data on the geographical extent of stocks of these species, and the high likelihood that there are cross-border stocks, the current project proposed to provide evidence on which to base sustainable management by extending data collection into neighbouring English waters of Liverpool Bay to more complete picture of the following:

- Spawning locations (bass)
- Population biology (bass and thornback rays)

- Stock structure and movement (bass and thornback rays)

As such, the project had the following specific aims:

- Collect post-larval ('0-group') bass recruits from estuaries identified through liaison with fisher Local Ecological Knowledge interviews and through liaison with the North Western IFCA and then combine ageing of otoliths with retrospective oceanographic modelling to identify the most likely spawning locations
- Collect and analyse samples of bass and thornback rays through the local fishing industry to gather data on biological parameters such as size at maturity, length frequencies, sex ratios and age structures for both bass and thornback rays, highlighting any spatial variation
- Use stable isotope analysis of these samples to elucidate stock structure and movement patterns in Liverpool Bay and the wider Irish Sea, for bass and thornback rays

The project commenced in July 2019, with all fieldwork and fish sample collection undertaken between August and November 2019. Government restrictions in place as a result of the Covid-19 pandemic (March 2020 onwards) prevented a small number of frozen thornback ray samples from 2019 being analysed in the laboratory, fieldwork in spring 2020, and the oceanographic modelling. However, a number of bass were collected on behalf of the project by a commercial fisher in spring 2020 as part of their normal fishing operations. These bass and ray samples will be processed when restrictions allow, and results from them, and from the oceanographic modelling, will be provided in an updated report at a later date.

2. Methods

2.1. Sample collection

0-group bass

As sampling specifically for 0-group bass in northwest England had previously been undertaken on a limited basis (Kelley, 1988), candidate locations in the region were identified from satellite imagery (Google Maps) based on known habitat preferences (e.g. saltmarsh and sandy shores of estuaries; Pickett and Pawson, 1994), along with data obtained from previous small fish surveys (Environment Agency, 2019) and engagement with stakeholders with local knowledge (Environment Agency, Wyre River Trust). Sampling was conducted under NWIFCA dispensation (no. 20264 SCI19) in daylight hours on foot, using a fine (3-7 mm) mesh seine net of approximately 4 m long by 1.5 m deep, towed across distances of 5-30 m in waters of 30-100 cm deep. Any 0-group bass caught were retained, measured, and weighed; other fish species were noted, and all fish and invertebrates were returned alive at point of capture.

Bass

Bass were sourced from one commercial fisher who targets bass using rod and line gear in Morecambe Bay, representing the northernmost extent of Liverpool Bay (Figure 3). Due to time and budget constraints, the fisher was asked to retain and freeze a size-structured sample of legally-sized bass from their normal fishing operations; these fish were therefore not necessarily representative of the size structure of the fished population. Undersized bass were collected under dispensation from NWIFCA (no. SCI250919) and the Marine Management Organisation (ref. 28/19), by chartering the commercial fisher's vessel. Undersized fish seized by the NWIFCA from enforcement work were also donated for analysis.

Thornback rays

Thornback rays were sourced from commercial otter trawlers operating in the Liverpool Bay and Morecambe Bay area, and by a gillnetter fishing in the mouth of the Dee estuary (Figure 3).

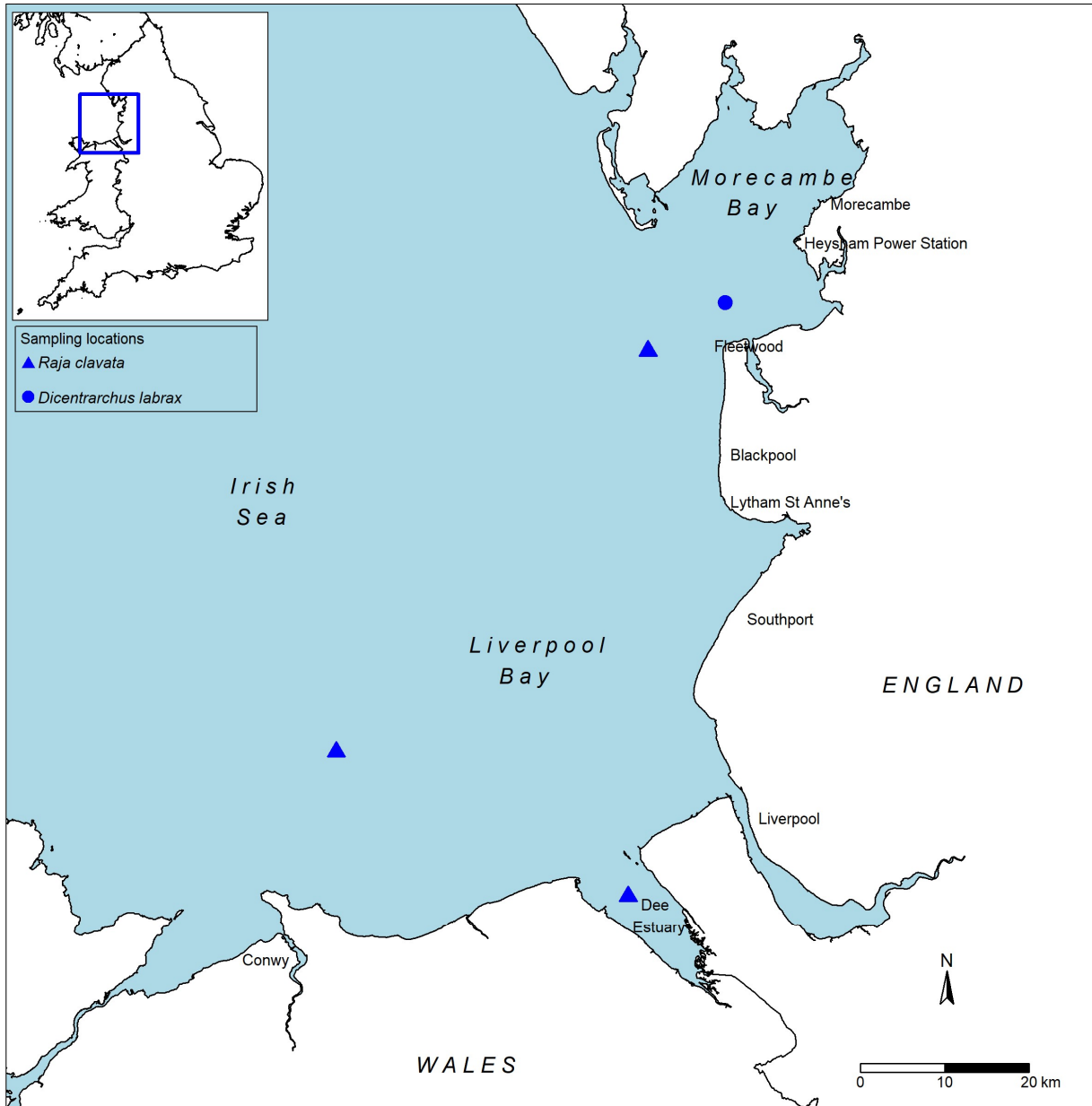


Figure 3. Map of Liverpool Bay study area, showing approximate locations where bass (*Dicentrarchus labrax*) and thornback ray (*Raja clavata*) samples were caught by commercial fishers.

2.2. Processing and analysis

0-group bass

Both sagittal otoliths were extracted, cleaned and dried. The right sagitta otoliths were weighed (mg) and mounted sulcus-side down (as this was flatter than on the distal side) on a microscope slide using Crystalbond® thermoplastic glue. Otoliths were ground using 5 μm and 3 μm lapping film consecutively, polished using cloths and 0.3 μm alpha alumina powder in solution and finally rinsed with distilled water (Morales-Nin *et al.*, 2010). Prepared otoliths were viewed using a Zeiss Axio Imager A1 and images were taken using an AmScope MU900 USB2.0 microscope. Images were captured at x100 magnification for the whole otolith and x400 for age readings. Age readings and measurements

were conducted in the FIJI version of Image J with the Object J plug in as per methods described by Denechaud *et al.* (2018). The dorsal axis was selected for readings as increments were generally clear there. The nearest possible axis to this was chosen when increments were poorly defined. Readings were conducted twice by the same person, first from core to edge and then from edge to core. The coefficient of variation (CV) was calculated between the two readings using the Chang Index (Chang, 1982), and a third reading was conducted if values were above the 10% limit of acceptability; otoliths were rejected if they still crossed this threshold after a third reading (Morales-Nin and Panfili, 2002). Since increment formation in *D. labrax* otoliths forms 2 days after hatch (Gutiérrez and Morales-Nin, 1986), 2 days was added to averaged age estimates to calculate the age of fish from hatching. A further 5.25 days were added to account for the average egg stage duration (Beraud *et al.*, 2018) and provide the estimated age of each fish. The birth date was then back-calculated from capture date using the estimated age estimate.

Bass & thornback rays

Individual fish were allocated a unique identification number tied to the skipper, vessel, location and date of capture. Total length (TL, bass and thornback rays) and disk width (DW, rays only) were recorded to the nearest mm; whole fish were weighed to the nearest g. For thornback rays, to compare the Cumbria Sea Fisheries MLS of 45 cm DW to TL of sampled fish, the DW-TL conversion $DW = (0.6572 * TL) + 0.909$ (McCully *et al.*, 2012) was used.

The liver and gonads of bass and thornback rays were excised and weighed separately to calculate hepatosomatic index (HSI) and gonadosomatic index (GSI) (weight of organ expressed as a % of whole body weight) respectively, for both bass and rays. Mesenteric fat (whitish lobe-like deposits in the main body cavity attached to other viscera, as compared to perinephritic deposits found longitudinally along the dorsal part of the body cavity) were also weighed from some bass to calculate a fat index (Pawson and Pickett, 1996).

Sex and maturity stage of bass were assessed by macroscopic examination of the ovaries and testes, and classification according to Pawson and Pickett (1996) and images of these stages from bass collected in Wales (Cambiè *et al.*, 2015). Sex of thornback rays was determined by presence or absence of claspers, with their maturity (immature or mature) and development stage (e.g. developing) assessed by macroscopic examination of reproductive organs according to the scale proposed by ICES (2013) for oviparous elasmobranchs. Clasper length (clasper tip to posterior margin of cloaca) and nidamental gland width were measured to the nearest mm for male and female rays respectively. Length at 50% maturity (L_{50}) was calculated using the R package sizeMat (Torrejón-Magallanes, 2019).

Bass were aged by scalimetry. Prior to photography under a dissecting microscope, scales were cleaned of mucus in distilled water by gentle abrasion with a nylon brush, with any that showed evidence of regrowth after damage being excluded. Estimates were based on two scales per fish from beneath the pectoral fin (A and B) by two readers (R1, R2) giving a minimum total of four age estimates per fish. To minimise bias readers made estimates independently of the other reader's estimate, and the length of the fish. In cases other than complete agreement on these four estimates, the following procedure was used. An average was taken in cases where 1) each reader had the same estimate for both scales, but this was one year different to the other reader (e.g. R1A5B5, R2A6B6 = 5.5) 2) both readers had the same one year difference between scales (e.g. R1A5B6, R2A5B6 = 5.5) and 3) where one reader had the same for both scales, but the other reader had different estimates for each scale,

within 1 and 2 years of this (R1A5B5, R2A6B7 = 5.75). In cases where one of the four estimates was one year different, the majority estimate was used. Where there was more than one year difference between readers one and two, two additional readers provided estimates of a single scale and when three readers agreed, this age was used. For cases where the range in estimates across the four readers was 3 years, an average was taken of the majority of values which were within 2 years of each other. Scales that had estimated ages spanning >3 years were excluded from the current study; a different set of archived scales will be re-examined at a later date to confirm ages. In order to be consistent with a bass study in Wales by Cambiè *et al.* (2015), who found the last scale annulus to be fully identifiable in July, June and July were considered as the months corresponding to the new age and first month of the new age of the fish, respectively. Therefore, a fish aged 4 years and caught in July was considered as 4.083 years of age (corresponding at 4 years and 1 month). The estimation of fish age including both years and months, allowed the use of the post-larval (0-group) bass recruit length data collected in the present study. Von Bertalanffy growth parameters were generated for males, females and for the combined dataset (mixed-sex) using the Fisheries Stock Assessment package (FSA) (version 0.8.3) (Ogle, 2009) in R Statistical software (Version 3.5.0) (R Development Core Team; www.r-project.org). Ageing of rays was not attempted, given recent evidence that suggests the long-accepted method of counting vertebral centra band pairs does not accurately record age (Harry, 2018; Natanson *et al.*, 2018).

Stable isotope analysis

Stable isotope analysis was performed on muscle, scales, and eye lenses (Table 1). White muscle (approximately 1cm³) was excised from the shoulder region for bass, and from the proximal part of the tail for rays, removing any skin. For scales in bass, as isotope data is only obtainable from the last (most recent) growth band (due to ‘under-plating’; Hutchinson and Trueman, 2006), this thin layer (0.44 ± 1.7 mm; Cambiè *et al.*, 2016) was trimmed off using a scalpel under a dissecting microscope, with multiple scales often needed to achieve the target sample weight (0.4 - 0.8 mg); trimmings were dried in paper prior to analysis. Eye lenses were prepared according to Simpson *et al.* (2019), i.e. removal of layers using forceps under a microscope, measurement of lens diameter before removal of each layer, and cleaning of dissecting equipment with ethanol between layers to minimise contamination. All samples for stable isotope analysis were freeze-dried, individually weighed, placed into pre-weighed tin capsules (Elemental Microanalysis, 8 x 5 mm), folded to remove air and to create a more uniform incineration surface for the mass spectrometer, and placed into a sterilised well-plate, with the location for each specimen in the well-plate recorded for later reference. Batches were couriered to the SEAPORT Laboratory (University of Southampton) for analysis of carbon and nitrogen isotopes using continuous-flow isotope ratio mass spectrometry (Isoprime100 isotope ratio mass spectrometer with Vario ISOTOPE select elemental analyser). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope ratios were recorded in delta notation, defined as parts per thousand (‰) deviations from the PeeDee Belemnite (PDB) international standard for carbon and air for nitrogen according to the equation:

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

Where X is $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ and R is the ratio of the heavy isotope to the light isotope ($^{15}\text{N}:^{14}\text{N}$ or $^{13}\text{C}:^{12}\text{C}$) in the sample and reference material.

Table 1. Sample size for bass (*Dicentrarchus labrax*) and thornback ray (*Raja clavata*) stable isotope analysis

Species	Tissue	Male	Female
Bass	Eye lens	14	15
	Muscle	14	15
	Scales	25	25
Thornback ray	Eye lens	15	15
	Muscle	15	15

To test whether the eye lenses grow linearly over the lifetime of bass, the relationship between eye lens outer diameter and body length was tested with a regression. Sequential samples of eye lens proteins recovered from eye lenses were analysed to generate isotopic time series (life history profiles). To characterise temporal patterns in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values across eye lens diameters, Generalized Additive Mixed Models (GAMM) were run in R with the mgcv package (Version 1.8-18). The weighted Akaike Information Criterion (wAIC) and the deviance explained was used to select the appropriate model. In the final model, sex and eye lens diameter were used as fixed effects and individual as a random effect to account for the repeated measures sample design. Eye lenses were also split into 3 phases, the core (the earliest days of the individual's life), the outer layer (the most recent period) and the remaining central lens tissue. Within-phase isotopic variability was quantified using Bayesian standard ellipse areas (SEAc) for each of the ontogenetic phases (core, lens and outer) using the SIBER package in R (version 2.14.1) (R Development Core Team, 2015; Jackson *et al.*, 2011). Isotopic niche area and overlap ($\%^2$) were estimated based on 100 000 posterior draws of the SEAc. A paired T-test was used to examine the difference in isotopic values between tissues.

Stomach contents of some bass and rays were identified using taxonomic keys (Hayward and Ryland, 1990) and enumerated where possible, in order to calculate frequency of occurrence (%O, i.e. stomachs that contained food in which a specific prey taxon was recorded, divided by the number of stomachs examined $\times 100$) and the percentage by number (%N; the total number of each prey taxon divided by the total number of enumerated prey items $\times 100$).

Archive samples for possible future analysis outside the present study were also retained, and included otoliths from bass, and clips of fins from both species for future molecular genetic analysis.

3. Results & Discussion

3.1. 0-group bass

Field sampling

Twelve locations in the Wyre, Ribble and Lune estuaries were surveyed during this study (Figure 4 and 5). Field sampling only recorded 0-group bass on the Wyre estuary at Shard Bridge, approximately 9 km upstream from the estuary mouth (Figure 4, Appendix 1), where 24 individuals were caught (30 - 52 mm TL, mean 37.6 ± 4.6 SD, Figure 5) at slack low water on the 9th of August 2019. 0-group bass were not recorded in any other location, although this may simply be due to patchy distribution, and/or poor weather conditions at the time of sampling.

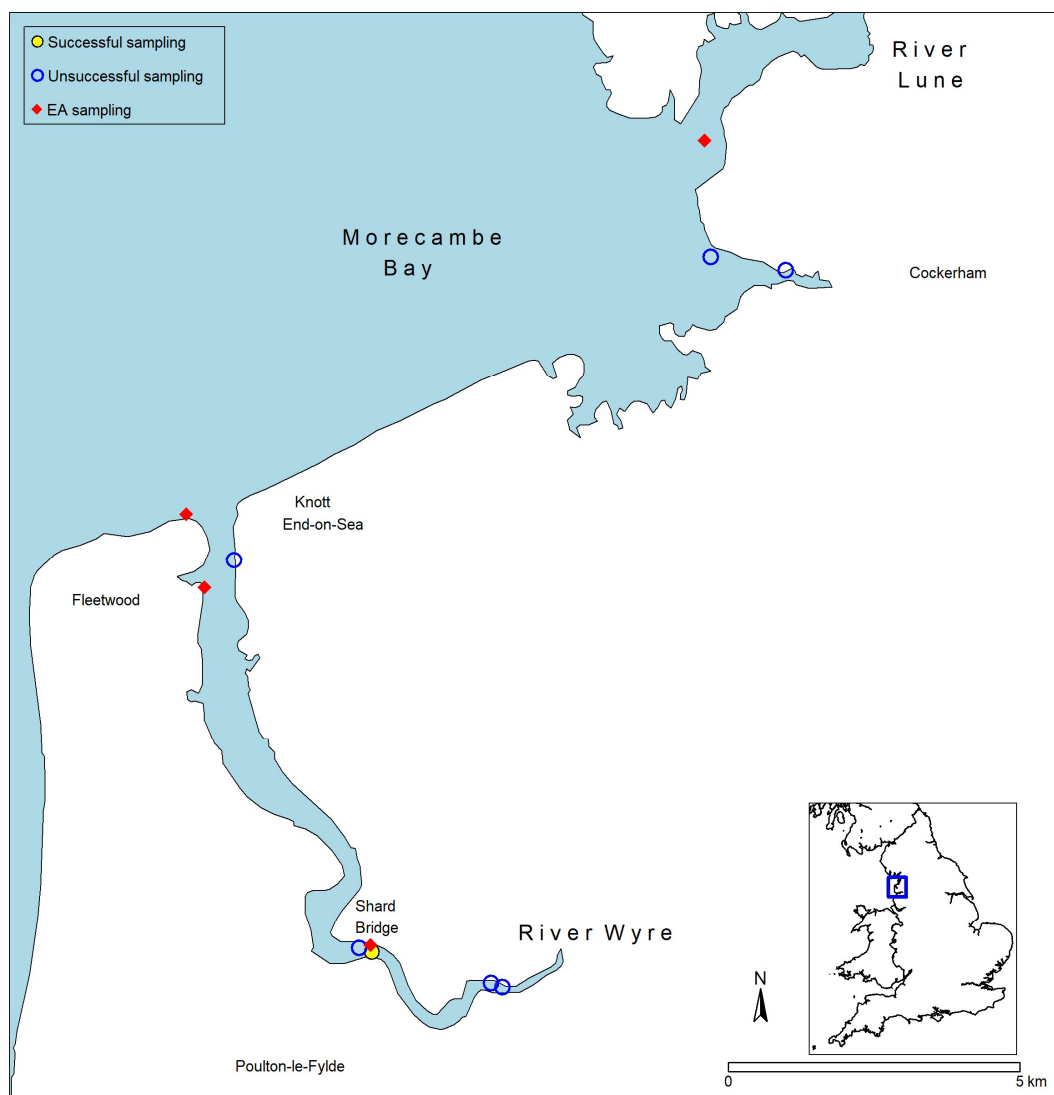


Figure 4. Sampling locations, and records of, 0-group bass *Dicentrarchus labrax* in the Wyre estuary (Lancashire). Circles indicate successful (yellow) and unsuccessful (blue) sampling in August 2019. Red diamonds indicate 0-group bass caught in previous small fish surveys in 2010 and 2012 (Environment Agency 2019).

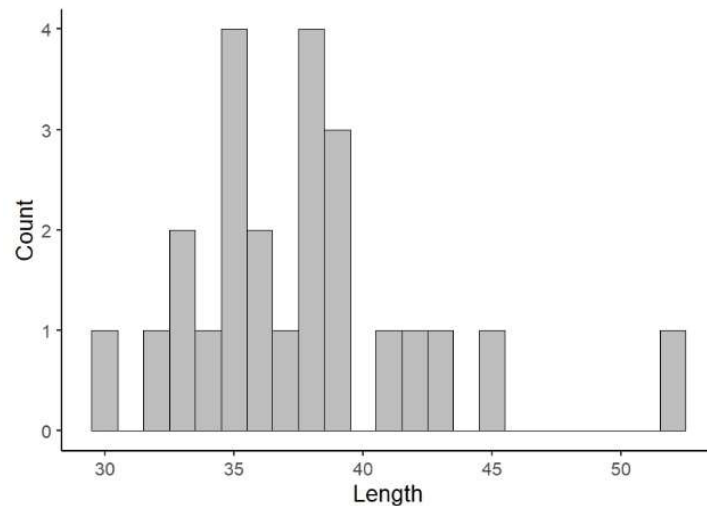


Figure 5. Length-frequency distribution of 0-group bass *Dicentrarchus labrax* caught in the Wyre estuary (Shard Bridge), Lancashire, 9th August 2019.

Data from previous small fish surveys (Environment Agency, 2019) also revealed a number of records of bass <50mm TL. These were mostly from Lytham on the Ribble estuary during sampling in the months of September and October (Figure 6) across a number of years (2004, 2008, 2010, 2012, 2017). The Ribble estuary has previously been identified as the northernmost extent of bass nursery areas in the Irish Sea (Kelley, 1988). The finding of 0-group bass in the Wyre estuary to the north, combined with other Environment Agency survey records of bass <50mm there (Shard Bridge (September 2010); Fleetwood dock and beach (September & October 2012) and local fisher knowledge (A. Bews pers. comm.) are therefore of interest, and suggest the estuary is worthy of further investigation as a bass nursery habitat.

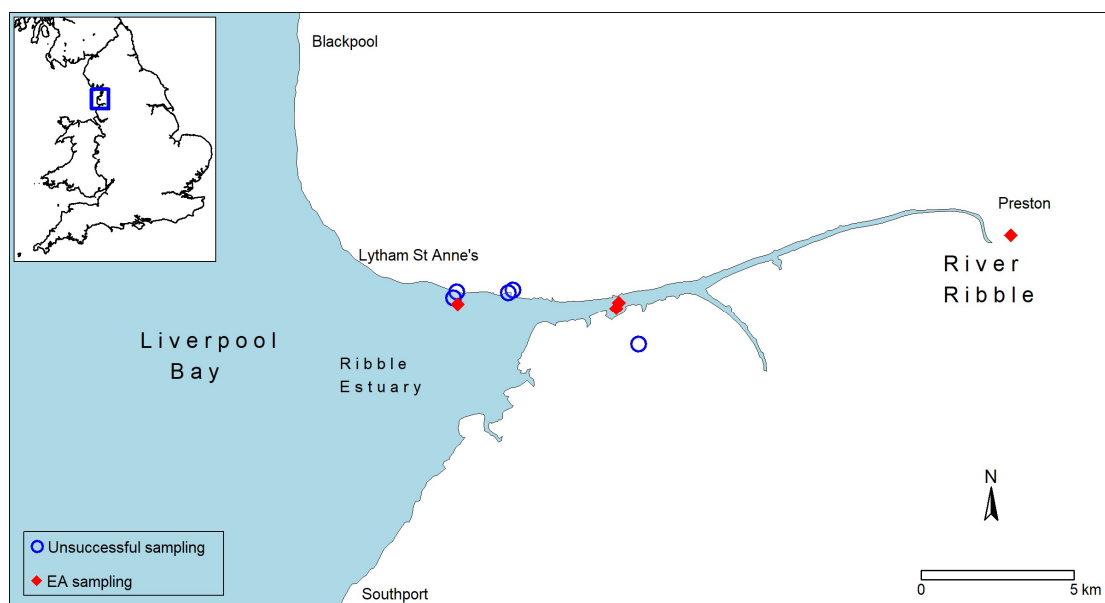


Figure 6. Sampling locations, and records of, 0-group bass *Dicentrarchus labrax* in the Ribble estuary (Lancashire). Blue circles indicate unsuccessful sampling in August 2019. Red diamonds indicate 0-group bass caught in previous small fish surveys from 2004-2017 (Environment Agency 2019).

Otolith ageing

Of the 24 0-group bass caught, 19 were aged successfully (Figure 7) with the remaining 5 bass rejected either as a result of damage during processing or falling outside the cut-off of 10% reading error. The mean age of 0-group bass found in the Wyre was 107 days with the youngest aged at 78 days and the oldest at 126 days (Table 2). The birthdates of fish were estimated to be between the 31st March – 18th May. 53% of fish were estimated to have birth dates across a 14-day period between the 4th – 19th April (Figure 8).

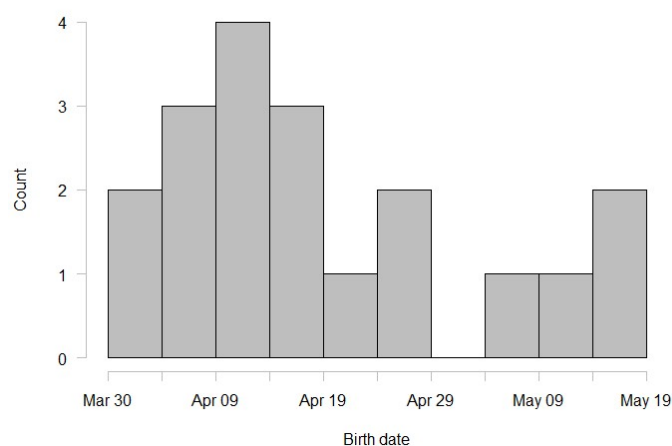


Figure 7. Frequency of estimated birth dates of 0-group bass *Dicentrarchus labrax* caught in the Wyre estuary (Lancashire) on the 9th August 2019.

Table 2. Estimated ages of 0-group bass *Dicentrarchus labrax* caught in the Wyre estuary (Lancashire) on the 9th August 2019.

Capture date	Estimated					
	Age (days)			Spawning date	Entering estuary	Time spent in estuary
	Min.	Mean	Max.			
9 th August 2019	78	107	126	18 th April 2019	14 th June 2019	56 days

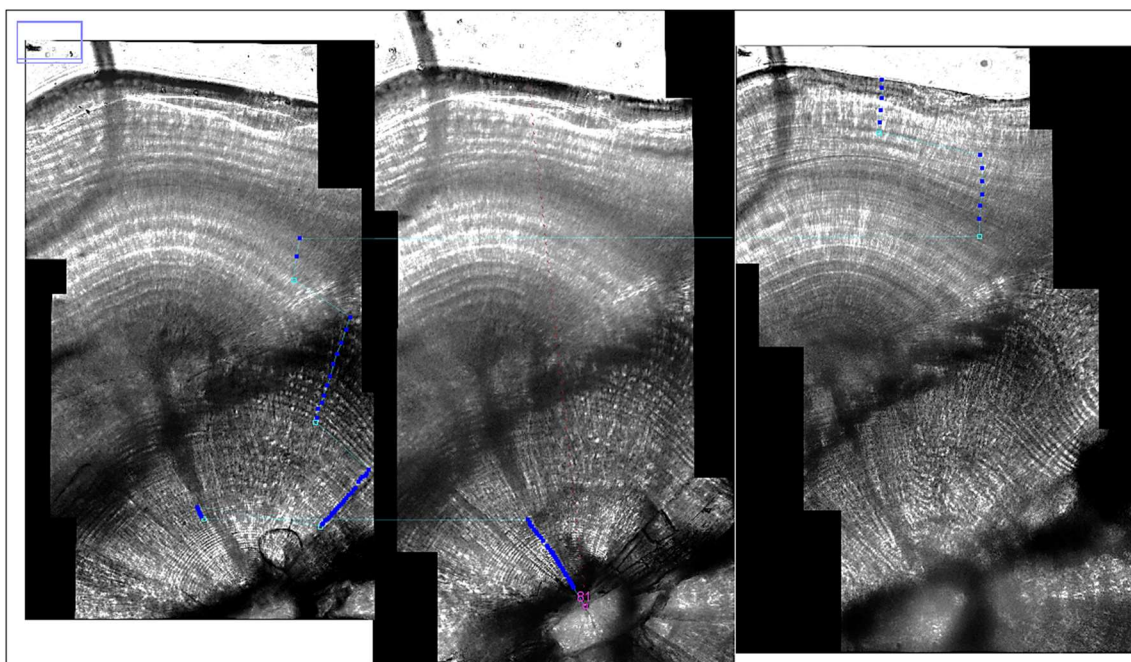


Figure 8. Sagittal otolith of a 91-day-old 0-group bass *Dicentrarchus labrax* caught in the Wyre estuary (Lancashire) on the 9th August 2019. Viewed at x400 magnification. The blue dots highlight the daily increments read from the core to the distal edge of the otolith.

3.2. Bass

Sample composition

All individuals were obtained from the outer Morecambe Bay area (Table 3).

Table 3. Details of fished bass *Dicentrarchus labrax* samples obtained for this study. ‘Sized’ and ‘undersized’ means above and below, respectively, the minimum conservation reference size (MCRS) of 42 cm total length.

Location	Date (2019)	Notes	N (sized:undersized)	♂	♀
Morecambe Bay (Heysham Power Station outflow)	5 th July	Confiscated by IFCA	14 (0:14)	2	12
Morecambe Bay	9 th Sept.–15 th Oct.	Commercial fishing (rod & line)	67 (65:2)	21	46
	20–21 st Oct.	Scientific fishing (rod & line)	28 (9:19)	8	20
	6 th Nov.		21 (5:16)	2	19
Total			130 (79:51)	33	97

A total of 130 bass were processed, comprising 51 individuals (39.2%) that were under the MCRS (333–419 mm TL) and 79 (60.8%) of legal size (420–657 mm TL)(Figure 9).

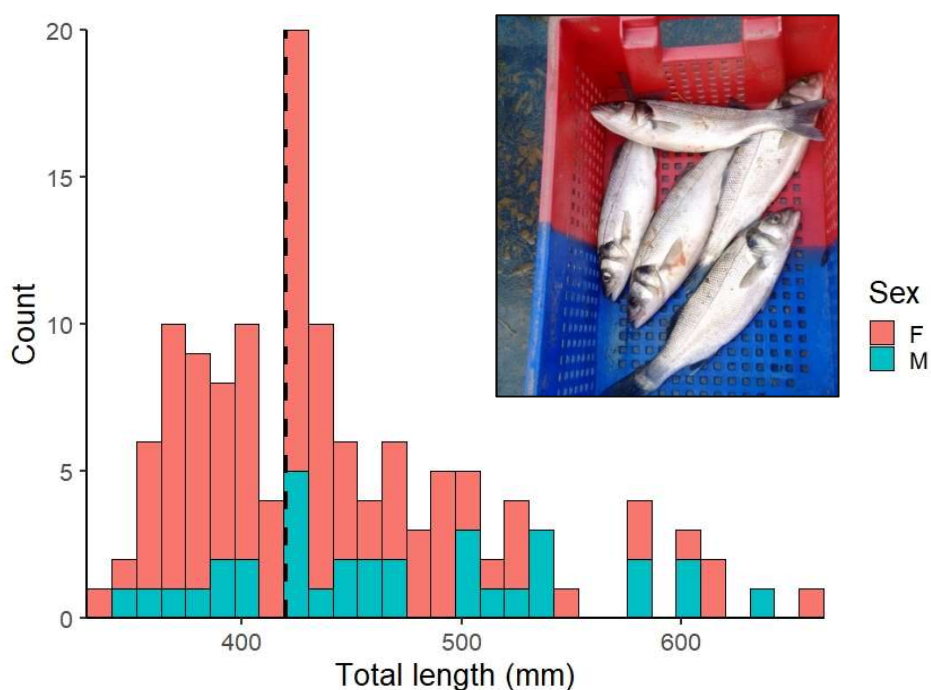


Figure 9. Size and sex composition of all fished bass *Dicentrarchus labrax* sampled from Morecambe Bay in 2019. Dashed line shows minimum conservation reference size (420 mm total length). Photo shows undersized bass caught under MMO/NWIFCA dispensation.

Sex ratio

There was a highly significant departure from parity towards females, which applied to the total sample (X^2 test = 31.51, d.f. = 1, $p < 0.001$), undersized (X^2 test = 21.35, d.f. = 1, $p < 0.001$), and sized fish (X^2 test = 12.17, d.f. = 1, $p < 0.001$). By month sampled, this bias towards females was highly significant in October and November (X^2 test = 14.29 and 13.67 respectively, d.f. = 1, $p < 0.001$), significant in July (X^2 test = 7.14, d.f. = 1, $p = 0.007$) but not in September (X^2 test = 2.27, d.f. = 1, $p = 0.13$).

The significant bias towards females in the present study is consistent with bass sampled year-round in North Wales by Cambiè *et al.* (2015), compared to a more equal sex ratio for bass in South Wales. These authors found the strongest female bias for North Wales to be in winter, which is consistent with bass samples from October and November for the present study. Possible reasons for this female skew include sampling bias, although Cambiè *et al.* (2015) investigated this and considered it unlikely. Temperature is known to affect sex ratios in bass under experimental conditions, with high and low temperatures early in development resulting in a bias towards males and females, respectively (Pavlidis *et al.*, 2000; Piferrer *et al.*, 2005). Cambiè *et al.* (2015) suggested that the predominance of females in the relatively cold waters of North Wales might therefore be a result of local spawning there, and it is possible that this can also explain the female bias in the Liverpool/Morecambe Bay area.

Length-weight

The length-weight relationship of bass from the Morecambe Bay, including Wyre estuary 0-group fish, is presented below (Table 4, Figure 10). The a and b parameters are broadly similar to a much larger, year-round study in Wales (Cambiè *et al.*, 2015).

Table 4. Length (cm)-weight (g) relationship ($W=aL^b$) for bass *Dicentrarchus labrax* from Morecambe Bay and Wales.

Study	Size	n	a	b
This study	Fished + 0-group	153	0.0227	2.8094
Wales* (Cambiè <i>et al.</i> , 2015)	Fished + 0-group	2541	0.0173	2.8671

*these authors found no significant difference between North and South Wales.

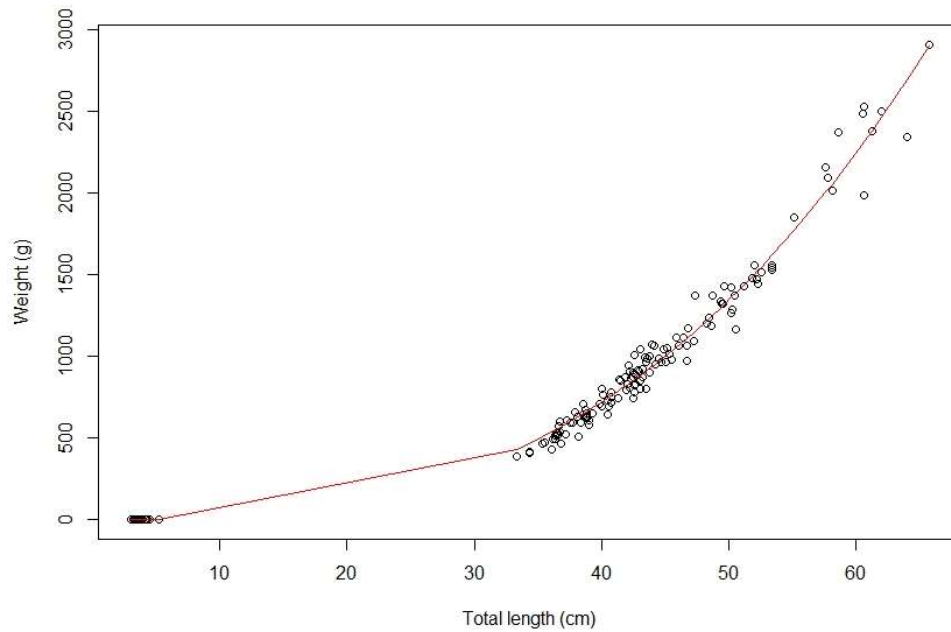


Figure 10. Length-weight relationship of combined male and female bass *Dicentrarchus labrax* from Morecambe Bay and the Wyre estuary in 2019.

Gonadosomatic index (GSI), hepatosomatic index (HSI) and fat index

GSI values ranged across an order of magnitude from 0.12 (518 mm TL male, October) to 1.43 (612 mm female, October). Females had higher GSI than males, and GSI values were broadly similar across the sampled months (Figure 11). Most GSI values were <1, and all <2, consistent with the latter being used as the demarcation between the spawning and non-spawning season in Wales (Cambiè *et al.*, 2015).

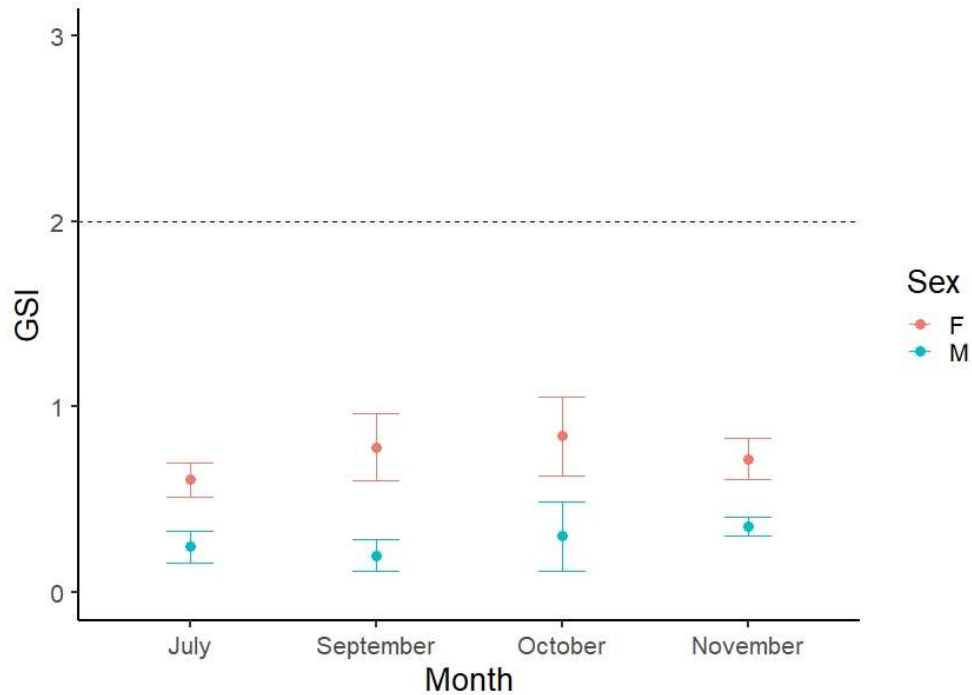


Figure 11. Gonadosomatic index (%) (mean with error bars indicating standard deviation) of bass *Dicentrarchus labrax* in Morecambe Bay across the sampling period of July-November 2019. The dashed line indicates the demarcation of higher GSI found in the spawning season of bass in Wales (Cambiè *et al.*, 2015).

There was a clear relationship between GSI and total length (Figure 12) with larger fish investing proportionally more in gonadal development. This was significant for females ($F_{1,25} = 31.75$, $r^2 = 0.54$, $p < 0.001$) and males ($F_{1,15} = 7.02$, $r^2 = 0.27$, $p = 0.018$) in September, and for females in October ($F_{1,36} = 84.01$, $r^2 = 0.69$, $p < 0.001$). Females always invested proportionally more than males for a given size. The difference between males and females was highly significant for September (ANOVA, $F_{1,15} = 91.37$, $p < 0.001$) and October (ANOVA, $F_{1,10} = 32.38$, $p < 0.001$). Small sample size constrained analysis in July and November. Overall, these findings are consistent with those from Wales (Cambiè *et al.*, 2015) and broadly support larger, more fecund fish, especially females, being more valuable to the sustainability of the fishery.

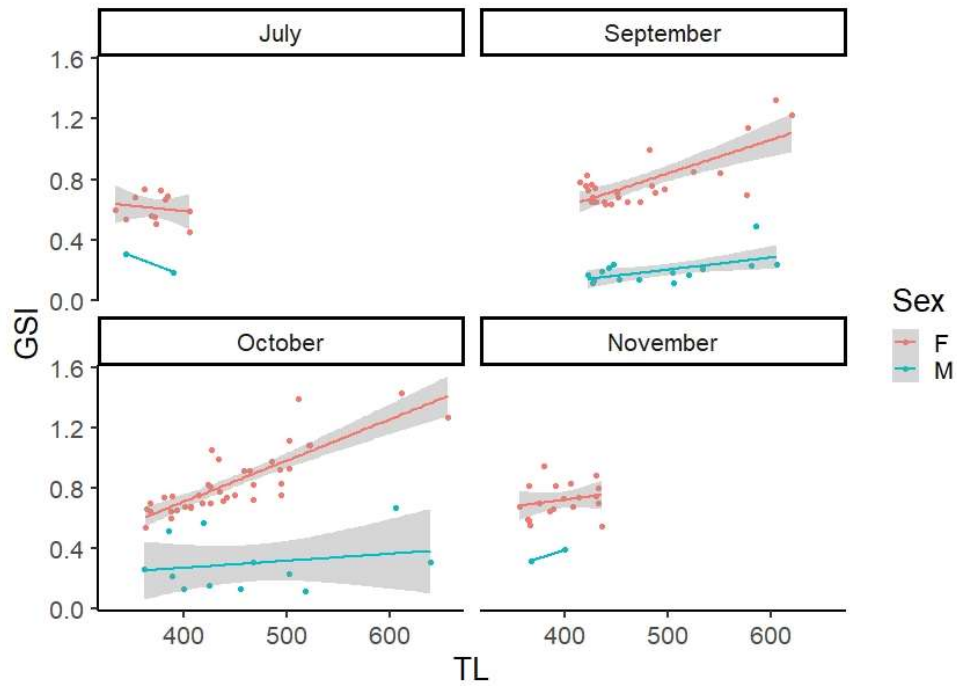


Figure 12. Total length (mm) of bass *Dicentrarchus labrax* against gonadosomatic index for fish caught in Morecambe Bay in July-November 2019.

There was less of a clear relationship between hepatosomatic index (HSI) and size (Figure 13), although females in September showed significant decreasing HSI with increasing size ($F_{1,25} = 7.69$, $r^2 = 0.20$, $p = 0.01$), when there was also a difference between sexes (ANOVA, $F_{1,15} = 6.03$, $p = 0.03$).

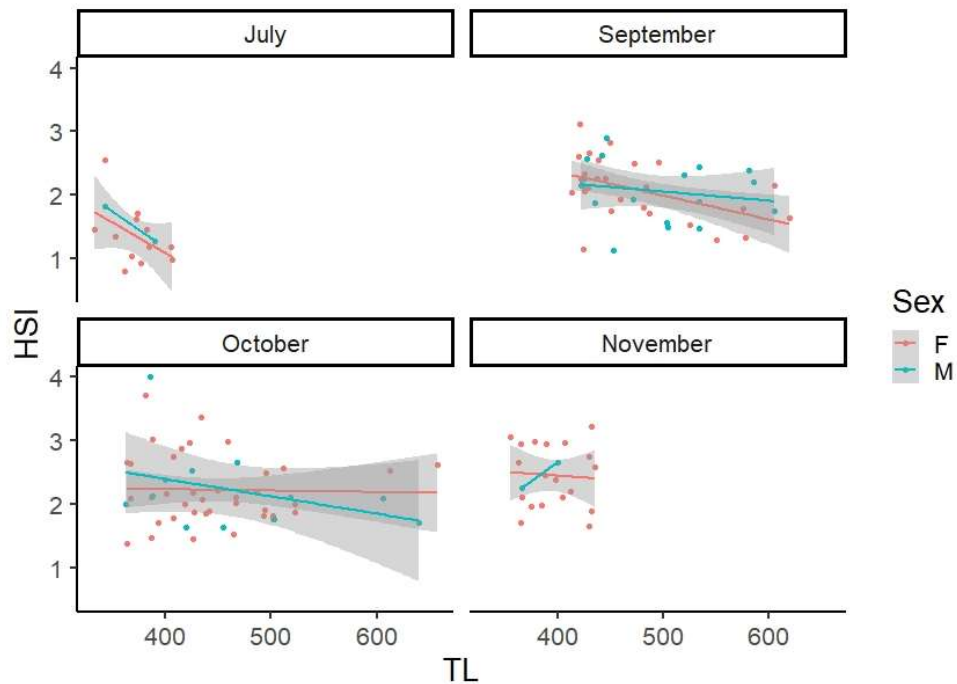


Figure 13. Total length (mm) of bass *Dicentrarchus labrax* against hepatosomatic index for fish caught in Morecambe Bay in July-November 2019.

Fat index (FI) data was available for 3 months (July, October and November) and varied widely (0.16 - 7.16; mean 2.69 ± 1.36 SD). There was a significant difference in FI amongst month (One-way ANOVA ($F_{2,82} = 10.65$, $p < 0.001$), with July having significantly lower FI than October and November (Tukey *post-hoc* test, $p < 0.001$).

Data from the current study is limited to months within the ‘feeding season’ of July-December identified for bass in Wales (Cambiè *et al.*, 2015). Further samples being obtained from Morecambe Bay during May 2020 will help provide data during the spawning season. Previous data on fat content in bass in the study region is limited to Pawson and Pickett (1996) who provided data on bass from a ‘northern region’ (comprising north Wales, Liverpool Bay, Morecambe Bay, Solway Firth and the southern North Sea). This found a rapid increase in fat content in the summer to highest levels around September, with the lowest levels during the spawning season (around April to May).

Maturity

Most bass were classed as stage III (developing, early); this included all male fish (343 - 640 mm TL) and the majority of females (333 - 576 mm TL). A small number ($n = 6$) of females were stage IV (developing, late), and were all larger (512 - 657 mm TL), with higher GSIs (>1) caught in September or October. Only two stage VII (spent) bass were recorded, both of which were large (578 and 605 mm TL) females with high GSIs (1.14 - 1.33) caught in September (Figures 14 and 15); these may represent individuals that spawned late in the season.

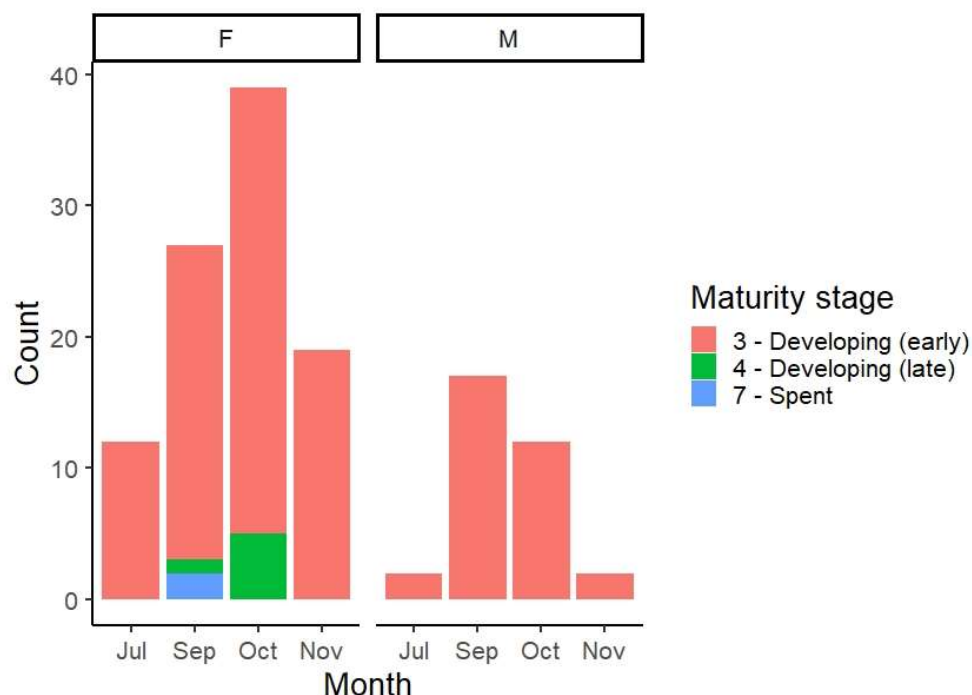


Figure 14. Maturity stages in female (left) and male (right) bass *Dicentrarchus labrax* sampled from Morecambe Bay, July-November 2019.



Figure 15. Examples of maturity stages in female bass *Dicentrarchus labrax* from Morecambe Bay, autumn 2019. L-R: Stage III (developing, early) AB4, 425 mm TL, September; Stage IV (developing, late) AB92, 612 mm TL, October; Stage VII (spent) AB24, 605 mm TL, September.

Age & growth

The fished bass had ages ranging from 4.3 years (343 - 425 mm TL) to 13.2 years (581 mm TL male), with most (70%) individuals aged at 5 years. A small number ($n = 5$) of mostly large fish had a high level of disagreement in estimated ages and were omitted from the present analysis; additional scales from these fish will be re-aged at a later date.

Von Bertalanffy growth curves using data from fished, sexed bass with unsexed Wyre estuary 0-group (Figure 16) and their associated growth parameter outputs (Table 5, including comparison with regional studies) are constrained by a lack of young and old fish. The available data do however indicate that females have slower growth and a larger asymptotic length compared to males (Figure 16a,b), with asymptotic lengths of both males and females broadly similar to that found for Wales (Table 5)(Cambiè *et al.*, 2015). Sexual dimorphism in growth rates appears to commence at around 6 years of age (Figure 16c), also similar to that found in Wales (Cambiè *et al.*, 2015).

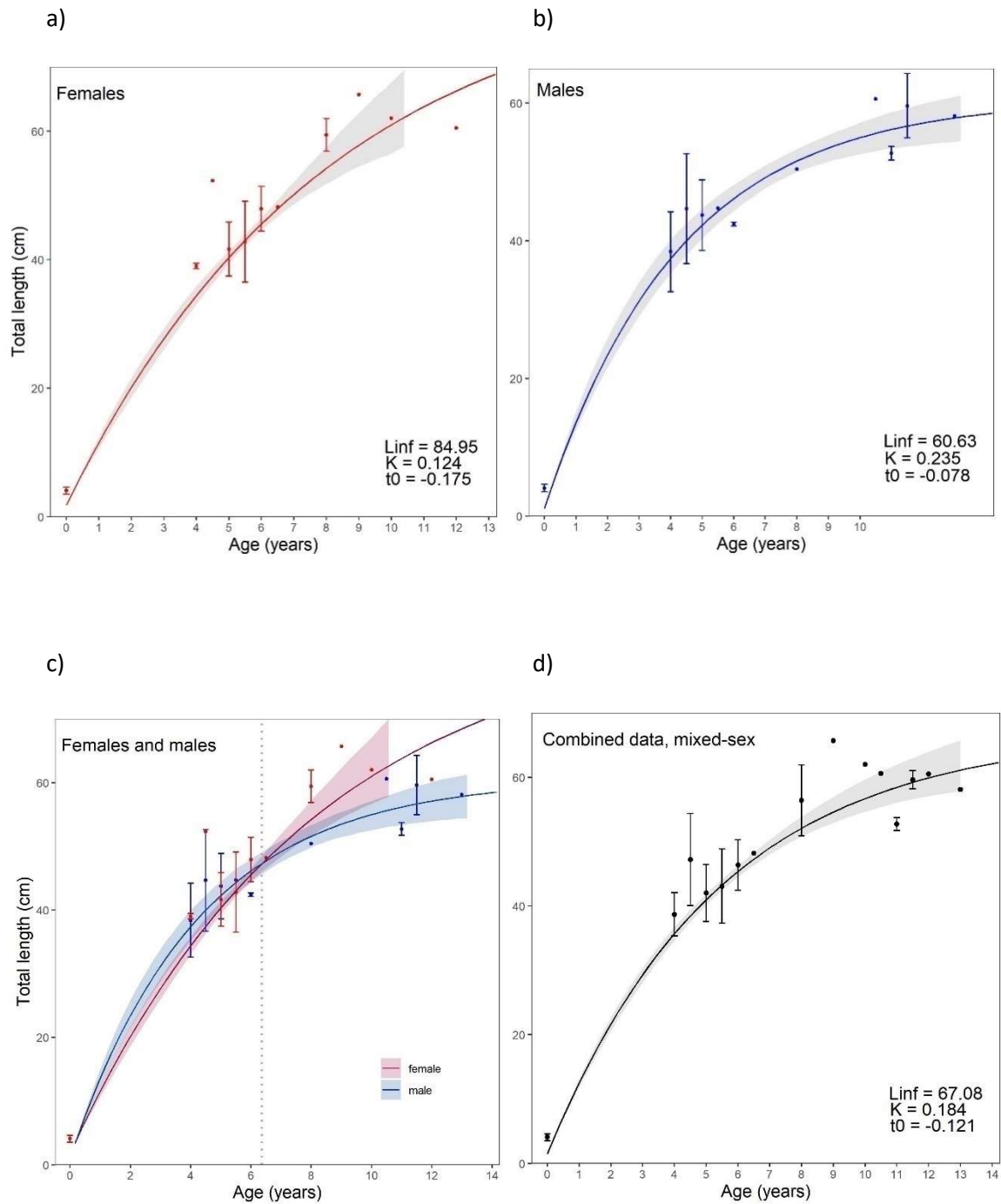


Figure 16. Von Bertalanffy growth curves for fished (Morecambe Bay) and 0-group (Wyre estuary) bass *Dicentrarchus labrax* collected in 2019. Points are mean values for each age class, with standard deviation as error bars. Shaded areas indicate 95% confidence limits.

Table 5. Von Bertalanffy growth parameters L_{∞} and K , and the growth performance index (Φ , Pauly and Munro, 1984) for *Dicentrarchus labrax* data from the present study (in bold) and others from different locations (adapted from Cardoso et al., 2015). M: males, F: females, C: combined.

Area	L_{∞} (cm)			K (y^{-1})			Φ			Ref.
	M	F	C	M	F	C	M	F	C	
Ireland	67.3	69.3	-	0.14	0.14	-	2.8	2.83	-	1
Irish Sea	63.3			0.16			2.81			2
UK all areas	53.8	54.9	-	0.23	0.24	-	2.83	2.87	-	3
UK East coast	55.1			0.38			3.06			3
UK West coast	69.9	68.5	-	0.13	0.14	-	2.8	2.82	-	4
UK (Irish Sea, Bristol Channel & Celtic Sea North)*	81.87			0.09			2.79			5
UK (Central and Southern North Sea) [†]	82.98			0.11			2.88			5
UK (Eastern English Channel) [“]	87.22			0.09			2.84			5
UK (Western English Channel) [§]	92.27			0.08			2.83			5
UK all areas	84.55			0.1			2.84			5
England (Morecambe Bay and Wyre Estuary)	60.63	84.95	67.08	0.24	0.12	0.18	2.94	2.95	2.92	6
Wales (North, Mid and South)	63.11	87.64	78.99	0.16	0.09	0.1	2.8	2.85	2.81	7
Netherlands (Dutch Wadden Sea)	83.5	114.6	102.4	0.11	0.08	0.09	2.88	3.02	2.97	8

¹Kennedy and Fitzmaurice (1972); ²Fahy *et al.* (2000); ³Pickett and Pawson (1994); ⁴Kelley (1988); ⁵ICES (2016); Correspond to ICES Divisions (*) VIIafg, (†) IVbc, (″) VIId, and (§) VIIe; ⁶This study; ⁷Cambiè *et al.* (2015); ⁸Cardoso *et al.* (2015).

Diet

A total of 94 bass stomachs were examined, mostly from October and November. Of these, 23 (24.5%) were empty, with 69 (73.4%) with identifiable contents (Table 6). The most important items were portunid crabs (likely *Carcinus maenas* or *Liocarcinus* spp.; 42.3 %O), fish [including unidentified remains (26.8 %O) and whiting *Merlangius merlangus* (14.1 %O)] and crangonid (brown) shrimp (22.5 %O).

Table 6. Stomach contents of bass *Dicentrarchus labrax* (n=94) from Morecambe Bay, mostly sampled in October and November 2019. Indet. = indeterminate.

Higher taxa	Taxa	Count of stomachs with prey type present	Sum of numbers of each prey type	%O	%N
Crustacea	Crustacea (indet.)	3	3	4.2	1.5
	Idoteidae (indet.)	1	2	1.4	1
	Crangonidae sp.	16	48	22.5	23.9
	Portunidae (indet.)	30	88	42.3	43.8
Pisces	Teleost (indet.)	19	27	26.8	13.4
	<i>Sprattus sprattus</i>	1	4	1.4	2
	<i>Merlangius merlangus</i>	10	24	14.1	11.9
	Triglidae (indet.)	1	1	1.4	0.5
	Ammodytidae (indet.)	2	2	2.8	1
Misc.	Digested remains	2	2	2.8	1
	Total	85	201	119.7	100

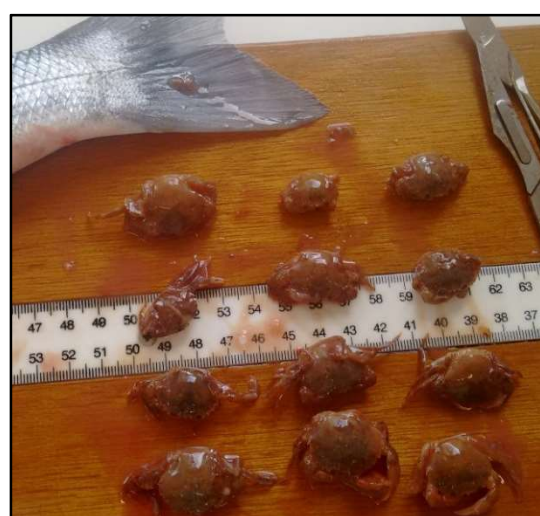


Figure 17. Examples of stomach contents in bass from Morecambe Bay, autumn 2019. (Left) Whiting, (right) portunid crabs.

Stable isotope analysis

The stable isotope analysis of bass eye lens was successfully applied for the first time. Eye lenses delaminated as expected. Between seven and eleven layers were removed from individual eye lenses, however only between five and seven were analysed (due to budget restrictions). In future it would be useful to analyse all the layers to investigate whether a finer resolution of bass life history is revealed.

Whole eye lens diameters varied linearly with total length ($r^2 = 0.162$, $p = 0.034$), though the relationship was noisy (Figure 18). The relationship between eye lens weight and total length had a higher r^2 value ($r^2 = 0.511$, $p < 0.001$) (Figure 18 **Error! Reference source not found.**). It is likely that eye lens weight was a more accurate measure of the total eye lens than diameter due to the difficulty measuring the diameter of viscous outer layers. Both these results indicate that eye lenses grow across the lifetime of the bass.

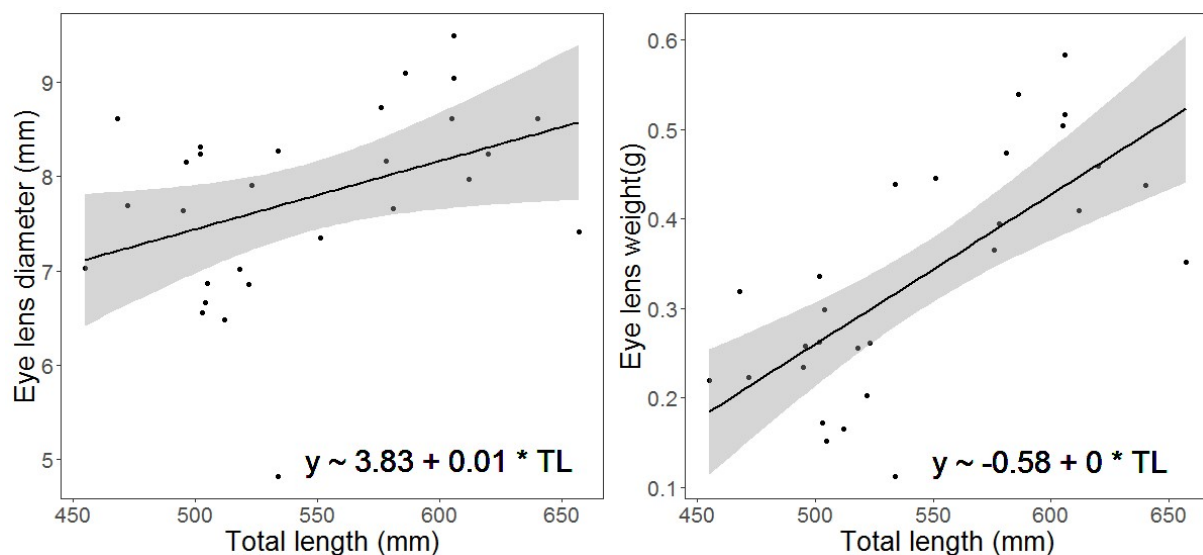


Figure 18. Left: Whole eye lens diameter (mm) and total length (mm) of individual bass *Dicentrarchus labrax* from Morecambe Bay caught in 2019. Right: Whole eye lens weight and total length of individual bass.

The samples as a whole showed significant change across the eye lens in $\delta^{13}C$ (Figure 19a), with $\delta^{13}C$ at the lowest in the cores, increasing until approximately 3 mm eye lens diameter, then plateauing, with a slight decline towards the outer lens ($p < 0.001$). This trend indicates spatial movements across the lifetime of these bass. There were however differing trends among individuals ($p < 0.001$) (Figure 19b), but there were no significant differences between sexes ($p = 0.77$) (Figure 21a) and total length was not significant ($p = 0.41$). The total deviance explained for the model was 56.9%. In the core of the eye lenses, individuals had varied $\delta^{13}C$, but all converged to more similar values across the eye lens, suggesting individuals aggregated to feed in the area from dispersed nursery areas.

Bass are thought to spend early life inhabiting coastal lagoons and estuaries (Dando and Demir, 1985). Once bass grow to mature phases (>32 cm), they move out of their inshore habitats to begin annual

spawning migrations to their offshore spawning sites (Pawson et al., 1987; Pawson and Pickett, 1996). The results of this study support these findings and provide a new method to explore the spatial ecology of bass.

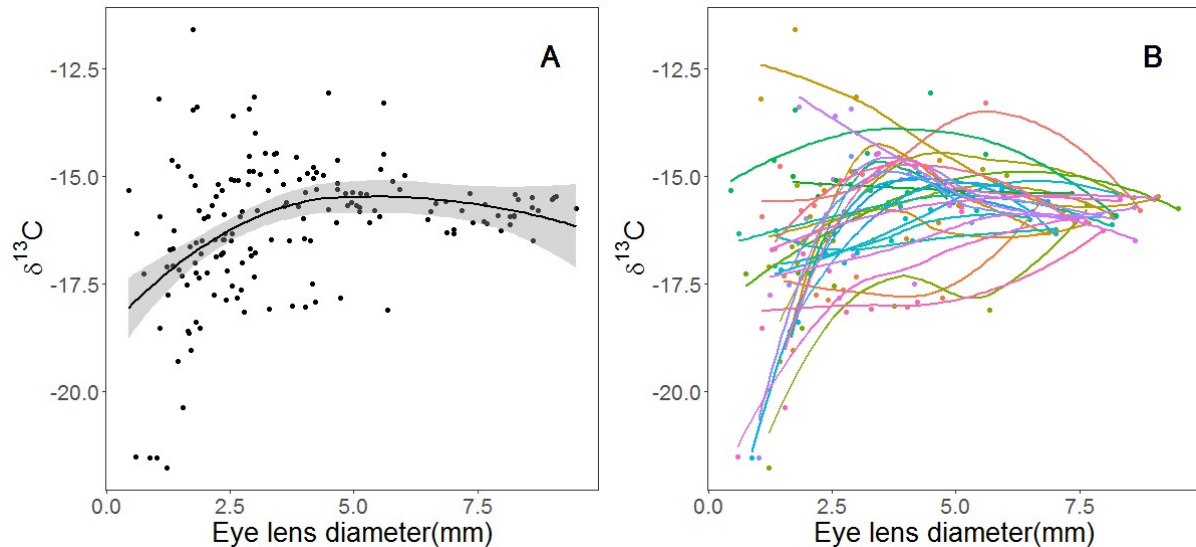


Figure 19. $\delta^{13}\text{C}$ across eye lens diameter for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019. A. sampled population trend B. Individual eye lens tracks.

For $\delta^{15}\text{N}$, values start lower in the core and increase across the eye lenses. The majority of individual tracks broadly show this trend ($p < 0.001$) (Figure 20a), but there were some individuals that differed, starting relatively high in the core, decreasing across the eye lens ($p < 0.001$) (Figure 20b). There was no significant effect of sex ($p = 0.50$) (Figure 21b) or total length ($p = 0.07$) and the total deviance explained for the model was 71.9%. The core $\delta^{15}\text{N}$ values show a similar pattern to $\delta^{13}\text{C}$, in that they are more varied early in ontogeny and then converge across the eye lens, feeding at the same trophic level and spatial location. Previous research found that $\delta^{15}\text{N}$ values become enriched as trophic level increases (Pinnegar and Polunin, 1999). The $\delta^{15}\text{N}$ values increasing across the eye lens diameter in bass implies that the trophic level of bass increases throughout their ontogeny. The increase in trophic level through the early stages of bass ontogeny, may reflect the mouth gape increase as the fish grows, allowing the consumption of larger prey, which are likely to occupy higher trophic levels (e.g. Costalago et al., 2012; June and Carlson, 1971).

As stated previously, for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values there were no significant differences in isotopic niche between sexes across the eye lens. During the first year of bass ontogeny they are sexually undifferentiated (Blázquez et al., 1995), but environmental factors, such as temperature can affect the synthesis of sex-determining hormones, therefore having a significant role in sex determination (Koumoundouros et al., 2002). We may therefore expect bass to exhibit spatial segregation between the sexes, especially during differentiation stages of their ontogeny. It is interesting that our results suggest a bias in sex ratio in catches towards female, but no difference in isotopic niche. It suggests that for this region there may have been more females present, but the males captured here occupied the same isotopic niche as females across their lifetime.

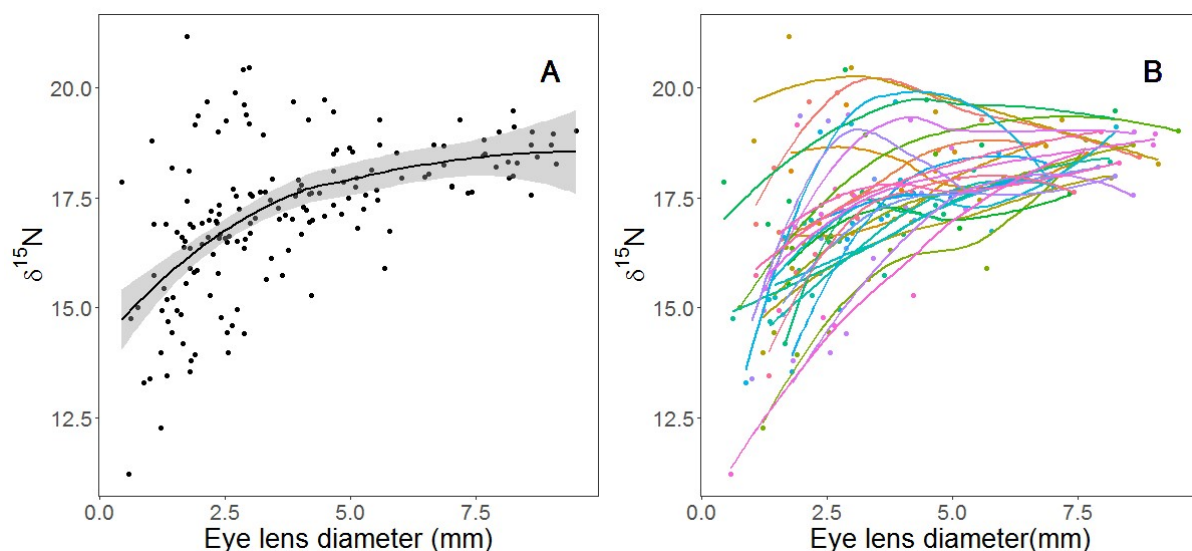


Figure 20. $\delta^{15}\text{N}$ across eye lens diameter for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019. A: sampled population trend. B: Individual eye lens tracks.

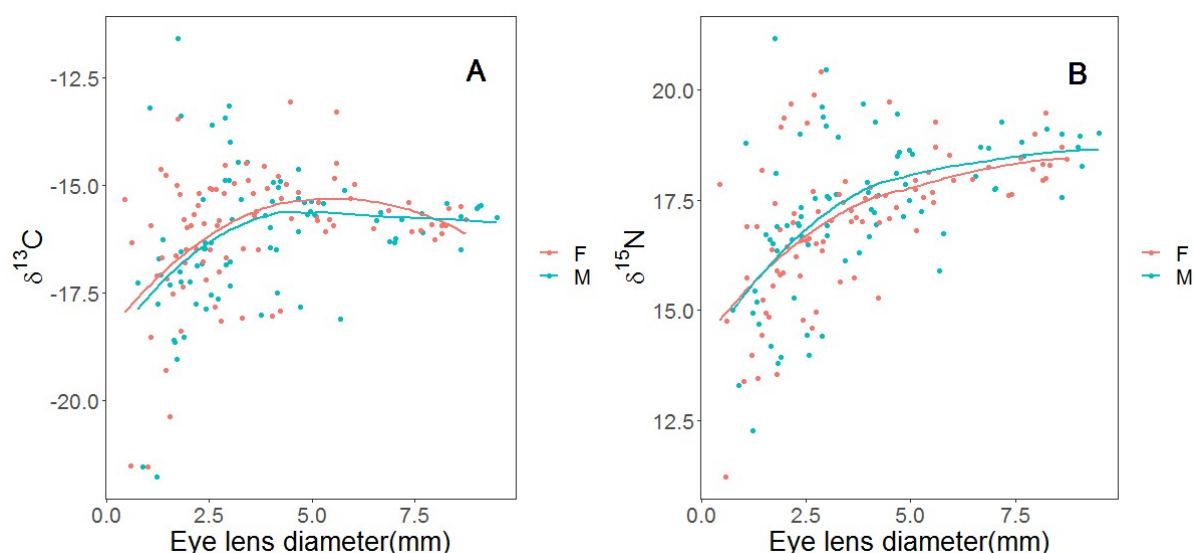


Figure 21. Stable isotope across eye lens diameter by sex for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019. A: $\delta^{13}\text{C}$. B: $\delta^{15}\text{N}$.

To further investigate the variation found in the cores compared to the outer lens, Figure 22 and Figure 23 examine the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to provide an isotopic niche width. The core of the lens has a significantly greater ellipse area (or isotopic niche width) than either of the other parts of the lens (outer layers and remaining eye lens) (Kruskal-Wallis = 10666, $df = 2$, $p < 0.001$). This suggests that the sampled bass originated from a wider trophic and spatial niche as juveniles, gradually occupying a narrower niche as they progress through ontogeny, where these individuals converged to feed.

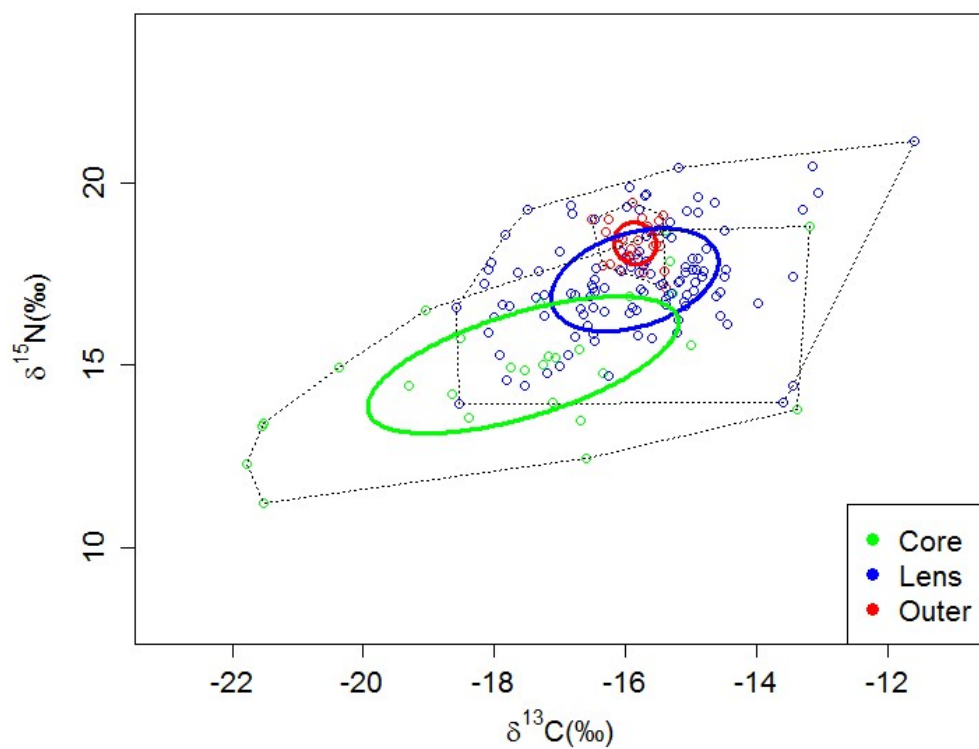


Figure 22. Isotopic niches of 3 phases of eye lenses (core, outer layer and the remaining central lens) for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019. Standard ellipse areas (solid lines) and convex hulls (dotted lines).

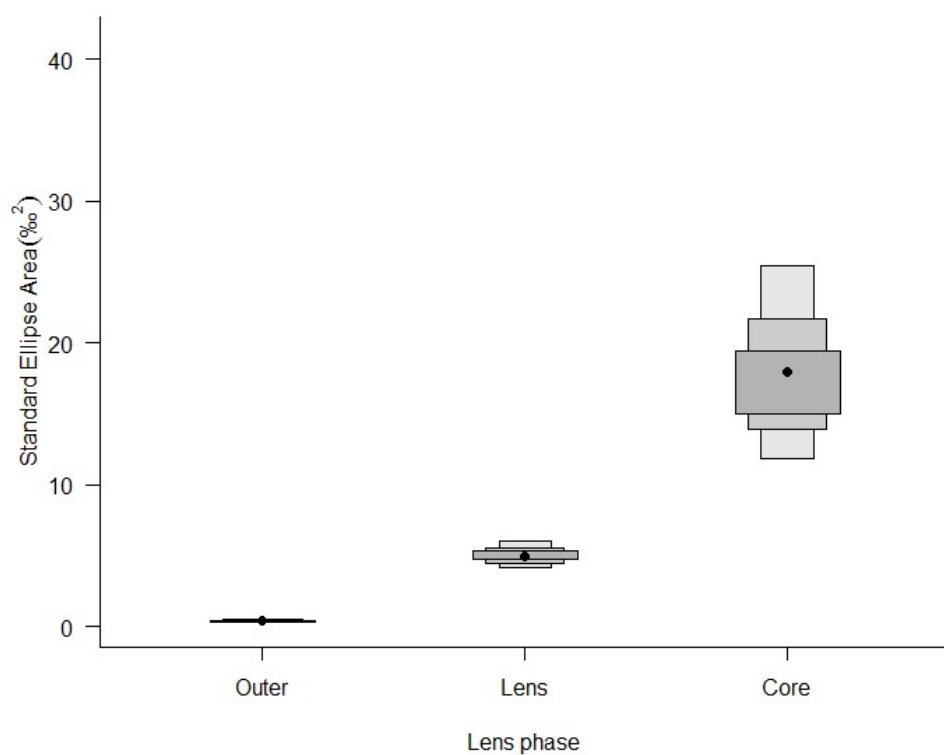


Figure 23. Difference in standard ellipse area among three lens phases for data presented in Figure 22.

Tissues, including scale, muscle and outer eye lens were taken from the same individuals and compared using a paired t -test. There were significant differences in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among tissue samples (**Error! Reference source not found., Error! Reference source not found.7**), except between muscle and lens $\delta^{15}\text{N}$. The difference is especially notable between scales tissue and the other tissue types. As tissues grow and turnover at different rates, the selection of different tissues for isotope analysis can provide information about an individual's trophic geography at various time intervals (Bavinck, 2011; Melnychuk and Walters, 2010). Muscle tissue for example can provide information about foraging location and trophic level over longer periods ranging from weeks to years (Horn, 1966; Whitehead *et al.*, 1984). Scales sampled here represent the last growing season of bass (Cambiè *et al.*, 2016). Muscle values here may therefore represent a longer phase in the life of the bass relative to the scales. Lenses were more similar to muscle, which may suggest that the outer lens is laid down over a longer period than scales.

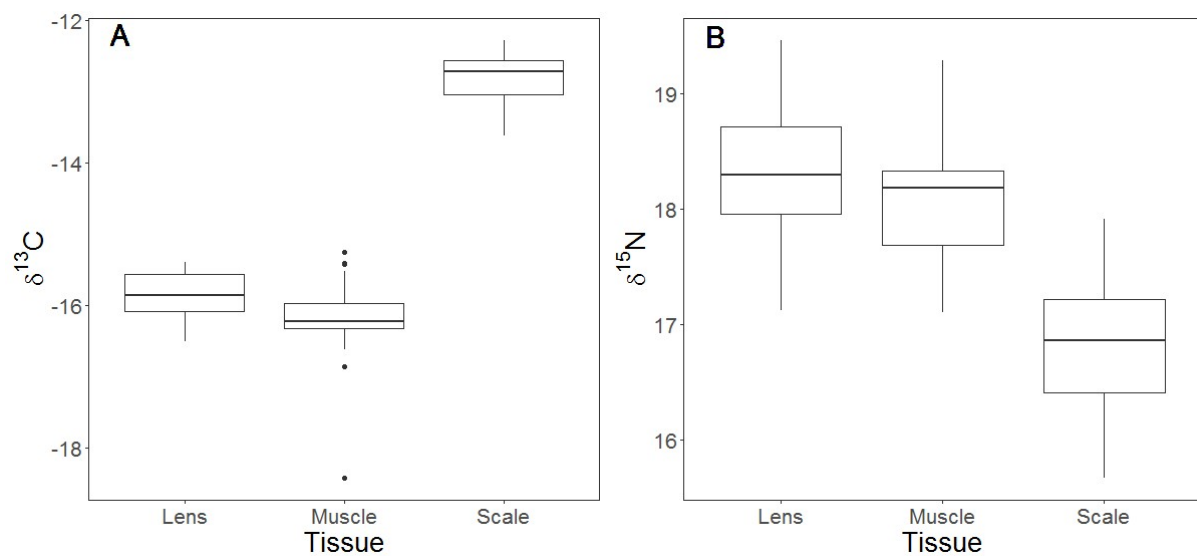


Figure 24. Differences in (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ between tissue types taken from the same individuals for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019.

Table 7. Paired t -test results comparing tissues from the same individuals for bass *Dicentrarchus labrax* caught in Morecambe Bay in 2019.

Tissue	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Scale and muscle	$t_{13} = 7.48, p < 0.001$	$t_{13} = -19.96, p < 0.001$
Muscle and lens	$t_{13} = -0.20, p = 0.84$	$t_{13} = 4.082, p = 0.001$
Scale and lens	$t_{13} = 4.95, p < 0.001$	$t_{13} = -29.00, p < 0.001$

3.2. Thornback rays

Sample composition

There was some difficulty obtaining thornback rays due to a paucity of trawlers operating in the area and a low market value. Individuals were obtained from three commercial fishers from three samples across the wider Liverpool Bay area (Table 8, Figure 3). This included whole individuals and those that had been ‘winged’ (pectoral fins removed for landing) leaving ‘backs’. (Note that the Dee Estuary individuals will be processed in the laboratory after Covid-19 restrictions are lifted and are not discussed further here). Total processed sample size was 152; however, over half of these were backs, limiting analysis of a number of key parameters (e.g. length-weight, GSI).

Table 8. Details of thornback ray *Raja clavata* samples obtained for this study.

Location	Date landed	Gear	N (whole: ‘backs’)	♂	♀
Liverpool Bay	6 th June 2019	Demersal trawl	93 (2:91)	35	58
Morecambe Bay	25 th September 2019	Demersal trawl	59 (36:23)	41	18
Dee Estuary	October 2019	Netting	~15 (~15:0)*	-	-
Total			152 (38:114)	76	76

*not yet processed.

Total length (TL) ranged from 283-879 mm, with a mean of 674 (\pm 98 SD) mm. Size and sex composition of the sample are presented in Figure 25.

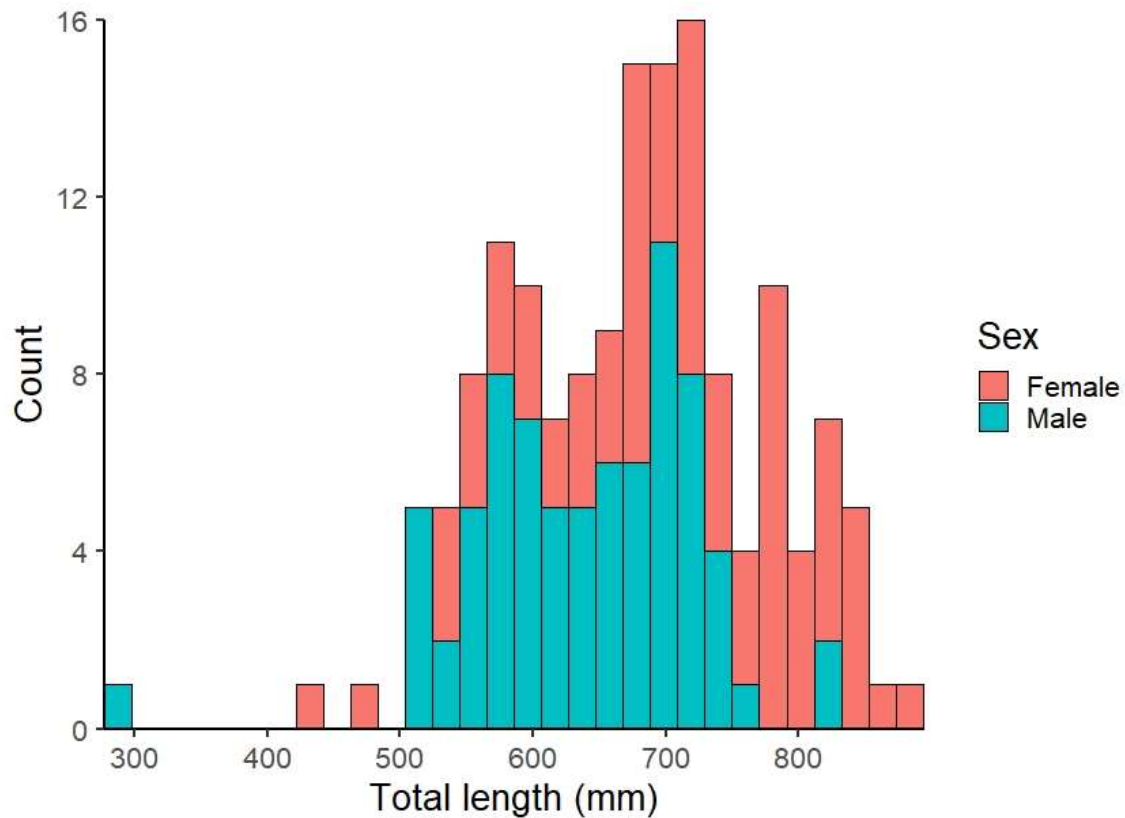


Figure 25. Length-frequency distribution by sex of combined thornback ray *Raja clavata* sample from the Liverpool Bay area, June & September 2019.

Sex ratio

Although the combined overall sample had the same number of males and females, there were significant departures from parity for each of the samples. These were in favour of females in Liverpool Bay in June (X^2 test = 5.69, d.f. = 1, p = 0.017) and males in Morecambe Bay in September (X^2 test = 8.97, d.f. = 1, p = 0.003). Sexual segregation is common in elasmobranchs and has been reported in adult *R. clavata*, when sampling is at a fine scale (Pawson, 1995; Rousset, 1990).

Maturity status

Most (60.5%) *R. clavata* individuals were classed as immature, with a greater proportion of females immature (68.4%) compared to males (52.6%) (Figure 26a). Based on ICES maturity stage, most immature females were stage 1 ('immature'), with mature females comprising equal numbers of 3a ('capable to reproduce') and those that were actively (3b) or recently (4a) egg-laying. Immature males comprised roughly equal numbers of immature (1) and maturing (2). Nearly all mature males were 3a ('capable to reproduce'), with two individuals 'active' (3b) (Figure 26b).

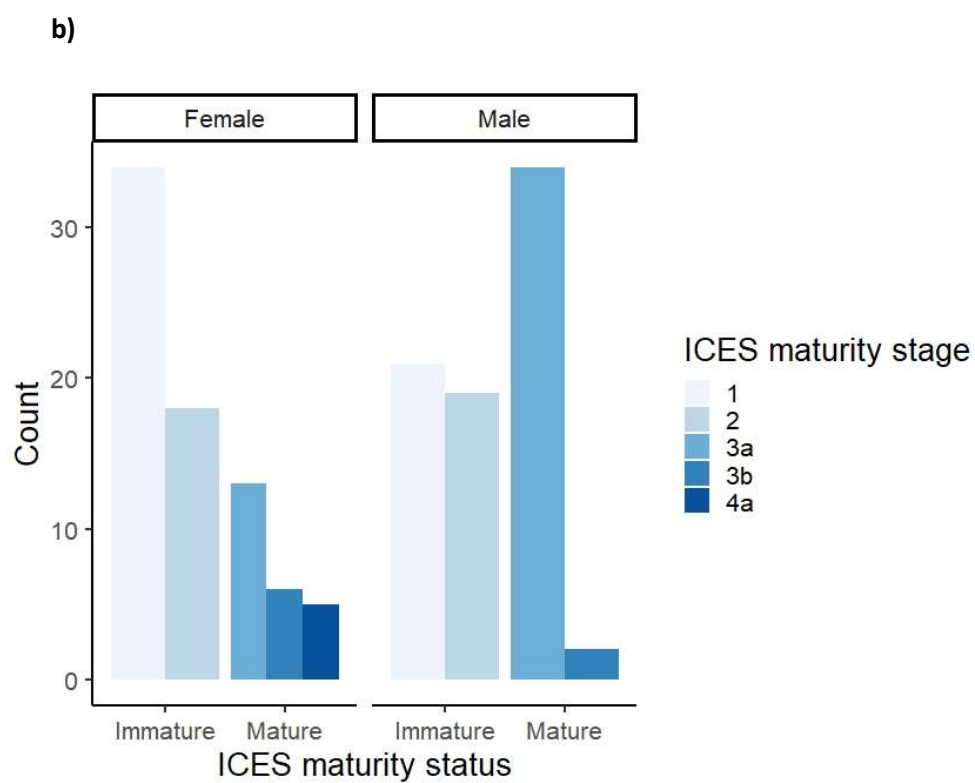
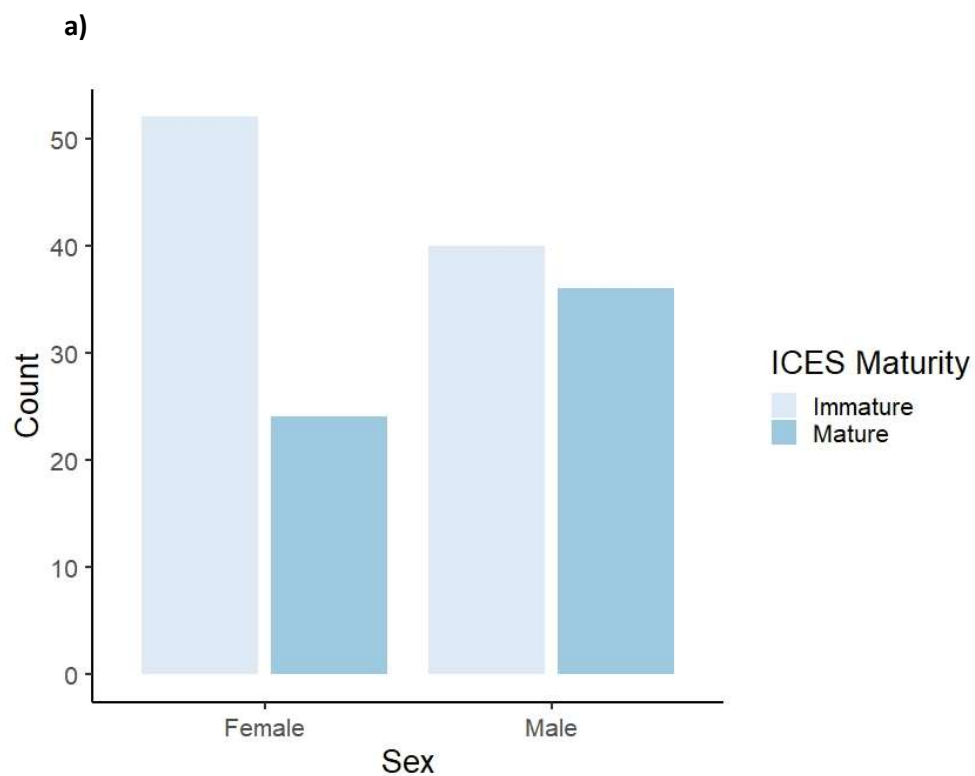


Figure 26. a) ICES Maturity status and b) ICES maturity stage of thornback *Raja clavata* from the Liverpool Bay area, June & September 2019, based on macroscopic examination of reproductive organs (ICES, 2013).

Size at maturity

For males ($n=76$), length at which 50% of individuals were deemed mature (L_{50}) based on macroscopic examination of gonads was 651 mm TL (95% CI 633 - 670; $r^2 = 0.65$)(Figure 27a). This corresponded reasonably well with the marked change in clasper length between 630 and 700 mm (Figure 27b).

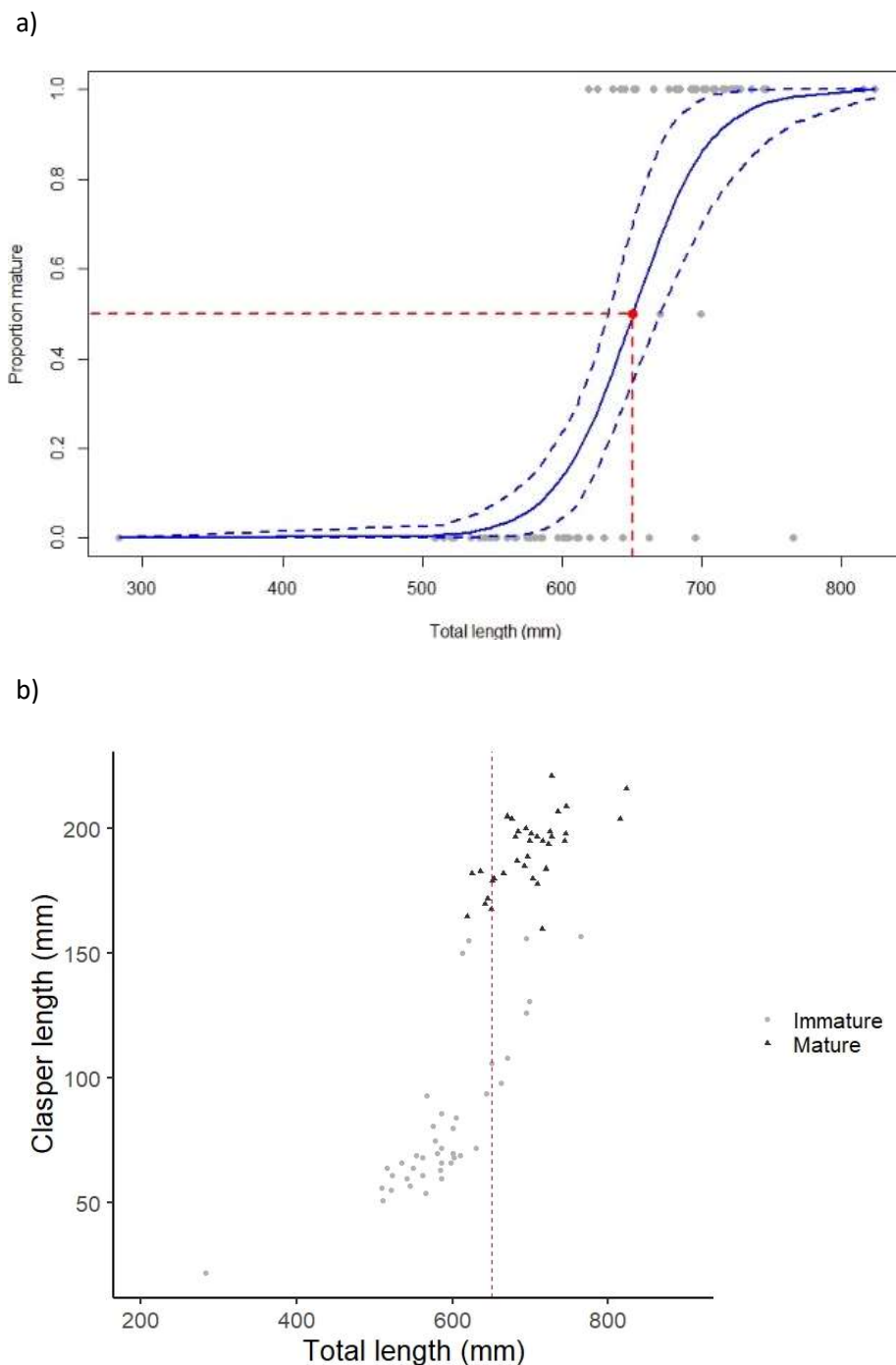


Figure 27. Maturity of male thornback ray *Raja clavata* from Liverpool Bay in 2019 a) Total length at 50% maturity ogive (L_{50}), based on macroscopic examination of gonads (ICES, 2013). $L_{50} = 651$ mm TL; $r^2 = 0.65$. Dashed blue lines indicate 95% confidence intervals (633 – 670 mm TL). b) Change in clasper length with total length. Maturity status (ICES, 2013) and L_{50} of 651 mm TL (dashed line) is indicated.

Females ($n = 76$) matured at a larger size than males, with an L_{50} of 771mm TL (95% CI 752 - 791; $r^2 = 0.70$) (Figure 28a), showing reasonable agreement with the increase in nidamental gland width between 700 and 800 mm (Figure 28b).

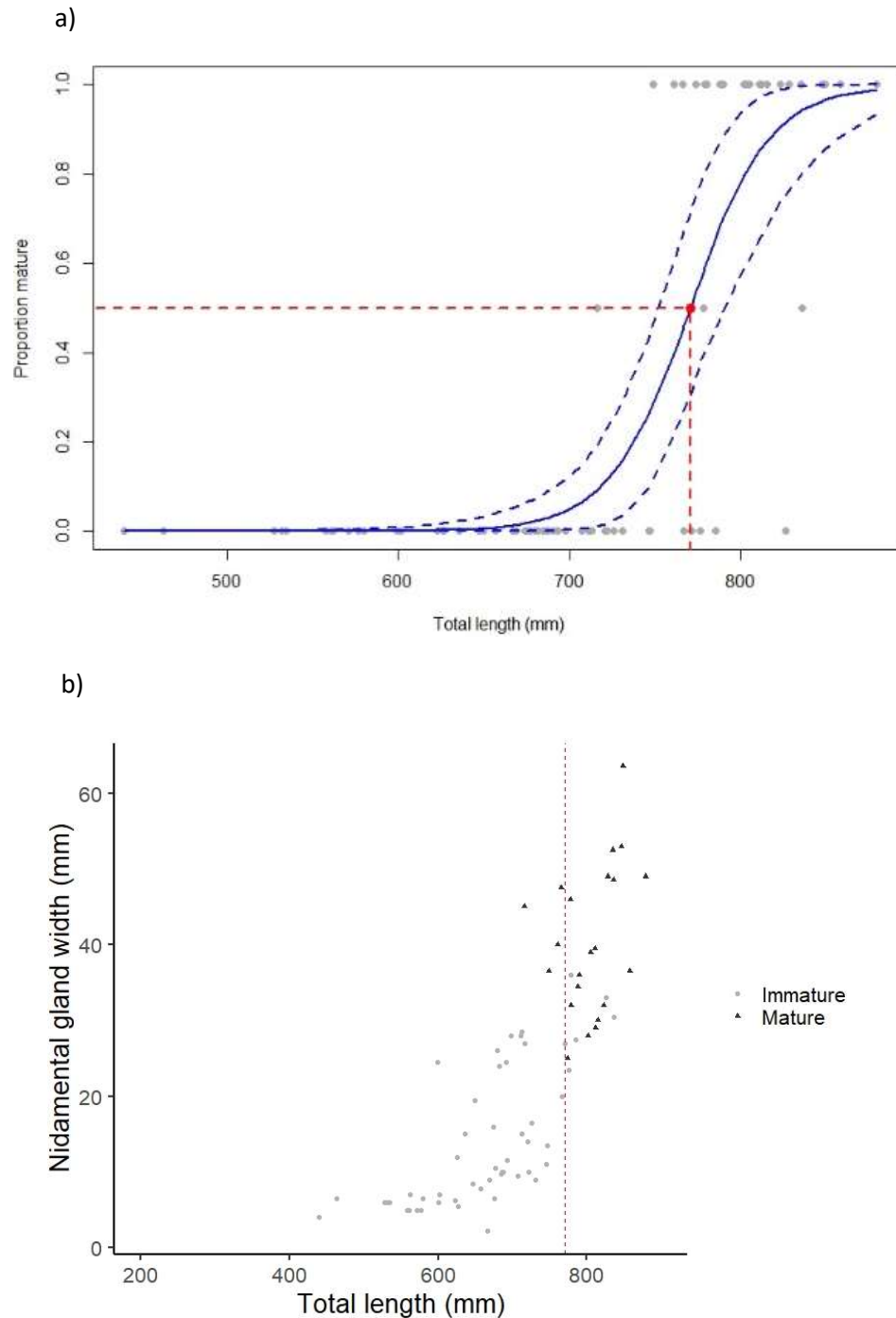


Figure 28. Maturity of female thornback ray *Raja clavata* from Liverpool Bay in 2019 a) Total length at 50% maturity ogive (L_{50}), based on macroscopic examination of reproductive tract (ICES, 2013). $L_{50} = 771$ mm TL; $r^2 = 0.70$. Dashed blue lines indicate 95% confidence intervals (752 – 791 mm TL). b) Change in nidamental gland width with total length. Maturity status (ICES, 2013) and L_{50} of 771 mm TL (dashed line) is indicated.

Table 9 compares results of the present study with regional work since the 1960s, including a large sample size collected by Cefas from the ‘Celtic Seas’ ICES ecoregion (inc. Irish Sea) (McCully *et al.*, 2012). Our estimate of L_{50} for males (651 mm TL) corresponds well to several studies from North Wales, Irish waters of the Irish Sea and the Celtic Seas ecoregion (Gallagher *et al.*, 2004; Lesser, 1967; McCully *et al.*, 2012). The smaller male L_{50} from other North Wales studies may be a result of smaller sample size (Cambiè, 2014; Whittamore and McCarthy, 2005). Our estimate of L_{50} for females (771 mm TL), as well as the size of the largest immature male, also agrees closely with McCully *et al.* (2012).

Table 9. Length at maturity (in mm TL) of thornback ray *Raja clavata* from the present study (in bold) and others in the Celtic Seas ICES ecoregion (including the Irish Sea).

Area	Sample size <i>n</i>		L_{50}		Smallest mature		Largest immature		Ref.
	M	F	M	F	M	F	M	F	
Celtic Seas (inc. Irish Sea)	2427	2368	666	782	560	470	760	900	1
Irish Sea	165	93	657	718	600 ^c	580 ^c			2
England/Scotland (Solway Firth)	271		600*	650*	-	-	-	-	3*
England/Scotland (Solway Firth)	-		620 [†]	710 [†]	-	-	-	-	4 [†]
Liverpool Bay	76	76	651	771	619	716	765	836	5
North Wales	390		648 [†]	635 [†]	-	-	-	-	6 [†]
North Wales (Red Wharf/Conwy Bays)	88		625	715	-	-	-	-	7
North Wales (Caernarfon Bay)	54	135	588	705	570 ^c	660 ^c	-	-	8 ^c
South Wales (Carmarthen Bay)	1019	1124	-	-	605	595	-	-	9

¹McCully *et al.* (2012); ²Gallagher *et al.* (2004); ³Nottage and Perkins (1983) *not L_{50} ; based on inflections in length/weight, assumed to be entry into adult growth and maturation; ⁴Williams *et al.* (1965); ⁵This study; ⁶Lesser (1967); ⁷Cambiè (2014); ⁸Whittamore and McCarthy (2005); ⁹Ryland and Ajayi (1984). [†] L_{50} calculated from disc width by Spencer (2019) using McCully *et al.* (2012). ^cVisual estimate from L_{50} plot.

Spawning

Females ($n = 6$, 761 – 849 mm TL) carrying egg-cases were only recorded in the June sample (Liverpool Bay). The seasonality of egg-laying in the Irish Sea remains a data gap for *R. clavata* (Ellis *et al.*, 2015), despite a number of reproductive studies in the area (Table 9). The commonly cited spawning season for *R. clavata* (e.g. February-September, peaking April to July; Ellis *et al.*, 2015) appears to be based on older work outside the Irish Sea (Clark, 1922; Holden, 1972; Ryland and Ajayi, 1984). Much of the eastern Irish Sea has been identified as an area of importance for young *R. clavata*, with high catch rates of juveniles (<180 mm TL) in the Liverpool Bay area (Ellis *et al.*, 2012, 2015).

Minimum landing size (MLS) in relation to maturity

The current MLS (Cumbria district) of 45 cm DW converts to a TL of 67.1 cm. This is similar to the L_{50} of 65.1 cm TL we found for males, but notably smaller than the L_{50} of 77.1 cm TL we found for females. The percentage of sampled rays that were at or above the MLS, but still immature, is presented in

Table 10. While a relatively small proportion of males (13.3%) were at or above the MLS but still immature, this was not the case for females (54.7%). This suggests that, based on the samples obtained, and assuming similar biology in *R. clavata* in the northern part of the NWIFCA district, the MLS may not be adequately protecting females.

Table 10. Proportion of sampled thornback ray *Raja clavata* from the Liverpool Bay area in 2019 that were immature in relation to the Cumbria Sea Fisheries Committee minimum landing size of 45 cm disk width (converted to total length of 67.1 cm using McCully *et al.*, 2012).

Sex	L_{50} (cm)	Number of rays that were \geq MLS but immature/Number of rays	% of sample \geq MLS that were immature
Males	65.1	Apr-30	13.30%
Females	77.1	29/53	54.70%

Diet

The stomachs of 61 rays (September, Morecambe Bay) were externally examined, with 46 (75%) appearing to have contents. To date, only 15 of these have been dissected, with 2 empty. Contents of the 13 stomachs with contents (Table 11) revealed crustaceans to be the most important (88.2 %N) major taxon, with crangonid shrimps and crabs (likely *Liocarcinus*) comprising the majority (30.5% and 28.8 %N, respectively). These preliminary data are in line with results of a large ($n=601$) study in the Irish Sea, which found *R. clavata* diet dominated by crustaceans, of which *Liocarcinus* crabs and *Crangon* shrimp were the most important (Ellis *et al.*, 1996).

Table 11. Stomach contents of thornback ray ($n=13$) from Morecambe Bay in September 2019. Indet. = indeterminate.

Higher taxa	Taxa	Count of stomachs with prey type present	Sum of numbers of each prey type	%O	%N
Polychaeta	<i>Arenicola</i> sp.	2	2	15.4	3.4
Crustacea	Crustacea indet.	5	6	38.5	10.2
	Crangonidae sp.	8	18	61.5	30.5
	Paguridae (indet.)	1	5	7.7	8.5
	Portunidae (indet.)	8	17	61.5	28.8
	<i>Corystes cassivelaunus</i>	5	6	38.5	10.2
Mollusca	Mollusca indet.	2	3	15.4	5.1
Pisces	Gadidae (indet.)	1	1	7.7	1.7
Misc.	Digested remains	1	1	7.7	1.7
	Total	33	59	253.8	100

Stable isotope analysis

Thornback ray eye lenses delaminated successfully as expected based on the work of Simpson *et al.* (2019). On average 5.8 layers were delaminated from each eye lens.

The samples as a whole show little change across the eye lens in $\delta^{13}\text{C}$ (Figure 29a), suggesting little overall trend in movement across the lifetime of these thornback rays. There were however some changes across the eye lens when examining individuals (Figure 29b). In the core of the eye lenses, individuals had varied $\delta^{13}\text{C}$, but all converged to more similar values across the eye lens, which suggests feeding in similar location. From around 3 mm lens diameter the variation among individuals decreases. There was a significant difference between sexes ($p = 0.003$). When sex is considered in the model, there is a significant influence of eye lens diameter on $\delta^{13}\text{C}$ ($p = 0.003$), but no individual effect ($p = 0.49$). Overall the deviance explained by the model was 21.9%.

Habitat partitioning between juveniles and adults is often missed in tagging studies and fishing surveys due to limitations on the size of individuals that can be tagged, and because neonates can escape through fishing nets with wider meshes. Our understanding of the distribution of juvenile skates is therefore limited (Ellis *et al.*, 2005). Skates are known to be oviparous and are thought to deposit eggs in nursery areas, where the juveniles remain until adulthood. Locations of skate nurseries around the UK have been proposed to be broadly in shallower waters than adult distributions (Ellis *et al.*, 2005). The trends found in the current study support a shift between juvenile and adult foraging habitat. The significant difference in $\delta^{13}\text{C}$ between sexes and the trends illustrated in **Error! Reference source not found.** indicates that this difference occurs in the earlier part of the eye lens, but converges at around 5-6 mm eye lens diameter. This would suggest that males and females are broadly feeding at the same trophic level across ontogeny, but there may be greater spatial differences between them in their earlier life. Sexual segregation in elasmobranchs is well documented in a number of species including white sharks *Carcharodon carcharias* (Domeier and Nasby-Lucas, 2012), catsharks *Scyliorhinus canicula* (Wearmouth *et al.*, 2012) and blacktip reef sharks *Carcharhinus melanopterus* (Mourier *et al.*, 2013). There is conflicting evidence for sexual segregation in Rajidae (Martin *et al.*, 2010; Rousset, 1990; Steven, 1933), however this study supports sexual segregation. To make further conclusions concerning sexual segregation during the early life of thornback rays, future research could increase the sample size of eye lenses to further investigate this.

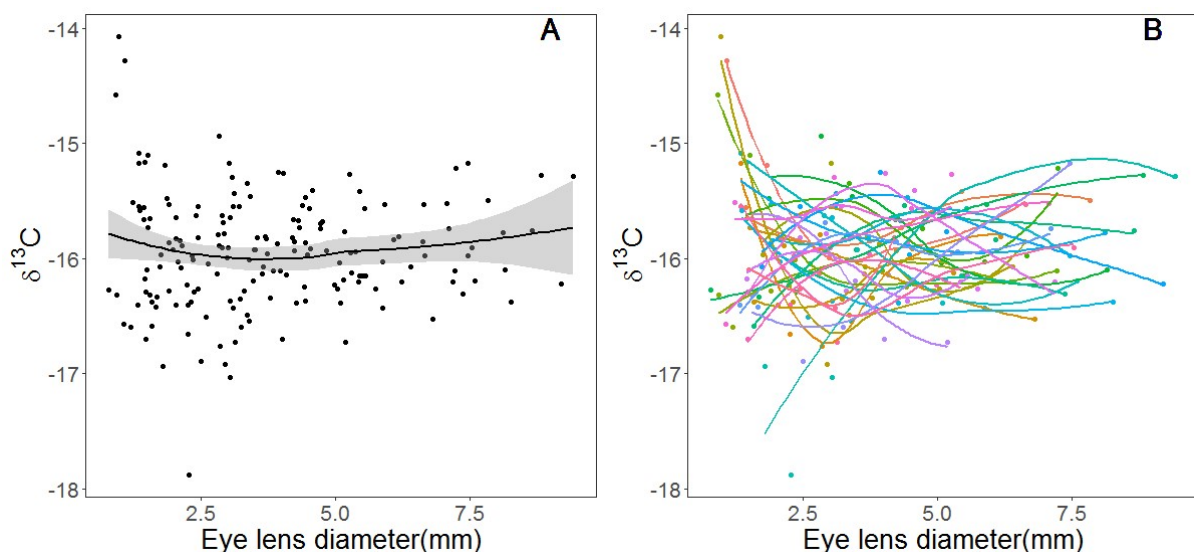


Figure 29. $\delta^{13}\text{C}$ across eye lens diameter of thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019. A: sampled population trend B: Individual eye lens tracks.

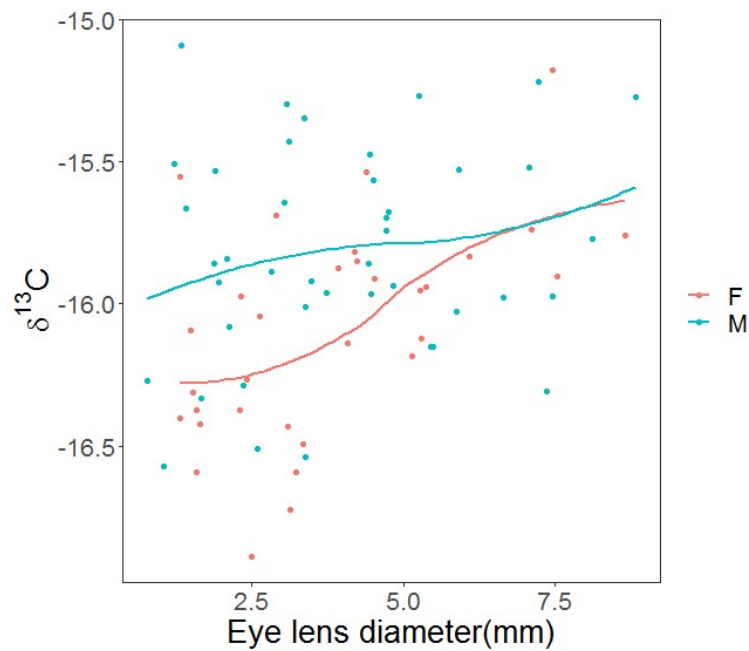


Figure 30. $\delta^{13}\text{C}$ across eye lens diameter by sex of thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019.

For $\delta^{15}\text{N}$, values start higher in the core and decrease across the eye lenses, before increasing in the outer sections of the lens ($p < 0.001$). The majority of individual tracks broadly show this expected trend (Figure 31a) previously found in thornback rays off Plymouth (Simpson *et al.*, 2019). There were some individuals that differed ($p = 0.002$), starting relatively high in the core, increasing post core and then decreasing across the eye lens (Figure 31b). The core $\delta^{15}\text{N}$ values are similar to $\delta^{13}\text{C}$, in that they are more varied and then converge across the eye lens. There was no significant differences between sexes ($p = 0.28$) (Figure 32 **Error! Reference source not found.**). Juveniles are often excluded in dietary studies, especially newly hatched individuals, as they may be missed by commercial fisheries and may be too small to perform reliable stomach contents analysis (Francis, 1984; Wheeler, 1969). Juveniles are often analysed as broad groups (Ellis *et al.*, 1996) often simply before and after maturity (Farias *et al.*, 2006), which potentially misses ontogenetic shifts in the early life of a skate. Ontogenetic shifts in diet are reported for skates; for juveniles, mysids and amphipods are important, but with increasing size and age there is a shift from such smaller prey items to larger crustaceans, fish and cannibalism (Ajayi, 1982; Farias *et al.*, 2006; Francis, 1984; Holden and Tucker, 1974; Quinn and Brodeur, 1991). The use of eye lenses here captures the earliest days of the individual's life, providing a greater understanding of this important stage in development.

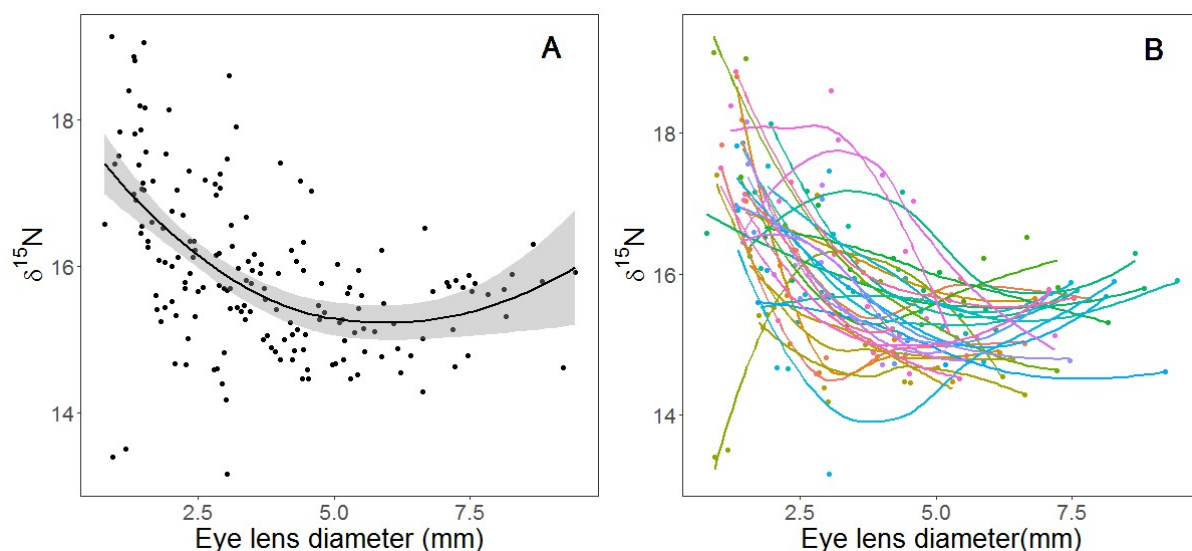


Figure 31. $\delta^{15}\text{N}$ across eye lens diameter for thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019. A: sampled population trend B: Individual eye lens tracks.

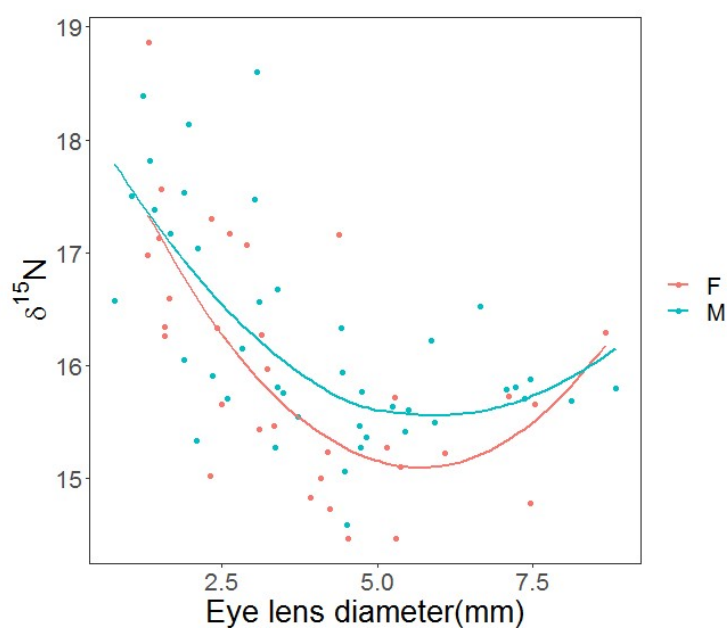


Figure 32. $\delta^{15}\text{N}$ across eye lens diameter by sex of thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019.

To further investigate the variation found in the cores compared to the outer lens, Figure 33 and Figure 34 examine the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to provide an isotopic niche width. The core of the lens has a significantly greater ellipse area (or isotopic niche width) than either of the other parts of the lens (outer layers and remain eye lens) (Kruskal-Wallis chi-squared = 10594, df = 2, $p < 0.001$). These results suggest that individuals occupying a similar isotopic niche as adults came from more varied isotopic niches as juveniles and that there is low overlap between these niches. This suggests an ontogenetic movement between nursery or juvenile grounds to adult foraging grounds.

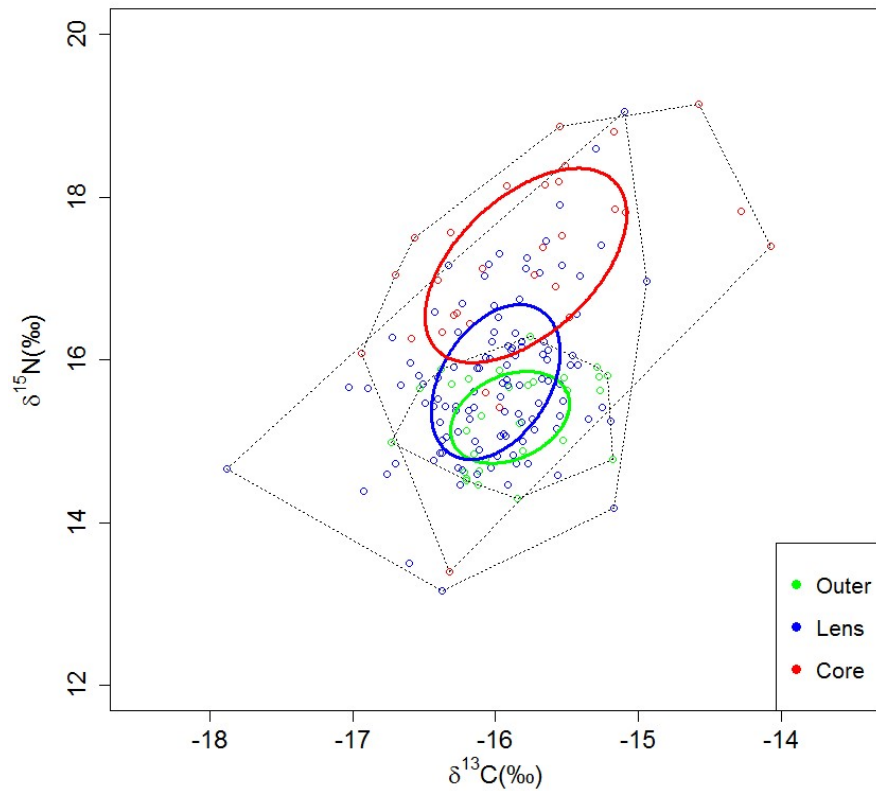


Figure 33. Isotopic niches of 3 phases of eye lenses (core, outer layer and the remaining central lens) for thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019. Standard ellipse areas (solid lines) and convex hulls (dotted lines).

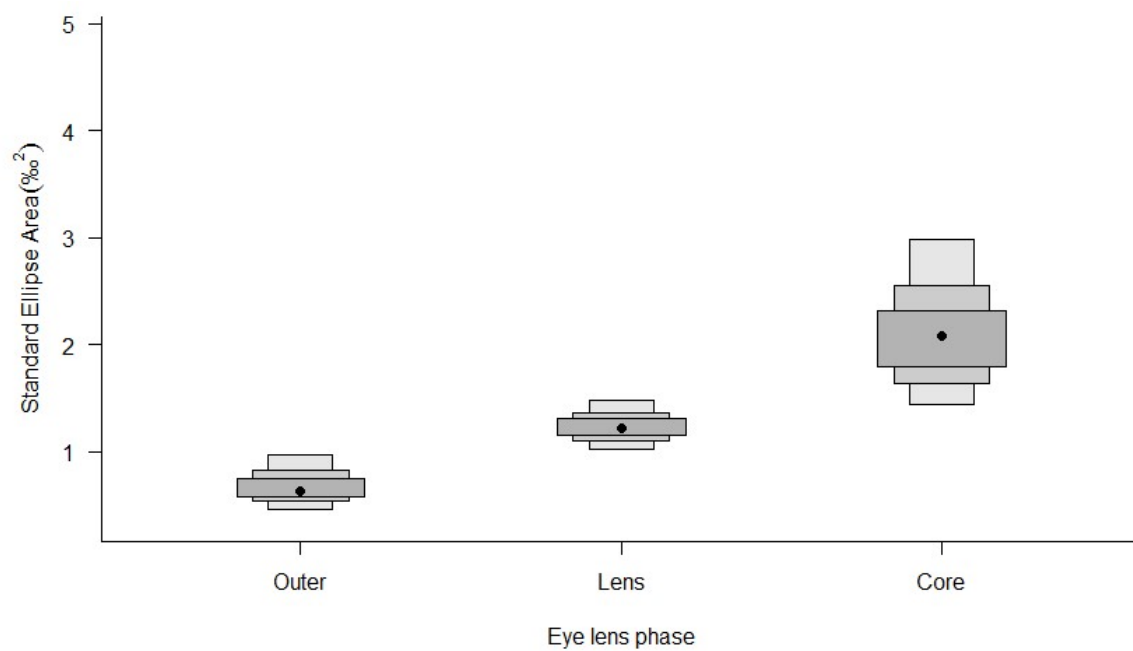


Figure 34. Difference in standard ellipse area among three lens phases for data presented in Figure 33.

There were no significant differences in $\delta^{13}\text{C}$ between muscle and outer lenses ($t_{12} = -0.29$, $p = 0.78$), but there were differences in $\delta^{15}\text{N}$ ($t_{12} = -8.79$, $p < 0.001$). This may suggest outer eye lenses are laid down over different time frames, but also suggests that foraging habitat has not changed but trophic level has after the outer layers of the eye lenses were formed.

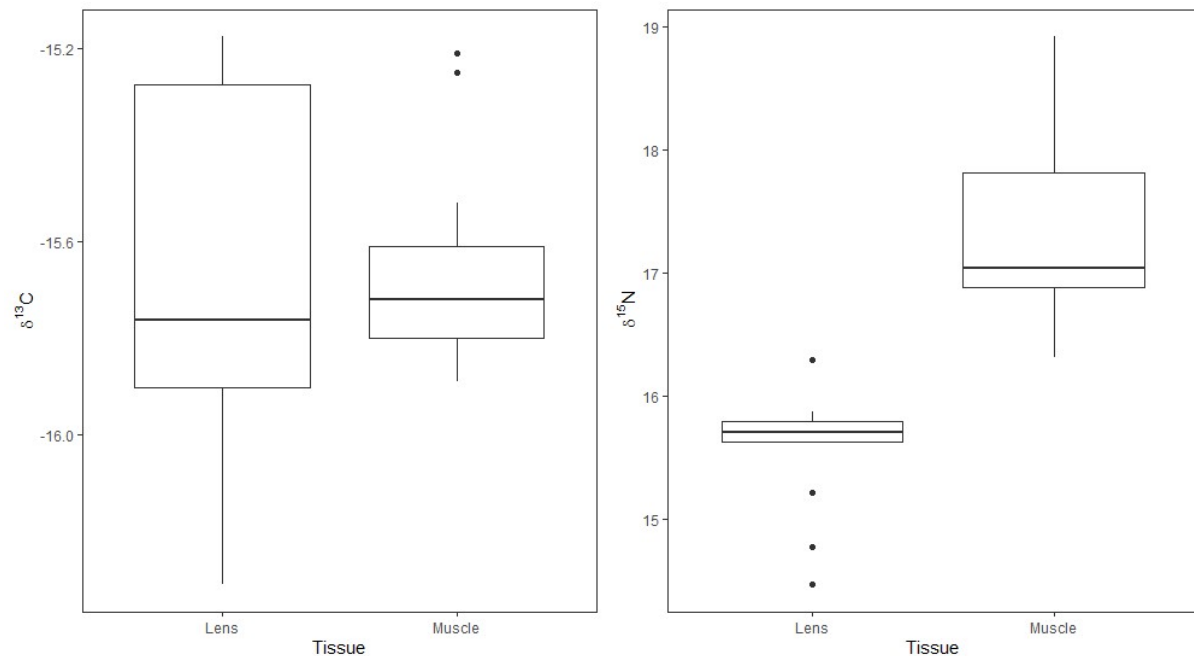


Figure 35. Differences in (Left) $\delta^{13}\text{C}$ and (Right) $\delta^{15}\text{N}$ between tissue types taken from the same individuals of thornback ray *Raja clavata* sampled in the Liverpool Bay area in 2019.

In future research, bass and rays isotope values should be compared to other regions (e.g. Welsh samples) to examine the differences and similarities among regions and assess the stock structure. The data collected here may also be applied to isoscape mapping to determine the most likely foraging habitat for these species at different developmental stages. This work is currently in progress and will be published in due course.

Acknowledgements

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Appendices

Appendix 1. Table of 0-group bass sampling details for this study.

Details of 0-group bass (*Dicentrarchus labrax*) surveys in and around the Ribble, Wyre, and Cocker Estuaries conducted in August 2020.

Estuary	Date	Location	Access	Position of sampling	Tidal state	Habitat	No. tows	Bass recorded	Notes
Ribble	07/08/2019	Hesketh Out Marsh	Via RSPB warden	53.718824, -2.888303	HW	Saltmarsh creek	5	None	Abundant gobies & shrimp
	30/08/2019	Lytham (foreshore)	Public from road	53.734388, -2.978531	HW	Salt marsh at top of sandy shore	3	None	Sampling difficult due to high winds and detritus in water; few gobies & shrimp
		Lytham (main drain)	Public from road	53.734803, -2.950665 53.734095, -2.952962	HW+2	Saltmarsh creek	3	None	
		Lytham (foreshore)	Public from road	53.732562, -2.980128	HW+3	Sand/muddy sand/mussel scar	3	None	Few gobies & shrimp; 1 small flounder
Cocker	09/08/2019	Pilling embankment	Public	53.942712, -2.932079	Reconnaissance. Good: short walk from car park to water's edge (sand against saltmarsh)				
		Pilling Amenity area	Public	53.937772, -2.893146	Reconnaissance. Not good. very long walk over saltmarsh to water; public car park				

Appendix 1. (Continued) Table of 0-group bass sampling details for this study.

Estuary	Date	Location	Access	Position of sampling	Tidal state	Habitat	No. tows	Bass recorded	Notes
Wyre	08/08/2019	Knott End/Preessall	Public from car park	53.922389, -2.997679	LW	Main estuary channel, mud, sand, mussel scar	3	None	Abundant shrimps, gobies & flounder
		Out Rawcliffe	Via landowner	53.856636, -2.927573 53.857235, -2.930611	LW+1	Main estuary channel, muddy sand/mud	5	None	2 elvers <i>Anguilla anguilla</i> ; abundant small flounder
		Skippool Yacht Club	Private	53.862604, -2.977717	Reconnaissance. Not good: steep muddy banks; private land				
		Wyre estuary country park	Public car park	53.880960, -2.980296	Reconnaissance. Not ideal: steep muddy banks; good access (car park)				
	09/08/2019	Shard Bridge	Public (park on roadside by gate on S bank before bridge; public footpath)	53.861987, -2.961630	LW	Main estuary channel, muddy sand/mud	3	24	Most bass in single tow, in slight pool. Mullet fry present.
				53.862626, -2.964992			3	None	Gobies & shrimp