

PLAICE (*Pleuronectes platessa* L.) NURSERY
HABITAT IN THE FORTH ESTUARY AND THE
FIRTH OF FORTH.

BY

JULIAN JOHN AUGLEY

Thesis Submitted to Napier University
in Partial Fulfilment of the Requirements for the Degree
of Doctor of Philosophy

School of Life Sciences
Napier University
Edinburgh

July 2007

Table Of Contents

List of Tables.....	iv
List of Figures	viii
Acknowledgements	xv
Abstract.....	xvi
Chapter 1. General Introduction: Plaice Nursery Habitat and Study Area	
1.1. General Introduction and literature review	- 2 -
1.2. Plaice biology	- 4 -
1.3. Nursery habitat.....	- 9 -
1.4. Plaice nursery grounds	- 14 -
1.5. Plaice investigations in the Firth of Forth	- 25 -
1.6. Aims and Objectives	- 26 -
1.7. Methods and Materials.....	- 28 -
1.7.1. Study Area: Firth of Forth and Forth Estuary.....	- 28 -
1.7.2. Study Sites	- 31 -
1.7.3. Sediment Characteristics	- 31 -
Chapter 2. Efficiency and Selectivity of a 1.5m Riley Push Net	
2.1. Introduction	- 39 -
2.2. Methods and Materials.....	- 45 -
2.2.1. Site Description	- 45 -
2.2.2. Calibration	- 47 -

2.3. Statistical analyses.....	- 50 -
2.3. Results	- 54 -
2.3.1. Plaice Densities and Total Lengths	- 54 -
2.3.2. Dispersion Analysis	- 63 -
2.4. Discussion.....	- 64 -

Chapter 3. Growth Rates of Juvenile Plaice in the Forth Estuary and Firth of Forth

3.1. Introduction	- 69 -
3.2. Methods and Materials.....	- 75 -
3.2.1. Site Description	- 75 -
3.2.2. Fish Sampling	- 81 -
3.2.3. Cohort Analysis	- 87 -
3.2.3.1 Otolith Increments	- 89 -
3.2.4. Plaice Densities.....	- 94 -
3.2.5. Caging Experiments.....	- 97 -
3.2.6. Statistical Analyses	- 98 -
3.3. Results	- 99 -
3.3.1. Length – Frequencies of plaice total lengths.	- 102 -
3.3.2. Cohort Analysis – Growth Rates	- 116 -
3.3.3. Otolith Increment Analysis	- 130 -
3.4. Discussion.....	- 144 -
3.5. Conclusion	- 169 -

Chapter 4. Effect of Salinity on Growth Rates of Juvenile Plaice

4.1. Introduction	- 171 -
4.2. Methods and Materials.....	- 174 -
4.2.1. Laboratory Growth	- 174 -
4.2.2. Statistical analyses.....	- 178 -
4.3. Results	- 180 -
4.3.1. Experimental Growth.....	- 180 -
4.4. Discussion.....	- 188 -
4.4.1. Field Growth (from chapter 3)	- 188 -
4.4.2. Experimental Growth.....	- 188 -
4.5. Conclusion	- 192 -

Chapter 5. Carbon Stable Isotopes in Estuarine Sediments and their Utility as Migration Markers.

5.1. Introduction	- 194 -
5.2. Methods and Materials.....	- 198 -
5.2.1. Site description.....	- 198 -
5.2.2. Sediment analysis	- 200 -
5.2.3. Otolith analysis.....	- 202 -
5.2.4. Statistical analyses.....	- 204 -
5.3. Results	- 205 -
5.4. Discussion.....	- 225 -
5.4.1. Sediment isotopic composition.....	- 225 -
5.4.2. Otolith isotopic composition	- 230 -

5.5. Conclusion	- 235 -
-----------------------	---------

Chapter 6. Summary and Conclusions

6.1. Summary and Conclusions	- 237 -
------------------------------------	---------

7. References.....	- 242 -
--------------------	---------

List of Tables

Table 1: Commercial landings of selected fishes into Northeast Atlantic ports (FAO Area 27), t=tonnes..	- 16 -
Table 2: Sieve mesh sizes and corresponding log transformed phi (Φ) values for sediment particle analysis.	- 37 -
Table 3: Sediment grain size parameters extracted visually from cumulative frequency curves (figures 4 – 7). ΦX is the diameter in phi notation of the X th percentile. These values were used in equation 2 to estimate σ_1 , which is inclusive graphic standard deviation. Wentworth descriptions taken from Bale & Kenny (2005). <i>na</i> means the sediment had a high percentage of silt/clay, precluding calculation of σ_1 .	- 37 -
Table 4: Gear deployment schedule.	- 49 -
Table 5: Randomised block ANOVA of density estimates using drop trap and push net at Blackness in May 2005 and Juen and July 2006. Time is the blocking (random) factor.	- 55 -
Table 6: Analysis of plaice dispersion during calibration trials. $\bar{x}$ is sample mean, s^2 is sample variance, v is degrees of freedom (number of samples – 1), D is dispersion coefficient, <i>Dispersion</i> is a descriptive from Fowler <i>et al.</i> (2004).	- 63 -
Table 7: Summary of sediment characteristics (taken from chapter 1) N/A = Median $\Phi < 62 \mu\text{m}$. * Limekilns sediments are highly variable, see text for description.	- 75 -
Table 8: Latitudes and longitudes assigned to each site for SeaWifs/MODIS-Aqua Sea Surface Temperature (SST) data extraction.	- 89 -
Table 9: Summary of catch composition of push net hauls in 2005. Corrected densities calculated using calibration data in Chapter 2.	- 114 -
Table 10: Summary of catch composition of push net hauls in 2006. Corrected densities calculated using calibration data in Chapter 2.	- 115 -
Table 11: Growth rates calculated by change in median total lengths of push net hauls in 2005.	- 117 -
Table 12: Growth rates calculated by change in median total lengths of push net hauls in 2006.	- 117 -

Table 13: Regression parameters for ln-transformed TLs' (y), measured from push net samples. * indicates significant r^2 . Values of x are days (no of days since 15 th May 2005 and 30 th May 2006, respectively)..	- 120 -
Table 14: Parameters from Tukeys multiple comparisons test of 2005 ln-transformed TLs' from push-net samples. SE is standard error; q is the test statistic (calculated using SE and b coefficients' of the relevant site in Table 13). * indicates significant differences at $\alpha = 0.05$, ** indicates significant differences at $\alpha = 0.001$ ($k = 4$, $DF_p = \infty$ (actual number is 675)).	- 121 -
Table 15: Parameters from Tukeys multiple comparisons test of 2006 ln-transformed TLs' from push-net samples. SE is standard error; q is the test statistic (calculated using SE and b coefficients' of the relevant site in Table 13). * indicates significant differences at $\alpha = 0.05$, ** indicates significant differences at $\alpha = 0.001$ ($k = 4$, $DF_p = \infty$ (actual number is 1565)).	- 121 -
Table 16: Parameters from between years comparisons of growth estimates at each site, from t-tests of homogeneity of regression coefficients using ln-transformed TLs'. t is the test statistic, v is degrees of freedom, and p is the significance level of the test..	- 121 -
Table 17: Site comparisons of growth rates estimated by push net lengths with growth rates estimated by otolith increment analysis. All TLs. Were ln-transformed and regression coefficients compared for differences using t-tests. No significant differences were found between slopes of regression estimated using different methods..	- 142 -
Table 18: Increment analysis of Blackness 2005 otoliths. Growth rate _{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.	- 130 -
Table 19: Increment analysis of Limekilns 2005 otoliths. Growth rate _{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.	- 130 -
Table 20: Increment analysis of Silver Sands 2005 otoliths. Growth rate _{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.	- 131 -
Table 21: Increment analysis of Gosford Bay 2005 otoliths. Growth rate _{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.	- 131 -
Table 22: Growth rates of 0+ plaice from other areas of its range. lf = change in length – frequency distributions over time, oi = otolith microincrement aging, it = individual tags.....	- 155 -
Table 23: Total length in mm of plaice at the start of the experiment. Rows highlighted in yellow indicate mortality by the end of the experiment.	- 180 -

Table 24 Comparison of %C and $\delta^{13}\text{C}$ values analysed on the NIWA Thermo-Finnigan Deltaplus mass spectrometer compared to reported NIST values. The $\pm$ values represent 1 standard deviation.	- 202 -
Table 25 Precision data for repeat analysis of urea standards during sample batch analyses. The $\pm$ values represent 1 standard deviation.	- 202 -
Table 26: ANCOVA was used to test for differences between locations. Percent fines (by weight) was used as a covariate. The first ANCOVA included an outlier from Portobello; the second ANCOVA excluded this outlier. The results indicate that there is a gradient in $\delta^{13}\text{C}$ with distance down the estuary, and that finer sediments are depleted in $\delta^{13}\text{C}$	- 217 -
Table 27 % Marine vs. terrestrial organic carbon inputs, Inputs of marine organic carbon in equation 6 were assigned $\delta^{13}\text{C}$ values of -22‰ (top), -21.88‰ (middle) and -19‰ (bottom). Inputs of terrestrial carbon were assigned a value of -28‰ . Sites in italics indicate sandy sediments.	- 220 -
Table 28: $\delta^{13}\text{C}$ values of various ecosystem components from published literature. Note the wide variability in values, even from locations that are relatively close.	- 228 -

List of Figures

Figure 1: Map of Plaice spawning areas, adapted from Coull <i>et al.</i> (1998).	- 7 -
Figure 2: Larval-juvenile transformation of plaice (from Fishbase).....	- 8 -
Figure 3: Map of the study area with the main four sites marked.	- 30 -
Figure 4: Cumulative frequency curve for the sand fraction (> 0.062 mm diameter) Blackness sediments. Each line is a single replicate. Blackness sediments were mostly silt/clay; therefore no further analysis of particle size distribution was possible for the sand fraction.	- 33 -
Figure 5: Cumulative frequency curve for the sand fraction (> 0.062 mm diameter) Limekilns sediments. Each line is a single replicate. Two of Limekilns sediments were mostly silt/clay, therefore no further analysis of particle size distribution was possible for these sand fractions. The curves for the remaining three were used to estimate phi- values for median particle size estimation.....	- 34 -
Figure 6: Cumulative frequency curve for the sand fraction (> 0.062 mm diameter) of Gosford Bay sediments. Each line is a single replicate. The curves for the sand fraction were used to estimate phi- values for median particle size estimation and particle dispersion.....	- 35 -
Figure 7: Cumulative frequency curve for the sand fraction (> 0.062 mm diameter) of Silver Sands sediments. Each line is a single replicate (only 4 samples were available from Silver Sands). The curves for the sand fraction were used to estimate phi- values for median particle size estimation and particle dispersion.....	- 36 -
Figure 8: Map of the Firth of Forth. All work in this study was carried out at Silver Sands and Blackness.....	- 46 -
Figure 9: Drop trap in use at Blackness.....	- 51 -
Figure 10: Push net at Silver Sands.	- 52 -
Figure 11: Lift net deployed at Blackness.....	- 53 -
Figure 12: Densities of plaice caught at Blackness in May 2005. Striped bar is mean drop trap density ($n = 14$), clear bar is mean push net density ($n = 4$). Number above the bar is % efficiency relative to the drop trap.....	- 56 -

Figure 13: Densities of plaice caught at Blackness in June 2006. Striped bar is mean drop trap density ($n = 5$), clear bar is mean push net density ($n = 3$) and solid bar is lift net density ($n = 2$). Number above the bar is % efficiency relative to the drop trap.	- 57 -
Figure 14: Densities of plaice caught at Blackness in July 2006. Striped bar is mean drop trap density ($n = 5$), clear bar is mean push net density ($n = 1$). Number above the bar is % efficiency relative to the drop trap.....	- 58 -
Figure 15: Densities of plaice caught at Silver Sands in June 2006. Striped bar is mean drop trap density ($n = 10$), clear bar is mean push net density ($n = 4$). Number above the bar is % efficiency relative to the drop trap.....	- 59 -
Figure 16: Length – frequencies of plaice caught at Silver Sands in June 2006. Clear bars are push net total lengths (mm; $n = 135$), solid bars are drop trap total lengths (mm; $n = 35$).	- 60 -
Figure 17: Length – frequencies of plaice caught at Blackness in May 2005. Clear bars are push net total lengths (mm; $n = 38$), solid bars are drop trap total lengths (mm; $n = 32$).	- 61 -
Figure 18: Length – frequencies of plaice caught at Blackness in June 2006. Clear bars are push net total lengths (mm; $n = 66$), solid bars are lift net total lengths (mm; $n = 10$).	- 62 -
Figure 19: Map of the study area showing the sites sampled for fish in 2005 and 2006. Skinflats was abandoned in June 2006 as no fish had been caught there. The Forth bridges are marked to show the lower limit of the estuary.	- 76 -
Figure 20: Blackness sampling locations. Top: Low tide. Bottom: Flood tide.....	- 77 -
Figure 21: Silver Sands sampling locations. Top: Low tide. Bottom: Flood tide.	- 78 -
Figure 22: Gosford Bay sampling locations. Top: High tide. Bottom: Low tide... ..	- 79 -
Figure 23: Limekilns sampling locations. Top: Low water. Bottom: High water.	- 80 -
Figure 24: Push net front, rear and in operation.....	- 83 -
Figure 25: Length measurements of plaice used in current study (Total Length), from the extreme posterior tip to the extreme anterior tip. Copyright FAO.	- 86 -
Figure 26: Apparatus used to prepare otoliths for microincrement analysis. The top figure shows the turntable with the glass disc and carborundum used for the initial grinding step. The bottom figure is a close up of a stub attached to the	

stylus arm in grinding position. The white material is silicon sealant used to keep the water/carborundum mixture on the glass disc..... - 95 -

Figure 27: Top figure is a SEM image of a prepared otolith. *N* is the Nuclear Primordium, which forms just before hatching, *L* corresponds to the larval phase of the individual, and *A* are accessory primordia, which form as the individual metamorphoses. The bottom figure is a close up of the same otolith; *D* is a single microincrement which corresponds to a single day. The light rings in the bottom figure are the protein organic matrix..... - 96 -

Figure 28: Plots of mean water temperature recorded at each site in 2006. - 100 -

Figure 29: Plots of monthly average SST extracted from SeaWifs/MODIS – Aqua satellite data. These data were used as mean temperatures in Fonds *et al.* (1992) growth model. - 101 -

Figure 30: Blackness 2005 length – frequency histograms. Numbered line is median total length. - 103 -

Figure 31: Limekilns 2005 length – frequency histograms. Numbered line is median total length. - 104 -

Figure 32: Gosford Bay 2005 length – frequency histograms. Numbered line is median total length. - 105 -

Figure 33: Silver Sands 2005 length – frequency histograms. Numbered line is median total length. - 106 -

Figure 34: Blackness 2006 length – frequency histograms. Numbered line is median total length. - 107 -

Figure 35: Limekilns 2006 length – frequency histograms. Numbered line is median total length. - 108 -

Figure 36: Gosford Bay 2006 length – frequency histograms. Numbered line is median total length. - 109 -

Figure 37: Silver Sands 2006 length – frequency histograms. Numbered line is median total length. - 110 -

Figure 38: Change in median total length (mm) at Blackness in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates..... - 122 -

Figure 39: Change in median total length (mm) at Limekilns in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates.....	- 123 -
Figure 40: Change in median total length (mm) at Gosford Bay in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates.....	- 124 -
Figure 41: Change in median total length (mm) at Silver Sands in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates.....	- 125 -
Figure 42: Change in median total length (mm) at Blackness in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates	- 126 -
Figure 43: Change in median total length (mm) at Limekilns in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates	- 127 -
Figure 44: Change in median total length (mm) at Gosford Bay in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates	- 128 -
Figure 45: Change in median total length (mm) at Silver Sands in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates	- 129 -
Figure 46: Scatterplot of individual fish age in days against total length in mm from Blackness in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 23$	- 134 -
Figure 47: Scatterplot of individual fish age in days against total length in mm from Limekilns in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 19$	- 135 -
Figure 48: Scatterplot of individual fish age in days against total length in mm from Silver Sands in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 20$	- 136 -

Figure 49: Scatterplot of individual fish age in days against total length in mm from Gosford Bay in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 13$.	- 137 -
Figure 50: Scatterplot of individual fish settlement date against growth rate from Blackness in 2005. $n = 23$.	- 138 -
Figure 51: Scatterplot of individual fish settlement date against growth rate from Limekilns in 2005. $n = 19$.	- 139 -
Figure 52: Scatterplot of individual fish settlement date against growth rate from Silver Sands in 2005. $n = 20$.	- 140 -
Figure 53: Scatterplot of individual fish settlement date against growth rate from Gosford Bay in 2005. $n = 13$.	- 141 -
Figure 54: Histogram of settlement date frequency distributions at each site calculated using otolith increment analysis from fish caught in 2005.	- 142 -
Figure 55: Scatterplot of density vs. growth rates estimated by push net samples. The fitted line plot shows a significant logarithmic relationship.	- 143 -
Figure 56: Layout of experimental tanks. Diagram (a) is a plan view of each shelf, diagram (b) is a side view of the apparatus, showing position of shelves in relation to each other. Numbers are unique tank identifiers, numbers in parentheses are salinities.	- 177 -
Figure 57: Scatterplot of wet weight and dry weights of chopped <i>Nereis virens</i> , the food source for plaice in the current study. Regression slope and regression equation fitted by least squares method.	- 182 -
Figure 58: Boxplot of % weight changes during minimum ration feeding regime (F1). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from $q_2 - q_3$. Asterisk above salinity 25 indicates an outlier.	- 183 -
Figure 59: Boxplot of % weight changes during <i>ad libitum</i> feeding regime, using the weight measured on 19 th July 2006 as the initial weight (F2 period 1). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from $q_2 - q_3$.	- 184 -
Figure 60: Boxplot of % weight changes during <i>ad libitum</i> feeding regime, using the weight measured on 11 th August 2006 as the initial weight (F2 period 2). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from $q_2 - q_3$.	- 185 -

Figure 61: First period (29 th -31 st August 2006) consumption of chopped ragworm as dry weight (g) per day.....	- 186 -
Figure 62: Second period (3 rd -4 th September 2006) consumption of chopped ragworm as dry weight (g) per day..	- 187 -
Figure 63: Map of the Forth Estuary and the Firth of Forth. Bridges are marked for delineation of physical features (see section 2.1). Arrows mark intertidal sampling sites. Letters in parentheses after the site name indicate whether otoliths (o) or sediments (s) were sampled at that site. Rivers at approximate point of discharge into the estuary are marked in italics: Forth (<i>F</i>), Teith (<i>T</i>), Allan (<i>A</i>), Devon (<i>D</i>), Black Devon (<i>BD</i>), Carron (<i>C</i>) and Avon (<i>Av</i>).....	- 199 -
Figure 64: Cumulative frequency curve of sediments from Portobello.	- 206 -
Figure 65: Cumulative frequency curve of sediments from Blackness Sandy..	- 207 -
Figure 66: Cumulative frequency curve of sediments from Blackness Silty.	- 208 -
Figure 67: Cumulative frequency curve of sediments from Limekilns Sandy. ..	- 209 -
Figure 68: Cumulative frequency curve of sediments from Limekilns Silty.	- 210 -
Figure 69: Cumulative frequency curve of sediments from Skinflats Sandy.....	- 211 -
Figure 70: Cumulative frequency curve of sediments from Skinflats Silty.....	- 212 -
Figure 71: Cumulative frequency curve of sediments from Torry Bay Sandy...	- 213 -
Figure 72: Cumulative frequency curve of sediments from Torry Bay Silty.	- 214 -
Figure 73: $\delta^{13}\text{C}$ measured at various locations in the Forth Estuary and Firth. Solid symbols indicate the sediment was predominantly muddy, outlined symbols indicate the sediment was predominantly sandy (however Torry Bay sandy sediments had intermediate silt/clay content), and Portobello sediments were sandy only. Distances from Kincardine Bridge are straight line distances (note that Blackness and Limekilns are actually the same distance from the bridge. This separation was necessary for visual clarity: Blackness is at the correct distance on this graph) Error bars are $\pm$ standard deviations. The single point at Portobello is a statistical outlier (see text).	- 215 -
Figure 74: Correlation analysis of organic content vs. $\delta^{13}\text{C}$. Pearson's product moment correlation values of r (- 0.550) and p (<0.001) suggest there is a negative linear relationship.....	- 218 -

Figure 75: Boxplot of $\delta^{13}\text{C}$ of plaice otoliths caught at each site. The solid lines in each box are medians, the circles are means. Numbers of otoliths for each site are as follows: Blackness, $n = 3$, Limekilns, $n = 4$, Silver Sands, $n = 4$, Gosford Bay, $n = 5$ - 222 -

Figure 76: Boxplot of $\delta^{18}\text{O}$ of plaice otoliths. The solid lines in each box are medians, the circles are means. Numbers of otoliths for each site are as follows: Blackness, $n = 3$, Limekilns, $n = 4$, Silver Sands, $n = 4$, Gosford Bay, $n = 5$ - 223 -

Figure 77: Scatterplot of $\delta^{13}\text{C}$ vs. $\delta^{18}\text{O}$ recorded in plaice otoliths ($n = 16$). Circles are Blackness otoliths, diamonds are Limekilns otoliths, squares are Gosford Bay otoliths and triangles are Silver Sands otoliths. Line is least squares fit. Pearsons product moment correlation values of r (0.949) and p (<0.001) indicate a very strong, positive linear relationship between the two isotopes. The two data points below the y-axis reference line and to the left of the x-axis reference line are from Blackness otoliths. - 224 -

Acknowledgements

These endeavours are never completed in isolation, I cannot possibly thank or acknowledge all those that allowed me the privilege of completing a PhD, however, the following people were especially important. I would like to thank Katrina Drennan, Marina Mocogni, Linda Wood, Chris Edmans, Sandra Dunbar, Bill Brownlees, Kim Kellett, Dr. John Kinross, the ERI, Thurso, and Margaret Stobie for help in the field and the laboratory. Alex Ford and Lorraine Quinn for help in the laboratory and with ideas and the nights out.

Special thanks must go to my supervisors Dr. Teresa Fernandes and Dr. Alastair Lyndon for their unwavering support during the last four years, and to Susi for understanding and patience. To Dr. Mark Huxham, I owe so much and without whom I could not have done this.

Finally, thanks to my parents, especially my father who is as responsible as I am for allowing this work to be completed, for their unconditional support and love during the last ten years.

Abstract

The nursery function of the Forth estuary and Firth of Forth for juvenile plaice was investigated using several methods. Growth rates of juvenile plaice in the estuary were compared with growth rates of juvenile plaice in the Firth of Forth. Growth was estimated for comparisons between sites because growth is expected to indicate the quality of a particular habitat as a nursery. Growth was estimated at four sites in 2005 using two methods and in 2006 using a single method: 1) Tracking changes in length – frequency distributions of newly – settled plaice over time was used in both years and 2) Plaice were aged to the day using otolith microstructure in 2005 only, and the ages were regressed against length to estimate growth rates as a ‘ground – truthing’ of method 1). The growth rates of plaice were found to be similar between estuarine and outer firth sites, but lower at all four sites in 2006 than 2005. A temperature – dependent model was used to compare maximum growth rates with estimated growth rates to determine if plaice were growing optimally, and growth was less than optimal at all four sites in both years, with some evidence of density – dependence. A push net was used to sample plaice for length measurements at each site on each sampling date, and efficiencies of the net were estimated to determine actual densities of plaice and whether the net was length selective. No length selectivities were found, however, the efficiency of the net was less than 100 % and lower on muddy sites than on sandy sites. The effect of salinity on growth of juvenile plaice was assessed in a laboratory experiment. Plaice were found to grow faster at salinity 25, next fastest at salinity 30 and lowest at salinity 35.

Assessment of consumption rates showed that the differences were likely to be caused by the effects of salinity on physiological performance of fish. A new method of inferring contribution of juvenile plaice from each habitat to adult recruitment, using stable isotopic composition of plaice otoliths was assessed. Plaice from the estuary could not be distinguished from outer firth plaice on the basis of otolith isotopic composition on the small scale used here, however, the work provides a baseline for this area in larger scale studies. Sediment isotopic composition showed differences between the estuary and outer firth, with differences between sediment types and a detectable influence of marine photosynthesis.

Chapter 1

General Introduction: Plaice Nursery Habitat and Study Area

1.1. General Introduction and literature review

*“Flatfishes have their eyeballs
On one side or the other,
Depending on the whereabouts
Of the eyeballs of their mother.*

*And father, too, would have a say
In how their eyeballs got that way.*

*It matters not which side they're on
And how the fishes got 'em.
They're glad to have their eyes on top
Instead of on the bottom.*

*For if they had to get around or
Swim with eyes below, they'd flounder.”*

- New Scientist, 1983

Plaice (*Pleuronectes platessa*) belong to the extant order of fishes Pleuronectiformes, commonly known as flatfishes, family Pleuronectidae (Right-eye flounders). In the UK they are an important commercial species and, together with cod (*Gadus morhua*), whiting (*Merlangius merlangus*) and haddock (*Melanogrammus aeglefinus*) accounted for 48% of the demersal landings into the UK by UK vessels in 2003, with plaice landings of approximately 18 000 tonnes (DEFRA, 2003).

Newly-transformed juvenile plaice settle in very shallow water nurseries, usually coastal areas. Coastal areas are under great anthropogenic pressure, indeed they are one of the most pressurised aquatic habitats globally (Edgar *et al.*, 2000; Nybakken, 2001; Elliott and Cutts, 2004). Provision of nursery habitat for fish and other aquatic organisms is one of the most important roles of coastal habitats,

including estuaries and microhabitats within marine coastal areas. The identification of nursery sites in UK coastal waters and the characterisation of the biotic and abiotic factors that make these nurseries special are important for several reasons.

First, commercially important fish stocks in the North Sea and elsewhere in European waters are heavily exploited. Total Allowable Catches (TACs) of many species have been reduced over the last five years and will be further reduced, or even removed entirely, for many roundfish species in the near future (DEFRA, 2003). North Sea fish stocks, including plaice, have suffered increasingly high exploitation over the period that records have been kept and, while the biomass of the North Sea has remained constant, the species composition has altered drastically (Symes, 1998). Symes (1998) also states that the fisheries in other waters surrounding the UK are in a similar situation. This is likely to increase the exploitation of flatfish species, as has happened with sandeel (*Ammodytes* spp. and *Hyperoplus lanceolatus*), some of which rely heavily on inshore and estuarine nursery grounds. In conjunction with this, management of European and North East Atlantic fisheries has consistently failed to protect stocks or reduce the level of fishing mortality for nearly all teleost species (Serchuk *et al.*, 1996; Kell *et al.*, 1999).

Second, the Water Framework Directive (WFD) requires scientific and technical guidelines for 'good quality' status of surface waters (Environment Agency, 2002). Fish composition and abundance in estuaries must be taken into consideration when deciding principles of 'good quality' status of transitional and

coastal waters. The presence and quality of flatfish nursery areas directly affect these requirements of the WFD, and will impact reference conditions of estuaries. Hence implementation of the WFD for estuaries will require the detection, prediction and monitoring of fish, including flatfish nursery habitats (Environment Agency, 2002).

Third, increasing interest is focusing on the ecological restoration and modification of estuaries through managed retreat (in the face of predicted sea level rise) and the disposal of dredged material (Bolam & Whomersley, 2005). Land claim from coastal areas has been highlighted as a major impact on estuarine and coastal habitats, with an estimate of anywhere between "... 50%-80% of wetlands lost from European and North American Estuaries..." (Elliott and Cutts, 2004). Attempts at mitigating these losses require knowledge of the ecological functioning of the lost areas, including their role as potential nursery habitat. There has been no peer-reviewed work on how these activities might impact nursery areas (for example by changing sediment characteristics), and on how best to use such activities to create or enhance nursery sites.

1.2. Plaice biology

Plaice, as with most bony fishes, are gonochoristic and oviparous. Plaice spawning in the North Sea takes place from December through to June each year, with the highest intensity of spawning during the second week of January in the southern North Sea, with approximately 60 million females spawning at this time (Wimpenny, 1953). In the Irish Sea, spawning begins in January and ends in early

May (Nash, 1998). A map of recorded spawning areas in UK waters is shown in Figure 1 (Coull *et al.*, 1998). In UK waters, female plaice migrate to these spawning areas from various bodies of water surrounding the UK. It has been found that female plaice in the largest spawning area of the southern North Sea split into discrete sub-populations in summer during feeding and growth stages, and that these sub-populations mingle during the spawning season (Hunter *et al.*, 2004) suggesting that they are effectively a single breeding population. Each female plaice extrudes approximately 84 000, externally fertilised, pelagic eggs during the spawning season (Wimpenny, 1953). This high fecundity suggests that plaice, as is common with most teleosts, experience massive mortality before breeding. The actual stage at which this mortality occurs is still the matter of some debate and is explored in more detail later in the current work.

Immediately before the eggs are extruded, they detect the salinity of the surrounding water and alter their density so that it matches that of the water. Density is altered by reducing or increasing the egg diameter (Wimpenny, 1953). Thus, the eggs float at or near the surface of the water and are carried by prevailing surface currents. The time required for hatching, as is common amongst fishes (Dickey-Collas *et al.*, 2003), is dependent on water temperature. However, in the majority of the plaices range, most eggs hatch after approximately 28 days (Wimpenny, 1953). The larvae are also pelagic and cannot swim fast enough to overcome tidal currents (Ryland, 1963). In order to reach suitable habitat, it has been suggested that plaice larvae utilise tidal currents in such a way as to be transported towards their coastal nursery grounds (Fox *et al.*, 2006). The plaice larvae migrate vertically in the water column rhythmically synchronous with ebb

and flood tides; they move into the water column on flood tides and migrate to the seabed on ebb tides, thus are retained in the shallow areas they require as metamorphosed juveniles (van der Veer, 1986).

Larval plaice are morphologically similar to symmetrical teleosts for approximately 10 days, depending on temperature (Wimpenny, 1953) before the first signs of asymmetry appear. The eye on the left side of the upright-swimming larva migrates to the right side, the bone structure of the jaw becomes asymmetrical (see Figure 2) and the body deepens as a result of gut coiling. Most individuals have lost their swim bladder by the time they have completed the transformation to the adult, asymmetrical morphology. It is during late metamorphic stages that plaice settle onto the seabed and begin the benthic phase of their juvenile and adult lives (Gibson, 1973; Modin *et al.*, 1996).

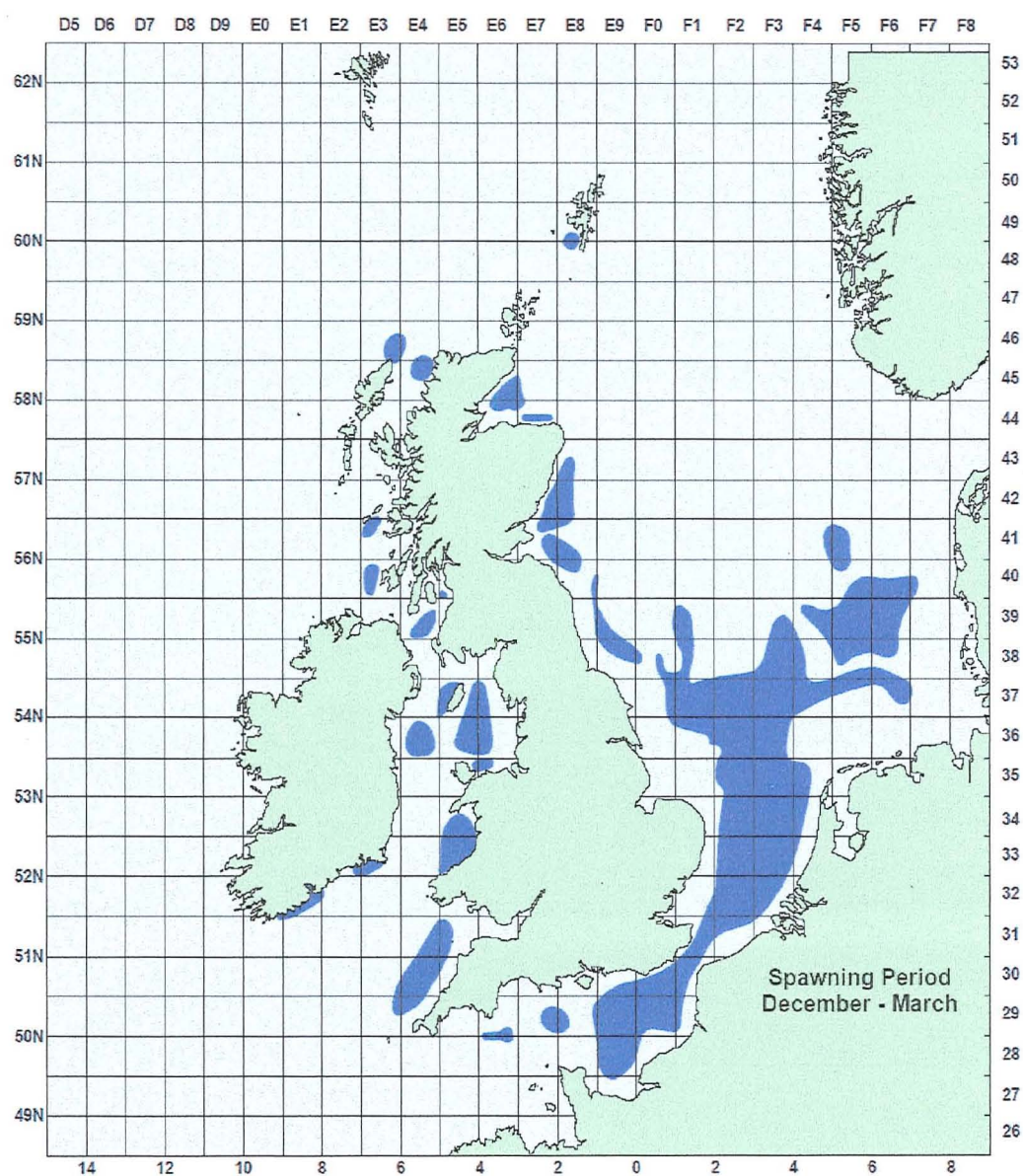


Figure 1: Map of Plaise spawning areas, adapted from Coull *et al.* (1998)

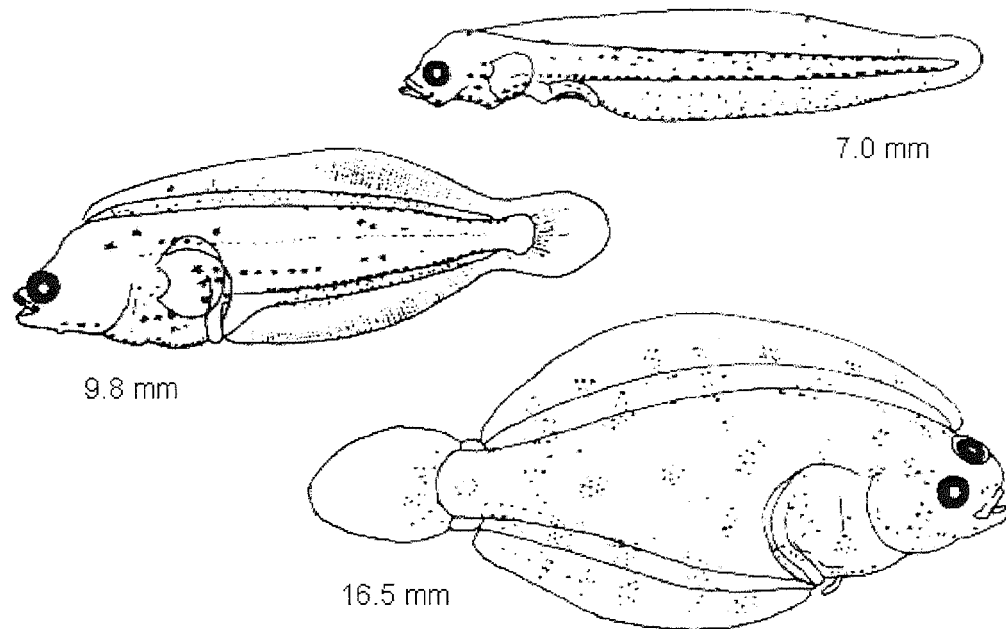


Figure 2: Larval-juvenile transformation of plaice (from Fishbase)

1.3. Nursery habitat

The nursery role has long been recognised as an important function of ecosystems. This recognition stems from the realization that many ecosystems export material, including biomass, to adjacent ecosystems (Gillanders *et al.*, 2003). A nursery is a habitat that is required by some species during a juvenile phase that may be spatially distinct from adult habitat, or a subset of adult habitat. In the past, a clear definition of nurseries has not been given, and nursery areas have been identified merely on the basis of being inhabited by juveniles (e.g. Pihl and van der Veer, 1992; Nash *et al.*, 1994a; Burke, 1995; Nagelkerken *et al.*, 2000a, b; Dorenbosch *et al.*, 2004), supporting higher densities of juveniles (e.g. Ellis and Gibson, 1995; de la Moriniere *et al.*, 2002), higher growth rates of individuals or lower mortality than structurally different, or spatially disjunct, nearby habitat occupied by juvenile conspecifics (e.g. Gibson, 1994; Gibson *et al.*, 1998), or a combination of these factors (e.g. Amara *et al.*, 2001)

These factors undoubtedly play a role in the quality of any particular habitat as a nursery. However, they do not take into account the role that migration from nursery habitat to adult habitat and recruitment to the breeding population (maturation) play in determining the quality of a particular nursery. Beck *et al.* (2001) proposed a definition that allows testable hypotheses to be formulated:

“A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur.”

Thus, determining the geographical origin of mature individuals, when they were juveniles, is paramount to measuring this contribution. The previously

mentioned papers do not measure contribution to adult stock; therefore, the habitats examined in those studies cannot be conclusively identified as nurseries under Beck *et al.* (2001) definition. While it is very likely that the habitats referred to in those papers were nurseries, without the aforementioned measurement of contribution to spawning biomass and comparison with other juvenile habitat, they cannot unambiguously be confirmed as such. The nursery definition provided by Beck *et al.* (2001) is also used in the current study, however, when citations of other papers that refer to nurseries have been made, that papers definition is used, and differences in definitions noted. Bailey *et al.* (2003) found a correlation between area of suitable juvenile habitat and spawning stock (breeding adult) biomass of Alaska plaice (*Pleuronectes quadrituberculatus*), however, although this habitat was undoubtedly juvenile habitat the importance of nursery areas (contribution to adult stock) within the juvenile habitat was not quantified in their study. Nagelkerken *et al.* (2000b) examined the fish species composition of Spanish Water bay in Curaçao, Netherlands Antilles, and concluded that juveniles of reef species that were found in this bay were nursery species, as opposed to the bay containing nursery habitats. Nagelkerken *et al.* (2000b) define nursery species as:

“...fish species which inhabit coral reefs as adults utilise mangroves, seagrass beds, and other shallow-water bay habitats as nurseries during the juvenile part of their life cycle (nursery species).”

This definition does not take into account differences in the contribution that different ‘...biotopes...’ in Spanish Water bay make to the adult reef populations. Further, Nagelkerken *et al.* (2000b) claim that one of their biotopes did not contribute very much to the nursery function of the bay as total abundance of

juveniles was low. Nevertheless, this biotope could have contributed the highest number of individuals per unit area to the adult population than any other biotope; however, this was not measured in their study. In addition Nagelkerken *et al.* (2000b) claim that fish densities, as well as surface area, must be considered when assessing nursery function of habitats, or the bay as a whole in their case. This statement could be construed as ambiguous: they do not say whether high or low densities are most important. From Beck *et al.* (2001) definition, low density habitats may provide a significant contribution to breeding habitats, merely by having high survival and providing safe migration routes, if the contribution per unit area is higher than that of other habitats that juveniles inhabit. Conversely, Nagelkerken *et al.* (2000b) mention surface area of high density biotopes and conclude that even though a habitat has a high density of juveniles, if the surface area of that habitat is low then it cannot contribute much to the nursery function of an area. Again, Beck *et al.* (2001) definition allows for low surface area of particular habitats to be considered as nurseries as it is possible for habitats with a low surface area (or volume) to contribute high numbers of juveniles per unit area to the adult population. Some of the habitat that juveniles are found in may be 'sinks'; i.e. juveniles will settle there but ultimately do not join the breeding population, hence, these habitats contribute little or nothing to the breeding population Beck *et al.* (2001).

Beck *et al.* (2001) definition applies only in cases where the adult and nursery habitat are spatially distinct i.e. there is some intervening "non-habitat" that must be traversed by juveniles to join the adult population. For example, juvenile and adult queen scallops (*Aequipecten opercularis*) prefer maerl beds to less

heterogeneous habitat, with no spatial separation of larval, juvenile or adult habitat (Kamenos *et al.*, 2004a, b, c); therefore, there is no disjunction between adult and juvenile habitat, which does not fit the nursery-role definition proposed by Beck *et al.* (2001). Also, as this definition relies on comparing all of the different habitats that juveniles of a particular species inhabit, nurseries are therefore a subset of juvenile habitat.

The contribution a nursery habitat makes to the adult population can be considered a measure of nursery quality: a greater contribution indicates higher quality nursery habitat. Nursery quality is likely to be a function of enhanced growth, reduced mortality/enhanced survival, reduced predation, reduced mortality during migration from the nursery (safe migration routes) or a combination of these (Gibson, 1994; Beck *et al.*, 2001; Gillanders *et al.*, 2003; Minello *et al.*, 2003; Ross, 2003).

In order to assess the contribution that a particular habitat makes to the breeding population, and assess whether that habitat is a nursery, it is necessary to track the migrations of juveniles from these habitats to the adult habitat. There have been various methods employed in the past to measure migrations, with varying success. These methods have been applied not only to juvenile ontogenetic migrations, but also movement of adults between suitable habitats as well as species other than fish, and these methods may also be suitable for tracking juvenile migrations. Gillanders *et al.* (2003) provide a comprehensive review of these methods for aquatic species, within the framework and limitations set by Becks' *et al.* (2001) nursery habitat definition. Gillanders *et al.* (2003)

categorise these methods as either intrinsic or extrinsic. Intrinsic methods usually involve some measure of the organisms' morphology, physiology or biochemistry e.g. abundances and distribution, size distributions, age structure, stable isotope composition of otoliths (Weidman and Millner, 2000; Augley *et al.*, 2007; Huxham *et al.*, 2007), elemental composition of otoliths (Gillanders & Kingsford, 2000; Spencer *et al.*, 2000; Rooker *et al.*, 2003; Kraus & Secor, 2005). Extrinsic measurements include: artificial tagging (either external such as fin clips or internal such as injected dyes or oxytetracycline marking of otoliths) or natural tags such as parasites (Chapman & Kramer, 2000; Gillanders *et al.*, 2003).

Some studies have inferred movement from juvenile to adult habitat using differences in size and/or age of individuals between different habitats (de la Morinière *et al.*, 2002; Mumby *et al.*, 2004). This type of measurement is indirect and relies on inference of movement, without actually measuring the individuals' movements. The preferred methods for tracking changes are direct measurements, i.e. following a known individual or group of known individuals from one habitat to another. However, this is logistically extremely difficult to do or even prohibitive for financial reasons. For example, Jones *et al.* (1999) used a mark-recapture study of over 10 million tetracycline-tagged damselfish, from eggs to adults, to measure recruitment and had impressive success. However, this method is labour-intensive and not easily carried out by most workers or concerned agencies, or indeed suitable for all species, as damselfish attach eggs to a substrate allowing easy marking by exposure to tetracycline, compared with pelagic eggs.

Traditionally, whole estuaries and coastal areas have been considered as nursery areas for many species of flatfish (Norcross *et al.*, 1995; Beck *et al.*, 2001; Gillanders *et al.*, 2003; Lazzari *et al.*, 2003). However, this general approach may not take into consideration fine scale heterogeneity and microhabitats within these relatively large landscapes e.g. it is common to find mangroves in close association with seagrass beds and coral reefs within tropical estuaries (e.g. Huxham *et al.*, 2004; Nagelkerken *et al.*, 2000a, Nagelkerken *et al.*, 2001, and references within these papers), and salt marshes in close association with mudflats (Nybakken, 2001; Minello *et al.*, 2003). It may be that such microhabitats provide varying quality of nursery areas for various species, and that a particular habitat type is of superior quality than others occupied by juveniles. Minello *et al.* (2003) tested hypotheses on the use of salt marshes as nursery habitat. They divided up marshes into 6 components and found differences in the nursery quality of these 6 components; salt marsh is just one component of estuaries (Nybakken, 2001). The assessment of these was further complicated by the physical and chemical features of the particular geographic area studied.

1.4. Plaice nursery grounds

There has been much work conducted on juvenile plaice (*Pleuronectes platessa*) habitat in many areas across its range, mostly, but not limited to, the southern North Sea, the Irish Sea, the English Channel and Scandinavian waters (Baltic Sea, Skagerrak and Kattegat) as well as laboratory-based work. The large body of published and unpublished literature is likely to be a result of the commercial importance of plaice in many European and Northeast Atlantic

countries (Table 1) and also because they are relatively abundant, easy to catch in shallow coastal waters and ubiquitous on most Western European coasts (Wimpenny, 1953; Gibson, 1973; Amara, 2004). Despite the large body of literature, there has been little or no investigation of plaice nursery habitat, within the constraints and definition of a nursery proposed by Beck *et al.* (2001). The work that has been carried out on plaice nurseries has concentrated on two questions: What are the important environmental (e.g. water temperature, sediment properties, wave exposure) and biological (e.g. growth rates, mortality and any density-dependence of these) variables influencing juvenile plaice settlement preferences, and how do these influence recruitment to adult populations? In this study, preference is taken as: "the likelihood of that habitat being chosen if offered on an equal basis with others or the degree to which one habitat is selected over others" (Gibson and Robb, 2000). Preferences may arise as a result of natural selection on behaviour, by habitats offering maximised growth, reduced predation, and reduced mortality, or some other condition resulting in increased survival; these preferences are likely to influence the habitat selection of juvenile plaice (as well as adults and other benthic taxa).

Table 1: Commercial landings of selected fishes into Northeast Atlantic ports (FAO Area 27), t=tonnes.

Species	1997 (t)	1998 (t)	1999 (t)	2000 (t)	2001 (t)	2002 (t)	2003 (t)
Plaice	121 421	103 586	113 421	113 233	110 907	99 204	92 906
Cod	1 328 176	1 158 203	1 025 406	877 150	884 785	852 088	810 341
Herring	2 249 400	2 148 649	2 129 642	2 103 709	1 645 085	1 612 384	1 661 342
Haddock	322 746	269 036	235 725	196 111	208 290	247 470	258 951
Greenland Halibut	41 790	34 506	47 571	43 108	47 190	44 523	48 187

Newly-transformed plaice settle on beaches in western and northern Europe at varying times of the year, depending on geographical location. The settlement periods for various areas of Europe are: UK coasts late April and early May (Wimpenny, 1953), Scandinavian coasts from late April to Mid-May (Pihl *et al.*, 2000; Wennhage & Pihl, 2001), the Wadden Sea from February until late May, peaking in April (van der Veer, 1986; van der Veer *et al.* 2000b), and French coasts of the Eastern English Channel from mid-March to late June (Amara and Paul, 2003). Plaice larvae are thought to use a combination of active and passive mechanisms for transportation to juvenile habitat (Wegner, *et al.*, 2003). Semi-active transport, or 'selective tidal transport' (De Veen, 1978; Rijnsdorp *et al.*, 1985), for juvenile plaice requires the plaice to vertically migrate in the water column in rhythm with tidal state; on flood tides they will move up into the water column and are transported shoreward, and they will settle on the sea floor during ebb tides in order to remain inshore. This semi-active transport is necessary as larvae and juveniles can only swim at a cruising speed of approximately 1 to 3

body lengths per second, insufficient to make headway against most coastal currents (Gibson, 1997). In areas with little or no tidal currents e.g. Baltic Sea, larvae will be transported by wind-induced or thermohaline currents (Gibson, 1997).

Once they have settled, metamorphosed juveniles show a remarkable along-shore homing ability and affinity with their selected habitat (Burrows *et al.*, 2004). This is especially remarkable as it has been discovered that initial habitat selection is partly determined by depth (Gibson, 1973; Gibson *et al.*, 2002). Sediment particle size also appears to influence habitat selection by juveniles. Gibson and Robb (2000) carried out field and laboratory investigations of sediment selection by juvenile plaice. They discovered that sediment selection was determined initially by burial ability, which is determined by body size (smaller fish bury more easily in finer sediments), and activity level. Activity level is controlled by irradiation levels as well as endogenous (internal) rhythms, while burial reduces activity (Gibson & Robb, 2000). Therefore, sediment selection has a behavioural basis at least in this laboratory-based study. In the same study, Gibson and Robb (2000) reported that fish choices in field experiments using trays with varying sediment particle diameter compositions, were similar to the choices in their laboratory experiments. The predominant sediment particle size used in these field experiments were as follows: fine: 0.25-0.5mm (52%), medium 0.5-1.0mm (59%) and coarse 2-4mm (44%) and 4-6 (26%). In the laboratory studies, sediment compositions were: fine <0.5mm, medium >0.5mm and <1mm, coarse >1mm and <2mm and very coarse 2-4mm. For both field and laboratory experiments, plaice

preferred the fine sediments notwithstanding non-significance of statistical testing of their data.

Sediment selection by plaice recorded in the laboratory in Gibson and Robb (2000) was observed in the absence of both predators and any food items. General flatfish, including plaice, settlement preferences may also be influenced by the presence or absence of these two factors, and/or by the composition of food and predator assemblages. Food composition may differ between two juvenile habitats, as a result of prey species identity or prey size differing between habitats. Likewise, the threat of predation may differ between two habitats as a result of the presence or absence of predatory species or as a result of the habitat altering predator foraging efficiency. Wennhage and Gibson (1998) carried out laboratory experiments on the settlement behaviour of newly-transformed (and laboratory-reared) plaice in the presence and absence of both food items (benthic meiofauna consisting mostly of harpacticoid copepods and nematodes, and *Artemia* nauplii) and predators (*Crangon crangon*). In both experiments, statistical differences ($p < 0.05$) in settlement were found between sediments with and without food (more plaice on sediments with food) and between sediments with and without predators (more plaice on sediments without predators).

In a similar study, Burrows and Gibson (1995) reported that the presence of predators also strongly reduced feeding success, by various mechanisms, the most important being an increase in time spent buried in the sediment (and, hence, inactive and not feeding). This would suggest that sediment particle composition plays a role in habitat selection partly as a means of reducing predation risk, as

suggested by Gibson and Robb (2000). Wennhage (2002) states that van der Veer and Bergmann (1987) found that predation risk from *C. crangon* on juvenile plaice increases with encounter rate (i.e. densities of both prey and predator). However, Wennhage and Pihl (2001) found consistent percentages (5-6%) of shrimp stomachs with plaice otoliths in them, regardless of plaice or shrimp density at the capture site. They suggest further work is required to elucidate and generalise interactions between these two species. Other species are also important predators of juvenile plaice: Ellis and Gibson (1995) examined stomach contents of piscivorous fish caught on Tralee beach on the west coast of Scotland. They found that cod (*Gadus morhua*), poor cod (*Trisopterus minutus*) and grey gurnard (*Eutrigla gurnardus*) preyed frequently on plaice, while bullrout (*Myoxocephalus scorpius*) although also a plaice predator, its importance could not be ascertained as only one individual was caught. Another important predator species of small plaice is the shore crab, *Carcinus maenas* (Gibson *et al.*, 1998).

Predation on 0-group plaice has varied effects on the juvenile population in nursery areas and on recruitment variability to breeding populations. While predation of plaice on nursery grounds is important to recruitment levels, it is unknown exactly what mechanisms control recruitment: whether nursery ground processes dampen variability or whether nursery ground processes generate variability. The authors van der Veer *et al.* (2000b) suggest that coarse regulation of recruitment occurs during the pelagic phase (eggs and larvae) and fine control occurs as a result of density-dependent mortality during early nursery ground phases (i.e. immediately after settlement). These authors also suggest that the observed high year-class strength of North Sea plaice after very cold winters is due

to some phenomenon affecting the pelagic phase. Temperature effects on mortality rates of the egg stage of fish have been suggested by other workers to control, or at least influence, recruitment variability (e.g. Dickey-Collas *et al.*, 2003; Bunn *et al.*, 2000). However, the mechanisms of temperature effects remain largely unknown, as measuring egg mortality rates, among other variables, is actually problematic in the field (Dickey-Collas *et al.*, 2003) and appears to be dependent on the species of fish (Bunn *et al.*, 2000). Nevertheless, plaice appear to exhibit density-dependent growth and mortality on juvenile habitat studied (Pihl *et al.*, 2000), which would be likely to dampen recruitment variability. Density-dependent mortality is likely to arise as a result of predation (Ellis and Gibson, 1995). Evidence for this comes from two studies: in contrast to van der Veer *et al.* (2000b), Nash *et al.* (1992, 1994a) provide evidence from two separate studies that after very cold winters, recruitment to the adult population was extremely high and that this was likely to be a result of low predator abundance on Port Erin bay, Isle of Man. Ellis and Gibson (1995) found that only cod (*Gadus morhua*) of 58mm or longer (usually I-group) preyed upon dab on Tralee beach, Scotland. This suggests that recruitment of cod in one year may affect recruitment of dab the next year, via increased or decreased predation pressure. It is not a large step to assume that this mechanism may also apply to plaice. Other marine teleosts experience massive mortality during the larval stages, suggested by Sette (1943) to be as a result of larvae being unable to find food after absorption of their yolk sac. Applying this logic to larval plaice, if they have been unable to find suitable habitat to settle on by the completion of metamorphosis (at approximately the same time as the yolk sac is completely absorbed), then they are unlikely to be able to find suitable food and, hence will perish.

Food preferences of juvenile plaice are fairly consistent across their range and in agreement with a benthic-feeding lifestyle (Poxton and Nasir, 1985). All food items in the following list refer to data from 0-group plaice caught in shallow water coastal areas. In the southern North Sea, polychaetes comprise the largest group eaten across all sizes of plaice, with molluscs making up the next largest prey group in juvenile plaice up to 5 cm (Gibson *et al.*, 1998; Piet & Rijnsdorp, 1998); in the northern North Sea annelids are also the most abundant prey group with molluscs, crustaceans and echinoderms also featuring in their diet (Rijnsdorp & Vingerhoed, 2001); polychaetes, crustaceans and molluscs dominated the diet of all sizes of plaice in the English channel (Amara *et al.*, 2001), in and around the Skagerrak and Kattegat coasts of Sweden, crustaceans, (*Corophium volutator* and sub-lethal predation of Balanidae cirri) and bivalve molluscs are the most important food items taken, with polychaetes making up a small part of the diet of all plaice caught by Gibson *et al.* (1998). These authors reported that meiofauna also made up a significant (in numerical terms at least) component of plaice diet, and made up the largest percentage of their diet when they fed at night. However, in an earlier study, Gibson (1973) claims that plaice feed mainly during the day and are visual feeders. In the studies carried out on diet composition and stomach contents of plaice, it has been found that plaice feed on the most abundant food items available, suggesting they are generalist and opportunistic feeders. In many studies, food does not appear to be a limiting factor for growth or numbers of settlers (Amara *et al.*, 2001, Fonds *et al.*, 1992) and, hence, not the cause of density-dependent growth, although it may affect distribution within a nursery area (Pihl and van der Veer, 1992)

Growth rates on juvenile habitat have been measured in several large juvenile plaice habitats in Europe, as well as several laboratory studies. The usual method of measuring growth of wild populations is to track changes in the average length of caught wild fish. This is dependent, however, on fishing gear being non-size selective or else, by using correction factors for biases and selectivities. Other methods employed include capture-recapture using individually marked fish (Nash *et al.*, 1994a) and measuring growth of captive fish (held in cages in the field) (Modin and Pihl, 1994). Growth rates of populations measured (as change in length per unit time) from field surveys range from 0 to 1.67 mm day⁻¹ in summer on Port Erin Bay, and 0.5 mm day⁻¹ in winter on the same beach (Nash *et al.*, 1994a, 1992), between 0.4 – 0.8 mm day⁻¹ in the Gullmar Fjord, Sweden (Modin and Pihl, 1994). Various average growth rates measured in wild populations include: 0.211 mm day⁻¹ in Port Erin Bay, Isle of Man, from 0.1 to 0.8 mm day⁻¹ in the Dollard estuary, Holland and 0.55 to 0.81 mm day⁻¹ in the Southern Bight of the North Sea (Jager *et al.*, 1993; Nash *et al.*, 1994a; Amara *et al.*, 2001). Although some authors suggest that growth rates are density-dependent (Pihl *et al.*, 2000; Modin and Pihl, 1994), others found no evidence for density-dependent growth (Nash *et al.* 1994a). Maximising growth rates may be an important mechanism for reducing predation on 0+ group plaice. *Crangon crangon* and *Carcinus maenas* are both important predators of small plaice, and plaice appear to have size refugia at 30mm for *C. crangon* and 50mm for *C. maenas* (van der Veer and Bergmann, 1987). This may explain why growth rates measured in numerous field studies (Berghahn *et al.*, 1995; Amara *et al.*, 2001; Amara and Paul, 2003; Amara, 2004) appear optimal (experimentally-derived maximum) when compared with, or even higher than (Nash *et al.*, 1994a) laboratory-based measurements (Fonds *et al.*,

1992; Glazenburg unpublished, 1983 and cited in Berghahn *et al.*, 1995). This phenomenon of size-selective predation (increased predation on smaller size classes) and, as a result, size-selective mortality (increased mortality of smaller size classes) is also known as the 'bigger is better' hypothesis (Litvak and Legget, 1992; Legget & Deblois, 1994). While this hypothesis does not apply to all predator-prey interactions (size-selection may result in higher mortality rates of larger size-classes), it is found to be applicable in many cases (Ellis and Gibson, 1995). Several workers have reported recording reduced and even negative growth rates during winter in field studies (Amara, 2004). Negative growth implies the average length of the fish becomes smaller over time. This may have two explanations: either individual fish shrink or they migrate and late settlers have not grown as fast for some other reason (either temperature or food availability). This observation (Amara, 2004) has been explained by plaice being visual foragers: during winter, there are less hours of daylight which may reduce the amount of food effectively available, however, there may also be a reduction in absolute prey availability as a result of seasonal variability in prey populations.

Other influences on settlement may include water temperature. Gibson *et al.* (1998) found that plaice on a microtidal beach made upshore and downshore migrations, and they suggest these could be related to temperature. Further evidence of this mechanism is provided by Fonds *et al.* (1992) who discovered that food consumption and growth of plaice are reduced above 20°C. These migrations may also be a means of predator avoidance (Gibson *et al.*, 1998), as many predatory fish migrate in and out of shallower water over diel timescales. Predator avoidance may also be one reason why plaice utilise the intertidal environment.

Apparent sediment selection may be a correlate of exposure of the beach and the effect of high energy waves on sediment particle composition affecting the animal burial ability, as well as the aforementioned predation and food factors (Pihl and van der Veer, 1992).

All of the aforementioned processes and influences operating on both the pelagic and nursery ground stages of plaice result in variability in recruitment to the adult population. However, plaice show an unusual (for teleosts) stability in numbers of recruits each year in many areas of their range. This has stimulated much work on early life stage processes in several areas of the UK and Europe. On the Isle of Man in the Irish Sea, Port Erin Bay has been particularly well studied, as have several beaches on the West Coast of Scotland. The Swedish west coast and, particularly, the Wadden Sea juvenile plaice are also very well studied. These studies have lead to several key findings regarding these early life stage processes, some of which have been mentioned in the preceding paragraphs. The key ecological and biological parameters and processes that have been measured in the European areas mentioned previously are: growth rates, mortality, density, food availability, presence of other species as potential competitors, predation and the physical and chemical properties of these habitats. While all of these measurements have been studied, there is still a lack of information that would provide a complete synthesis of plaice juvenile habitat. However, some important observations have been made regarding the relationship between pelagic and early-benthic stage processes and recruitment to the Spawning Stock Biomass (SSB). In the areas that are regularly monitored for fisheries management, it appears that recruitment to adult population is related to

temperature, at least during cold winters (van der Veer *et al.*, 2000b). However, recruitment is very stable over years, relative to other commercially harvested species, suggesting that some variability damping process operates on the pre-breeding life stages. Some workers suggest that these processes operate on the pelagic stages (e.g. Nash *et al.*, 1992, 1994a; Gibson, 1994); others suggest that the damping processes operate on the early benthic stages of plaice (e.g. Wimpenny, 1953; van der Veer, 2000b). Beverton (1995) suggests that North Sea plaice exhibit the characteristics of a population that has strong density-dependence during the juvenile (i.e. benthic) phase. If damping operates on the benthic stage, then, some aspect of the nursery grounds may be responsible for final numbers of recruits to adult populations. Beverton (1995) suggest the most likely candidate for this damping effect is the surface area of suitable habitat and subsequent effects of area on the degree of 'concentration' of pelagic larvae onto benthic habitat as benthic juveniles, and density-dependent mortality, i.e. nursery ground. Once again, this invokes Beck *et al.* (2001) nursery habitat definition: that contribution to adult habitat must be measured in order to determine the quality of a particular juvenile habitat and its qualification as a nursery ground.

1.5. Plaice investigations in the Firth of Forth

There have been few studies focused directly on juvenile plaice in the Firth of Forth and estuary. Early studies of natural populations were the results of sampling cruises along the whole of the Scottish east coast (e.g. Bowman, 1914, 1921). These studies sampled mainly in sub tidal waters at depths usually not less than 2 m, thus the early juvenile stages were not studied in these investigations.

Poxton and Nasir (1985) and Nasir (1981, 1985) examined juvenile plaice populations in the Firth of Forth (but not the estuary) and estimated total population size of 0+ group plaice to be approximately 2 million in 1979-1980. However, these workers based their estimates on sandy beaches in the Outer Firth only, therefore, the importance of the Forth estuary for juvenile plaice has not been investigated.

Dab (*Limanda limanda*), flounder (*Platichthys flesus*), cod (*Gadus morhua*) and whiting (*Merlangius merlangius*) have been caught in the Firth and estuary in reasonable numbers (Poxton, 1987; Elliott *et al.*, 1990; Greenwood *et al.*, 2002) and these species may be important predators and/ or competitors of plaice on the intertidal (Ellis & Gibson, 1995).

1.6. Aims and Objectives

The aims of the work presented here are 1) to determine whether the muddy intertidal areas in the Forth estuary are potential plaice nurseries 2) whether salinity affects growth of juvenile plaice and 3) whether stable isotopes can be used to identify nurseries as defined by Beck *et al.* (2001). These aims will be addressed in the following chapters; specific objectives for each chapter are listed here:

Chapter 2 – Efficiency and Selectivity of a 1.5 m Riley Push Net. In order for quantitative estimates of growth of juvenile plaice to be made using the push net, gear characteristics must be determined. In particular, whether the gear is size – selective and/or whether densities (abundance per unit area of habitat) are accurately measured, will be determined. The key hypothesis tested in this chapter

is: the 1.5 m push net is not as efficient as a putatively 100% efficient drop trap and is not size – selective. The results of this chapter are in preparation for submission for publication.

Chapter 3 – Growth Rates of Juvenile Plaice in the Forth Estuary and Firth of Forth. Growth rates of juvenile plaice were measured over two years at four sites. Growth rates were measured using two methods and compared with a growth model. The aim of this chapter was to use growth as an indicator of the potential quality of a particular habitat, to determine whether nurseries potentially may be found in the estuary. The aim is to test two single hypotheses: 1) the Outer Firth habitats are the only suitable habitat for newly-settled plaice, therefore, 0+ plaice growth rates in the Outer Firth will be higher than growth rates of 0+ plaice in the estuary. The second hypothesis is 2) growth rates of plaice on nursery grounds is limited, therefore growth rates of populations will be lower than maximal growth rates estimated from temperature – dependent models. The secondary aim of this chapter was to determine whether growth is an important influence on each habitats contribution to recruitment, once recruitment contribution had been determined (*Chapter 5*). The results of chapter 3 and chapter 4 have been submitted as a single manuscript to Journal of Sea Research for consideration for publication.

Chapter 4 – Effects of Salinity on Growth Rates of Juvenile Plaice. In light of the results from estimates of growth rates in the estuary, and the lack of previous published work on this subject, an attempt was made to determine what effects, if any, different salinities have on the growth of juvenile plaice. This was done in a

controlled experiment where fish were exposed to three different salinities. The effects of salinity on growth rates and feeding behaviour of 0+ plaice were assessed. The results of this chapter and chapter 3 have been submitted to Journal of Sea Research.

Chapter 5 – Carbon Stable Isotopes in Estuarine Sediments and their Utility as Migration Markers. The first main aim of this chapter was to determine whether a gradient in carbon stable isotopes exists in intertidal sediments in the Forth estuary. The second main aim of this chapter was to determine if stable isotopes of oxygen and carbon could be used to estimate the contribution of plaice from habitats in the Forth Estuary compared to the Firth of Forth. A secondary aim was to determine the effects of sediment particle size on stable isotopic composition of estuarine sediments. The results of this chapter have been published in Estuarine, Coastal and Shelf Science (Augley *et al.*, 2007). A reprint of this paper has been inserted at the end of the thesis.

Chapter 6 – Summary and Conclusions. The aim of this chapter was to summarise the previous chapters in order to identify the contribution made by the thesis.

1.7. Methods and Materials

1.7.1. Study Area: Firth of Forth and Forth Estuary

The Forth estuary begins in the city of Stirling, and runs for approximately 40 km to the Forth road and rail bridges at Queensferry (McLusky, 1987). This body of water is enclosed on three sides and a salinity gradient is measurable as far as the bridges (Webb & Metcalfe, 1987). The estuary can be divided into three sections: the upper section with salinities at around 5 ppt (Webb & Metcalfe, 1987), from the head at Stirling to Alloa, the mid – section from Alloa to Bo' ness and the lower section from Bo' ness to the mouth of the estuary at the road and rail bridges (McLusky, 1987). These physical delineations were adopted by the Royal Society of Edinburghs' 1987 Symposium on 'The Natural Environment of the Estuary and Firth of Forth', and reported by McLusky (1987). Eastward of the bridges is the Firth of Forth, which is a semi enclosed body of water with no measurable dilution by freshwater inputs (Figure 3). The firth continues eastward until it gradually merges with the Northern North Sea, past the Isle of May (McLusky, 1987). The estuary is characterised by predominantly silty sediments with a high organic content and typical estuarine benthic fauna (McLusky, 1987), while the Outer Firth is characterised by coarser sediments and a richer benthic fauna, although still impoverished compared to similar areas (Read, 1987).

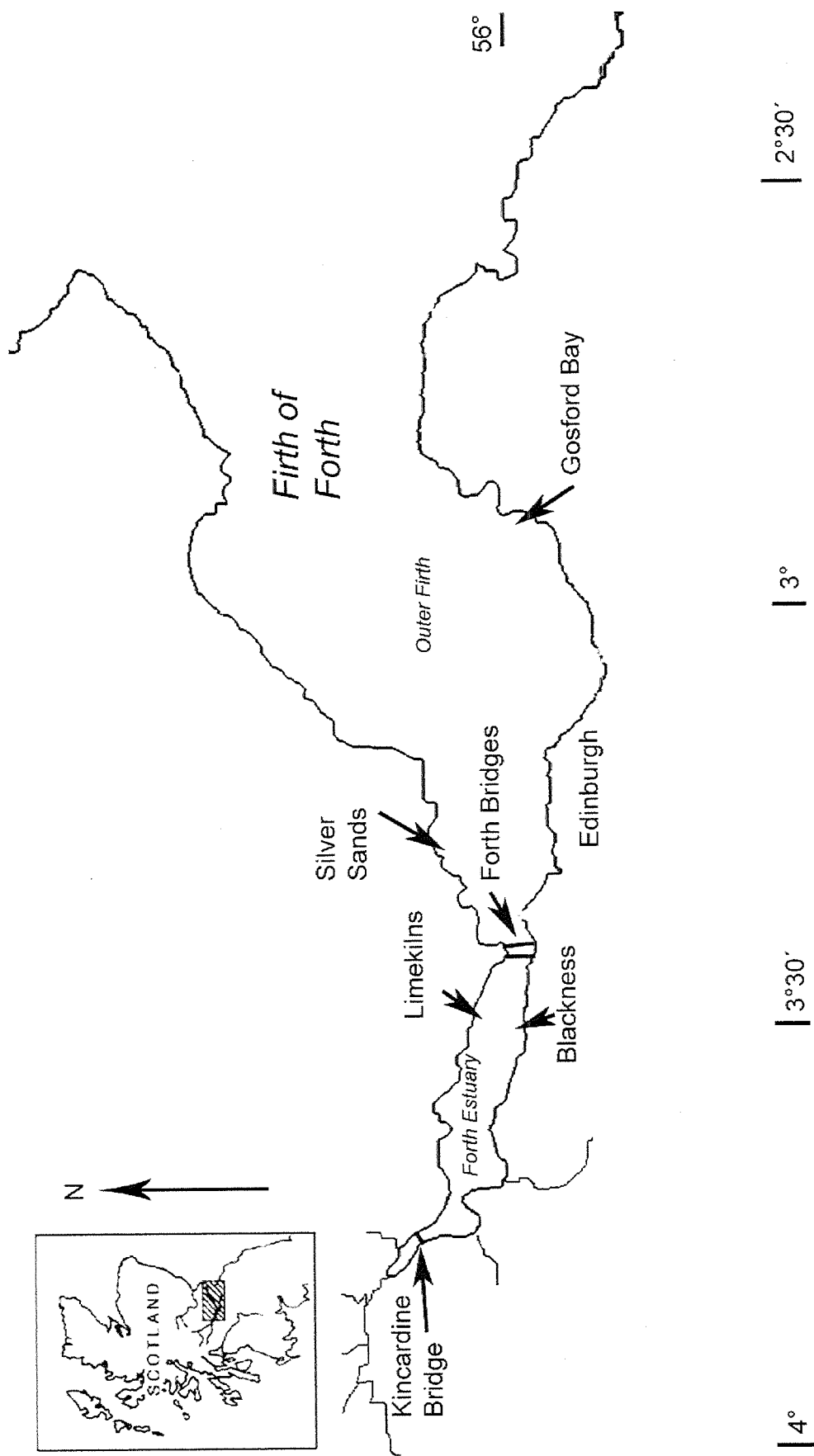


Figure 3: Map of the study area with the main four sites marked.

1.7.2. Study Sites

Four sites were selected for study of the juvenile plaice in the forth and estuary, Blackness and Limekilns in the lower estuary and Silver Sands and Gosford Bay in the Firth of Forth (Figure 3). The sediment characteristics, granulometric analyses and organic contents, were analysed for a general description of each site. Other sites were used for additional work; however, these are described in their respective chapters, only the sites selected for study of plaice growth are described here.

1.7.3. Sediment Characteristics

Sediments were sampled from Blackness, Limekilns, Silver Sands and Gosford Bay in July 2006 for granulometry and organic content determination. Five replicates were taken at low tide near to the waterline, within an area of approximately 100 m² at each site. The top 3 – 5 cm of each sediment was sampled by scraping and scooping up with a credit – card – sized piece of thin plastic. Granulometry was carried out as per Buchanan (1984) and Bale & Kenny (2005), and organic content was estimated by % weight loss on ignition at 475 °C for 4 – 6 hours. Sediment particle size distributions were estimated by first sieving the wet sample in a 63 µm sieve and measuring the difference in dry weight between pre- and post – sieved sample, as % fines. The remaining sand fraction was passed through a stack of graded sieves (based on the Udden/Wentworth Scale presented in Table 2, adapted from Bale & Kenny, 2005) and the amount of sediment retained by each sieve was weighed. Sieve mesh sizes were transformed to phi (Φ) notation (Table 2) using the following log-transformations:

$$\phi = -\frac{\log_{10}(\text{diameter}, \text{mm})}{\log_{10} 2} \quad (1),$$

cumulative % finer weight of sediment was calculated from the weight of sediment retained on, and plotted against the Φ value for, each sieve, and used to estimate median particle diameter. Cumulative frequency curves for each site are shown in Figures 4 – 7; organic contents and values for other calculations extracted from these cumulative frequency curves, are shown in Table 3. The cumulative frequency curves were used to estimate median particle diameter and dispersion. Median particle diameter (M_d) was estimated visually by reading the Φ value on the x – axis where the cumulative frequency curve crossed the 50 % line on the y – axis (Bale & Kenny, 2005). This was possible for all of the sediment samples from Gosford Bay and Silver Sands only, and for three of the Limekilns samples where the silt/clay fraction accounted for less than 50 % of the sediment weight. Dispersion was calculated by visually estimating Φ values from the x – axis and substituting these values into equation 2:

$$\sigma_1 = \frac{\phi_{16} - \phi_{84}}{4} + \frac{\phi_5 - \phi_{95}}{6.6} \quad (2),$$

Where, σ_1 is inclusive graphic standard deviation and ϕ_X is the Φ value at the Xth percent cumulative weight (Table 3) (Bale & Kenny, 2005). It was possible to calculate σ_1 for Silver Sands and Gosford Bay only, as Limekilns and Blackness had silt/clay fractions greater than 5 % by weight.

Blackness

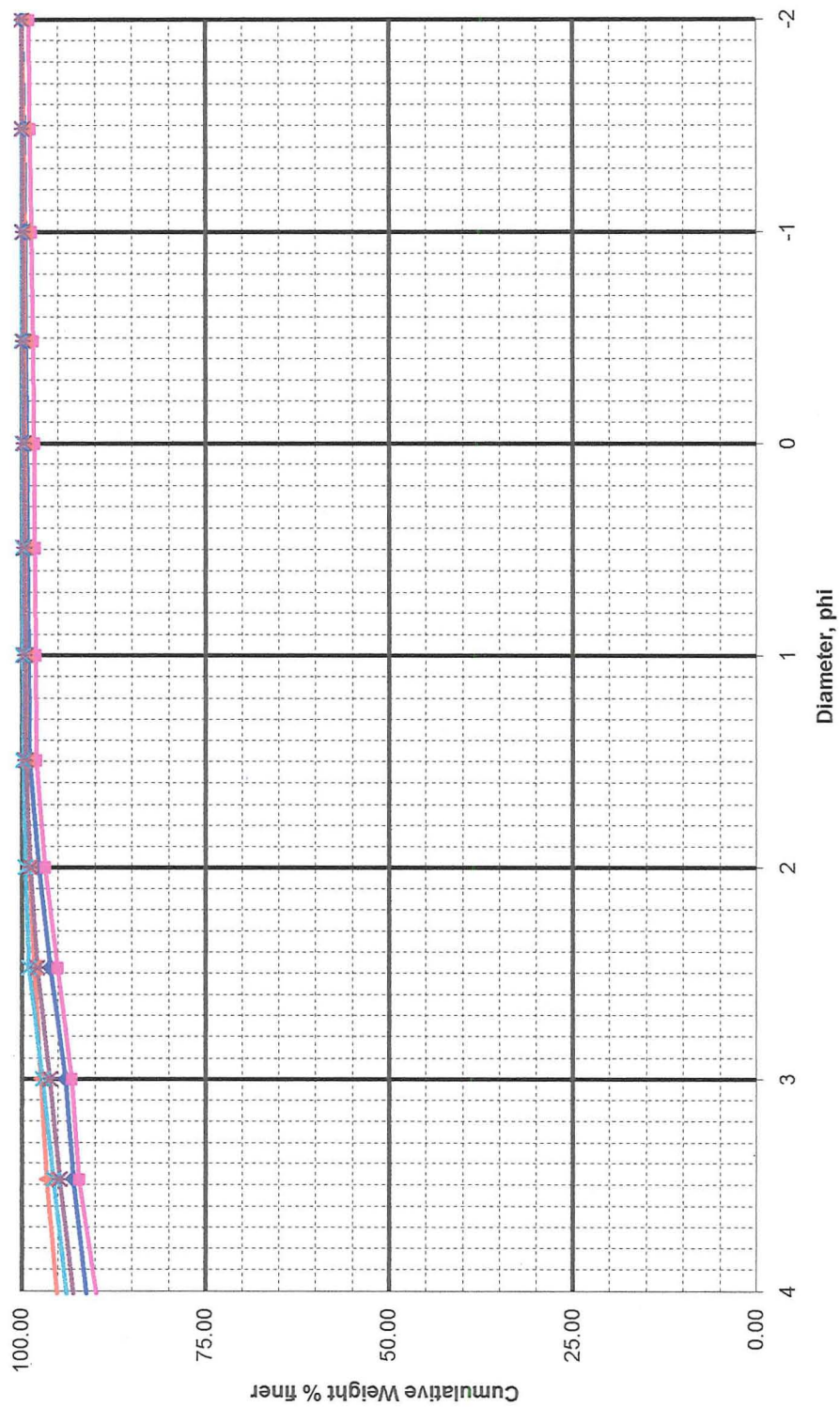


Figure 4: Cumulative frequency curve for the sand fraction (> 0.063 mm diameter) Blackness sediments. Each line is a single replicate. Blackness sediments were mostly silt/clay; therefore no further analysis of particle size distribution was possible for the sand fraction.

Limekilns

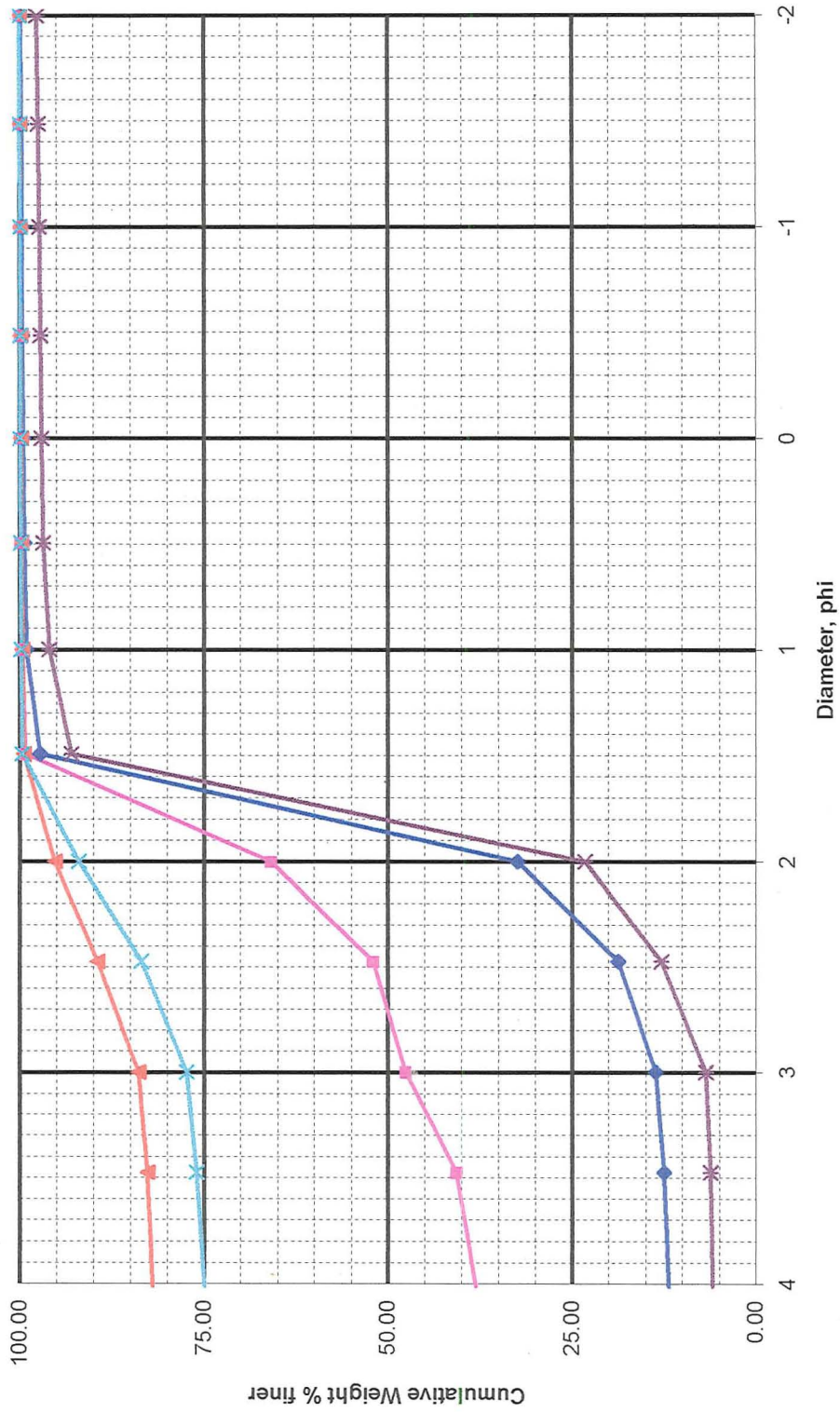


Figure 5: Cumulative frequency curve for the sand fraction (> 0.063 mm diameter) Limekilns sediments. Each line is a single replicate. Two of Limekilns sediments were mostly silt/clay, therefore no further analysis of particle size distribution was possible for these sand fractions. The curves for the remaining three were used to estimate phi- values for median particle size estimation.

Gosford Bay

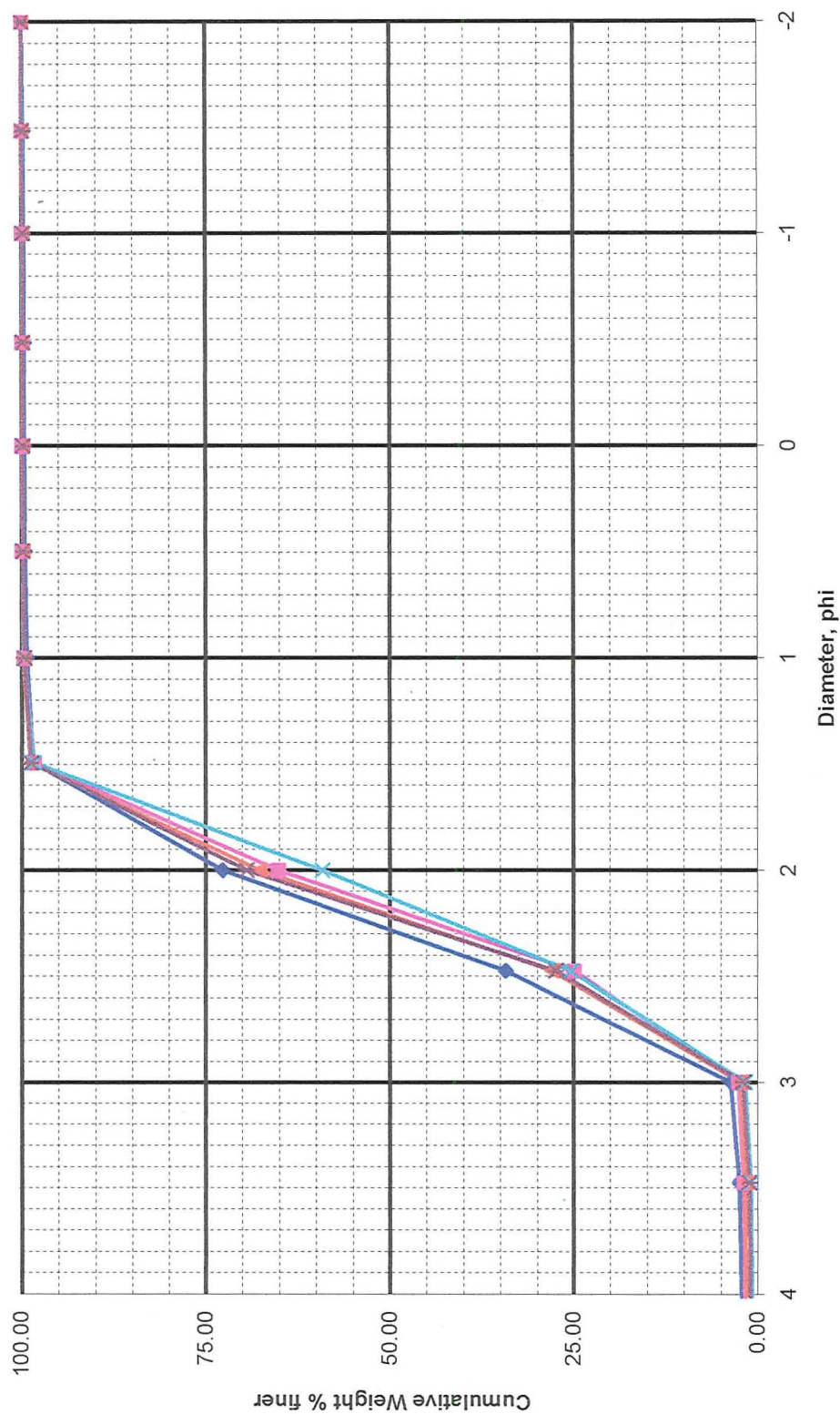


Figure 6: Cumulative frequency curve for the sand fraction (> 0.063 mm diameter) of Gosford Bay sediments. Each line is a single replicate. The curves for the sand fraction were used to estimate phi- values for median particle size estimation and particle dispersion.

Silver Sands

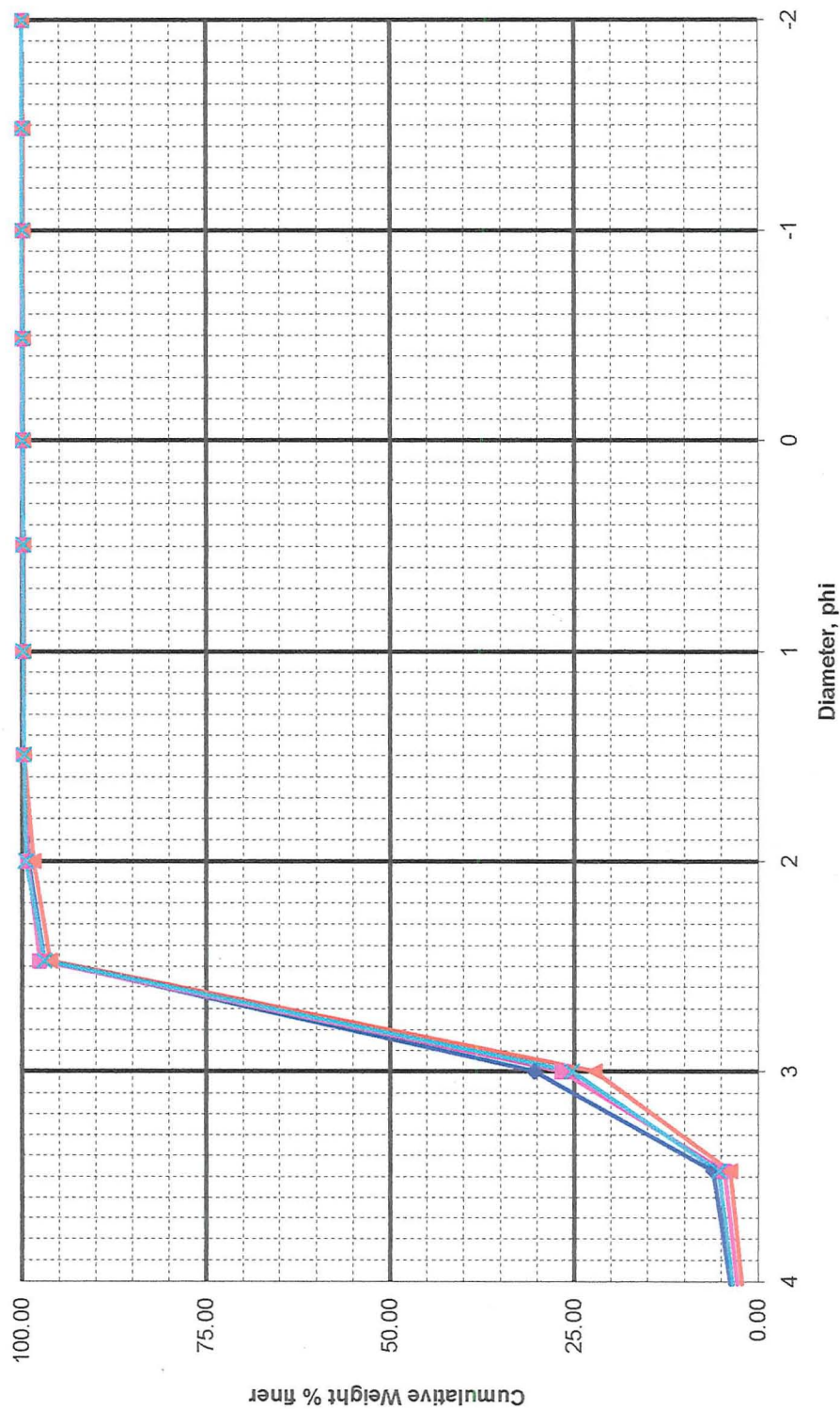


Figure 7: Cumulative frequency curve for the sand fraction (> 0.063 mm diameter) of Silver Sands sediments. Each line is a single replicate (only 4 samples were available from Silver Sands). The curves for the sand fraction were used to estimate phi-values for median particle size estimation and particle dispersion.

Table 2: Sieve mesh sizes and corresponding log transformed phi (Φ) values for sediment particle analysis.

Sieve Mesh Size (mm)	phi Φ
4.000	-2.00
2.800	-1.49
2.000	-1.00
1.400	-0.49
1.000	0.00
0.710	0.49
0.500	1.00
0.355	1.49
0.250	2.00
0.180	2.47
0.125	3.00
0.090	3.47
0.062	4.01

Table 3: Sediment grain size parameters extracted visually from cumulative frequency curves (Figures 4 – 7). ΦX is the diameter in phi notation of the Xth percentile. These values were used in equation 2 to estimate σ_1 , which is inclusive graphic standard deviation. Wentworth descriptions taken from Bale & Kenny (2005). *na* means the sediment had a high percentage of silt/clay, precluding calculation of σ_1 .

Site	Sample	$\phi 5$	$\phi 16$	$\phi 84$	$\phi 95$	σ_1	Md ($\phi 50$)	Wentworth Description	Organic Content (%)
Blackness	1	na	na	na	na	na	< 4.00	silt/clay	10.30
	2	na	na	na	na	na	< 4.00	silt/clay	9.35
	3	na	na	na	na	na	< 4.00	silt/clay	9.92
	4	na	na	na	na	na	< 4.00	silt/clay	10.45
	5	na	na	na	na	na	< 4.00	silt/clay	10.62
Limekilns	1	na	na	na	na	na	< 4.00	silt/clay	3.01
	2	na	na	na	na	na	< 4.00	silt/clay	8.38
	3	na	na	na	na	na	2.70	fine sand	10.42
	4	na	na	na	na	na	1.90	medium sand	8.87
	5	na	na	na	na	na	1.80	medium sand	2.05
Silver Sands	1	3.80	3.30	2.55	2.50	0.384	2.75	fine sand	1.21
	2	3.50	3.25	2.60	2.50	0.314	2.75	fine sand	1.27
	3	3.45	3.25	2.55	2.50	0.319	2.70	fine sand	1.25
	4	3.50	3.25	2.55	2.50	0.327	2.70	fine sand	1.36
Gosford Bay	1	3.00	2.80	1.75	1.55	0.482	2.30	fine sand	1.02
	2	2.95	2.75	1.70	1.55	0.475	2.20	fine sand	1.02
	3	2.95	2.70	1.70	1.55	0.462	2.20	fine sand	0.88
	4	2.90	2.70	1.70	1.55	0.455	2.10	fine sand	0.94
	5	2.95	2.70	1.75	1.55	0.450	2.20	fine sand	0.82

The sediment analyses shown in Figures 4 – 7 and Tables 2 and 3 indicate that Gosford Bay and Silver Sands are predominantly homogenous fine sand with low organic content, while Blackness is a typical muddy estuarine site with relatively high organic content and predominantly silty sediments. Limekilns, however, has highly variable, over the small scale measured here, sediment types, ranging from medium sand to typically estuarine muddy sediments. However, the organic content of Limekilns sediments is relatively high and comparable with Blackness. Thus, these sites represent, in terms of basic sediment properties, two habitat types: 1) Relatively high energy sandy marine sites (Silver Sands and Gosford Bay) and 2) Low energy muddy estuarine sites (Blackness and Limekilns). The first of these two site types has been considered typical plaice nurseries (Rae, 1970; Poxton & Nasir, 1985), providing a baseline for comparison with the estuarine muddy sites, habitat type 2 in the current study.

Chapter 2

Efficiency and Selectivity of a 1.5m Riley Push Net

2.1. Introduction

Gears most commonly used for sampling in fish and fisheries research often consist of some type of net or, more recently, enclosure gears. Nets can be either passive (stationary), such as fyke nets, koms, fyke-koms, and v-traps (e.g. van der Veer *et al.*, 1992; Rozas & Minello, 1997; Butcher *et al.*, 2005) or active (mobile), such as beam trawls, otter trawls, beach seines, purse seines and pelagic trawls (e.g. Kuipers, 1975; Kuipers *et al.*, 1992). Enclosure gears enclose a known area, such as drop traps, pop nets, stake nets, suction samplers and lift nets (e.g. Vance *et al.*, 1996; Orth & van Montfrans, 1987; Rozas & Minello, 1997; Huxham *et al.*, 2004). Each of these gear types has a specific application, dependant on many factors, e.g. the target species (i.e. whether pelagic, demersal or benthic), the habitat being surveyed (e.g. sediment type: gravel or mud, mangals, intertidal etc), the purpose of the capture attempt (i.e. whether a quantitative assessment of abundance is required or if the purpose is to obtain sufficient individuals for another type of measurement or tissue sample such as condition indices, stomach contents or physiological measurements (Miller *et al.*, 1992; Rozas & Minello, 1997). Whatever the type of gear deployed, some consideration must be given to the efficiency and selectivity of the gear if quantitative measurements of organism abundance and/or size are required (Kuipers 1975, Kuipers *et al.*, 1992; Rozas & Minello, 1997; Borg *et al.*, 2002). Efficiency can be defined as the percentage of the true density caught by any given gear. Selectivity is defined as a size-dependent efficiencies such that, for example, smaller fish are caught with a higher efficiency than larger fish. It must

be expected that fish, and indeed many other motile aquatic organisms, by their behaviour, life habits and evolution, will attempt to evade capture by these gears, whether mobile or stationary. The physical structure of the habitat occupied by the target(s) of the gear will also influence gear efficiency due to interference with the gears trapping mechanism, or by providing refugia from the gear. Thus, to make quantitative measurements of the true abundance of fishes, reduced or enhanced efficiencies and any size-dependant selectivity, caused by the target organisms' behaviour and life history, must be determined.

Push nets have been used for both commercial, subsistence and research purposes for many years, possibly as far back as the 16th Century. They are especially useful for catching fish and crustaceans in very shallow water areas that are not easily accessible by boat (Riley, 1971). The focus of the present study is the nursery function of intertidal habitats for juvenile European plaice (*Pleuronectes platessa*). The juvenile (especially the 0+ group) stages of this species are found in very shallow intertidal coastal and estuarine waters, making the push net particularly useful for capturing these stages of plaice.

Measurements required by this particular study include determinations of growth rates and densities on the intertidal habitats. Growth rates can be most easily estimated by recording the change in some measure of the central tendency of fish lengths, i.e. mean or median length, and densities can be easily estimated by recording the number of individuals caught in a known area. As stated previously, accurate measurements of these factors (densities and mean/median lengths)

rely upon determining the efficiencies and selectivity of the gear being deployed. For example, if the push net captures smaller individuals with a greater efficiency than larger individuals (i.e. larger individuals escape more readily than smaller individuals), measuring growth rates by recording the change in mean or median length over time, will tend to underestimate growth rates. Therefore, it is important to determine the efficiency and selectivity of the push net, before any firm conclusions or inferences about the population structure and dynamics on the sampled habitats can be drawn. Kuipers (1975) assessed the efficiency of a 2 m beam trawl used to capture plaice in the Balgzand in the Dutch Wadden Sea, and listed several possible means of gear evasion by plaice. These are: 1) escape underneath the beam trawl as it sweeps over the area occupied by that individual, 2) lateral escape whereby the fish escapes to either side of the net, 3) escape over the top of the net and 4) herding of fish by the net. These particular features of beam trawl gears may also affect push nets, with the exception of escape over the top of the net. The net is used in relatively shallow water and is only occasionally completely submerged, and when it is, it is not expected that plaice can or choose to escape over the top of the net (Kuipers, 1975). The same author also suggested another means by which a beam trawl may not capture fish with 100% efficiency: disturbance caused by the rope or ropes used to tow the trawl may cause fish to swim outside the area to be swept by the gear, thus reducing the numbers caught. Disturbance caused by tow ropes does not affect the push net used in the current study as the net is pushed from behind, and nothing disturbs the water column or sediment before the tickler chains attached

to the net, sweep the sediment in front of them. Wennhage *et al.* (1997) estimated the efficiency of 2 m and 1.5 m beam trawls by comparing densities of fish caught with the trawls, to densities of fish caught with a drop trap developed for intertidal areas, with efficiency close to 100% (Pihl & Rosenberg, 1982; Rozas & Odum, 1987). In comparison to Kuipers (1975), Wennhage *et al.* (1997) estimated the efficiency of the beam trawls as approximately 10 %, compared to the 33% commonly used for this gear type. Thus, the efficiency of putatively identical gear types may vary according to small differences in operating parameters, e.g. speed of tow/push, operator performance and conditions, such as substrate. Accordingly, for each investigation, it is preferable to estimate efficiencies for the gear in question (Wennhage *et al.*, 1997).

The objective of the current study is to assess the efficiency and length selectivity of a 1.5 m Riley push net, when used to assess densities and lengths of juvenile plaice. The investigation requires sampling by the push net in two different habitat types, broadly categorised as muddy and sandy. The efficiency of the net in these two habitat types may differ, thus a hypothesis is proposed for testing in the two habitat types. The hypothesis can be stated: a comparison of estimates of juvenile plaice lengths and densities between the 1.5 m push net and the drop trap will show that the drop trap estimates higher densities and different total lengths of intertidal phase 0+ plaice. The efficiency and selectivity of the net may depend on the particle size distribution of the sediment on which the net is used, therefore, efficiencies calculated for two habitats with different

sediment particle size distributions could differ. The statistical null hypothesis to be tested is: H_0 : There is no difference in the average total lengths and densities of plaice caught using a drop trap and a 1.5 m push net.

2.2. Methods and Materials

2.2.1. Site Description

Three sites were chosen for calibration of the 1.5m push net: Blackness in the Forth Estuary, and Silver Sands and Portobello, in the Outer Firth. However, at Portobello, rough waves caused a hole to appear in the sand at the base of the side of the trap that was facing the incoming waves. Thus, the trap is only suitable for use on sheltered shores or in relatively calm water, and only Blackness and Silver Sands had suitable conditions. The locations of Blackness and Silver Sands are shown in Figure 8. For a general description of the Forth Estuary and Outer Firth, see *1.6. Methods and Materials*.

Blackness is a sheltered intertidal mudflat in the lower estuary with predominantly silty sediments, i.e. median particle size is $<63\mu\text{m}$. Silver Sands is a sheltered sandy bay on the north shore of the Outer Firth, comprising fine sandy sediments with a median particle size of approximately 0.2 mm.

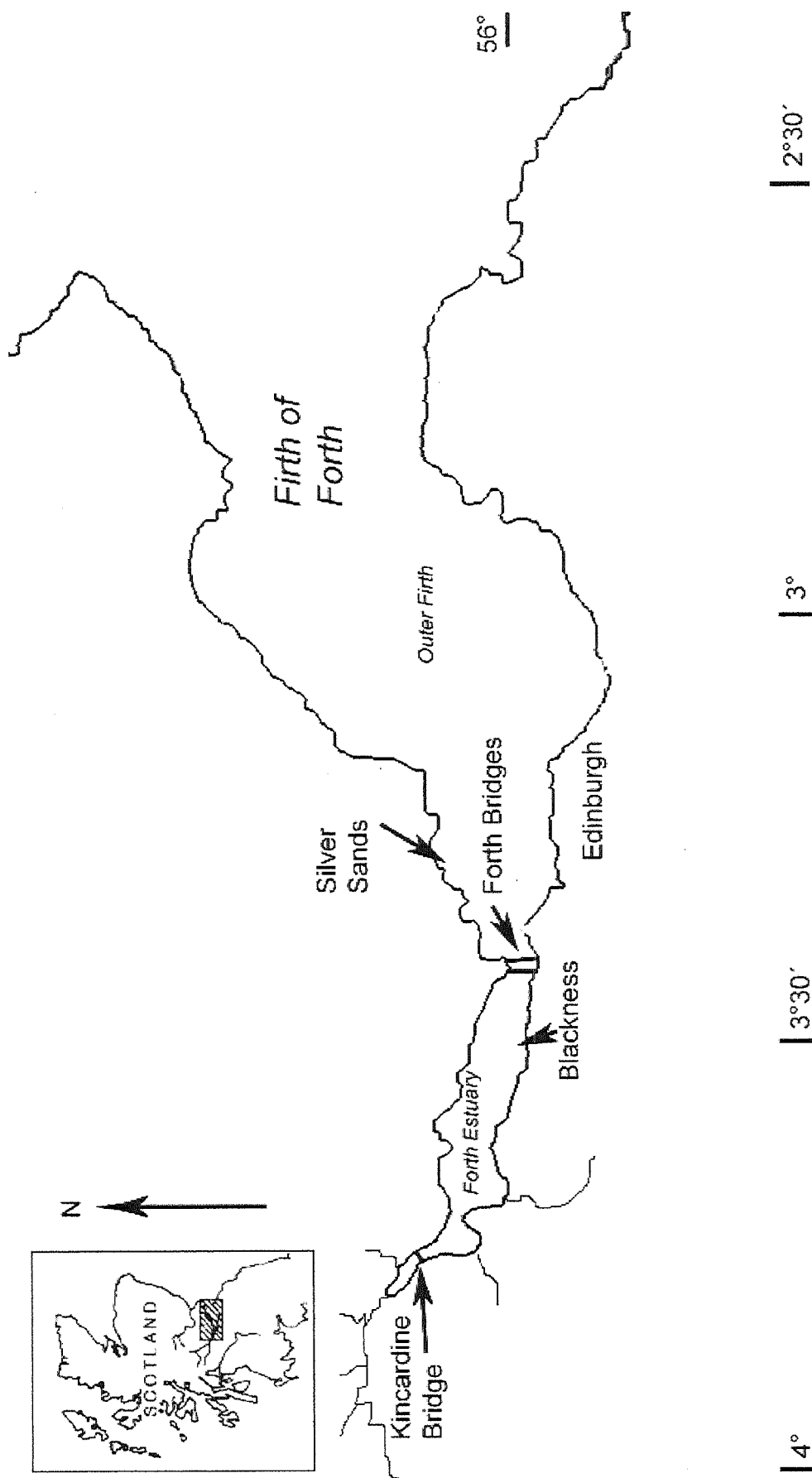


Figure 8: Map of the Firth of Forth. All work in this study was carried out at Silver Sands and Blackness

2.2.2. Calibration

In order to calibrate the push net, densities and lengths of juvenile plaice caught with the net were compared with the densities and lengths of juvenile plaice caught with a drop trap and a lift net. The lift net was used on two occasions at Blackness, once in June and once in July 2006. The drop trap was used on four occasions: Blackness in May 2005 and June and July 2006 and at Silver Sands in June 2006. Silver Sands was sampled only once as this was the only day with suitable conditions in the period selected for the attempt. The three gear types are shown in Figures 9, 10 and 11. An attempt was made to use a stake net as another non-selective, 100% efficient method, identical to that of Huxham *et al.* (2004). However, after one attempt with two replicate stake nets, sediment disturbance was too great and it was thought that the densities and lengths of plaice recorded by the stake net would not be accurate, as the sediment disturbance may affect the fishes' behaviour (Kuipers, 1975). The stake net was therefore not used to calibrate the push net.

The drop trap consists of a 1 m², square aluminium tube, similar to that deployed by Wennhage *et al.* (1997). This was deployed by placing the trap on the sediment, and fishing in the water inside the trap with a 1 mm² mesh hand net (Figure 9). The frame of the hand net was scraped three times rapidly through the surface sediment to disturb any trapped fish, causing them to swim into the water column, where they were captured by the net. The hand net procedure was carried out simultaneously by two operators with one hand net

each. The lift net is a 3 m x 3 m net with a mesh size of 5 mm. The net is attached to four 1.5 m wooden beams; two beams on opposing sides of the net (Figure 11). The lift net is laid out on the intertidal at least one tidal cycle before use, and the net and beams are pushed into the sediment to prevent the gear floating into the water column and being lost. Deploying the net disturbs the sediment slightly and allowing at least one tidal cycle before use allows some sediment to be redistributed over the net by wave action (Figure 11). When the lift net is submerged under the required depth of water on the flood tide, four operators simultaneously lift the net out of the water column and remove any fish that are caught on the mesh. This method requires a very careful approach: operators must approach the location slowly to avoid creating disturbance that causes fish to escape the area. Minimising disturbance is aided by attaching a rope and float to the beams, this allows the operators to approach the ends of the net perpendicular to the beams.

Table 4: Gear deployment schedule

Gear	Site	Date Deployed	Number of replicates
Drop trap	Blackness	May 2005	14
		June 2006	5
		July 2006	5
	Silver Sands	June 2006	10
Push Net	Blackness	May 2005	4
		June 2006	3
		July 2006	1
	Silver Sands	June 2006	4
Lift Nets	Blackness	June 2006	2

The gears were deployed simultaneously on each occasion: at Blackness in June 2006, the drop trap, lift nets and the push net were deployed along the shore at approximately the same depth (<1 m) and along a transect parallel to the shore (Table 4). This ensured that the area sampled was fished by only one gear type to minimise disturbance and allow an accurate measurement of ambient plaice density to be made. At Silver Sands, only the push net and drop trap could be used as this beach is more exposed than Blackness, precluding the use of the lift nets. The beach also has a high number of recreational visitors during the plaice nursery phase; therefore any equipment left unattended is likely to attract the attention of beach visitors.

2.3. Statistical analyses

Dispersion of plaice on each site was estimated by using the analysis presented by Fowler *et al.* (2004), on densities measured using the drop trap. An index of dispersion (D) is calculated using the following equation:

$$D = \frac{s^2}{\bar{x}} \quad (3)$$

Where, s^2 is the sample variance, and $\bar{x}$ is the sample mean. The index is then multiplied by the degrees of freedom to give chi-square statistic; this product is then compared to a scale given by Fowler *et al.* (2004) to determine whether the plaice dispersion is clumped, random or regular. This analysis would allow the investigation of underlying spatial variabilities obtained at each sampling occasion. For example, a highly clumped dispersion on the intertidal may mean that a larger sample size is required for the drop trap to accurately estimate lengths and densities of plaice.

Kolmogorov – Smirnov tests were used to test whether total lengths estimated by each gear type were different. To compare densities estimated by each gear type at Blackness, ANOVA was employed, using $\log_e((\log_e(x + 1)) + 1)$ – transformed densities, with gear as a fixed factor and time as a random factor. Interactions were compared using pairwise t – tests. At Silver Sands, densities estimated by drop trap and push net were compared using a two – sample t – test.



Figure 9: Drop trap in use at Blackness



Figure 10: Push net at Silver Sands



Figure 11: Lift net deployed at Blackness.

2.3. Results

2.3.1. Plaice Densities and Total Lengths

Densities of plaice caught using each gear type are shown in Figures 12 – 15. Efficiencies of each gear type are expressed as a percentage relative to the drop trap, which is assumed to be 100% efficient. On all occasions, the push net was less than 100% efficient with the exception of Blackness June 2006, where the net was greater than 100% efficient. At Blackness in June 2006, the lift net was also deployed and caught a higher density of plaice than both of the other gear types. The reasons for both nets' apparent high efficiencies, on this occasion, relative to the drop trap are discussed in 2.4. *Discussion*.

Densities estimated at Blackness using drop trap and push net only were not significantly different (Table 5). However, there was a significant interaction between gear and time, suggesting a difference in the efficiency of the net between times. This is due to a lower density recorded by the trap compared to the push net, a reversal of the expected trend. Pairwise *t* – tests indicate the net and trap estimates were significantly different in May 2005 ($t = 5.95$, $df = 14$, $p < 0.001$), but were not significantly different in June 2006 ($t = -1.31$, $df = 8$, $p = 0.225$). There were insufficient push net samples in July 2006 had for a pairwise comparison. Silver Sands, density estimates did not differ significantly between gear types ($t = -1.64$, $df = 9$, $p > 0.05$).

Length – frequencies are shown in Figures 16, 17 and 18. As all data could not be normalised, length – frequency data were tested for differences between gears at each site on each sampling occasion using the Kolmogorov-Smirnov non-parametric test to compare 2 independent samples. The trap length data from Blackness June 2006 and July 2006 were excluded from statistical testing as there were too few fish caught to satisfy the assumptions of the non – parametric test (Zar, 1999). The total lengths of plaice were not significantly different between the drop trap and the push net at Silver Sands ($Z = 1.05$, $N = 170$, $p > 0.05$) and at Blackness in May 2005 ($Z = 1.17$, $N = 70$, $p > 0.05$). However, the gear type with the highest median length was different at different sites; at Silver Sands, the drop trap recorded a higher median total length than the push net: at Blackness the push net recorded a higher median total length than the drop trap. Total lengths were not significantly different between the lift net and the push net deployed at Blackness in June 2006 ($Z = 0.76$, $N = 76$, $p > 0.05$). Thus, there is no evidence to suggest that total lengths estimated by the three different gear types are different; the net is thus non – size selective over the size – range of plaice caught for the current study.

Table 5: ANOVA of density estimates using drop trap and push net at Blackness in May 2005 and June and July 2006. Gear type is a fixed factor, time is a random factor. * significant at $\alpha = 0.05$

Source	DF	Adj SS	F	P
Time	2	0.38477	0.58	0.634
Gear	1	0.22446	0.78	0.462
Time*Gear	2	0.66508	3.89	0.031*
Error	31	2.64883		
Total	36			

Blackness May 2005

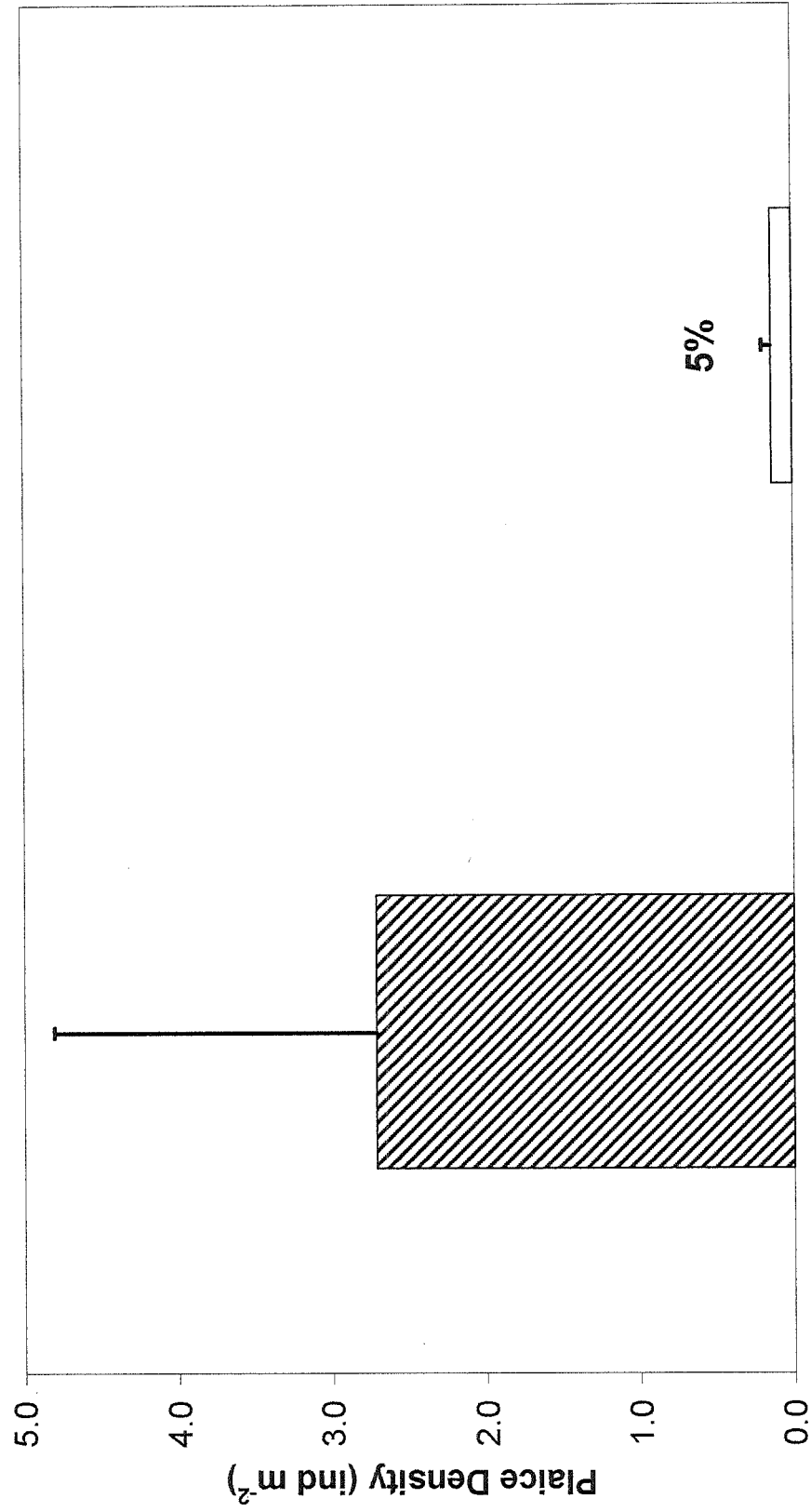


Figure 12: Densities of plaice caught at Blackness in May 2005. Striped bar is mean drop trap density ($n = 14$), clear bar is mean push net density ($n = 4$). Number above the bar is % efficiency relative to the drop trap. Error bars are $\pm$ S.E.

Blackness June 2006

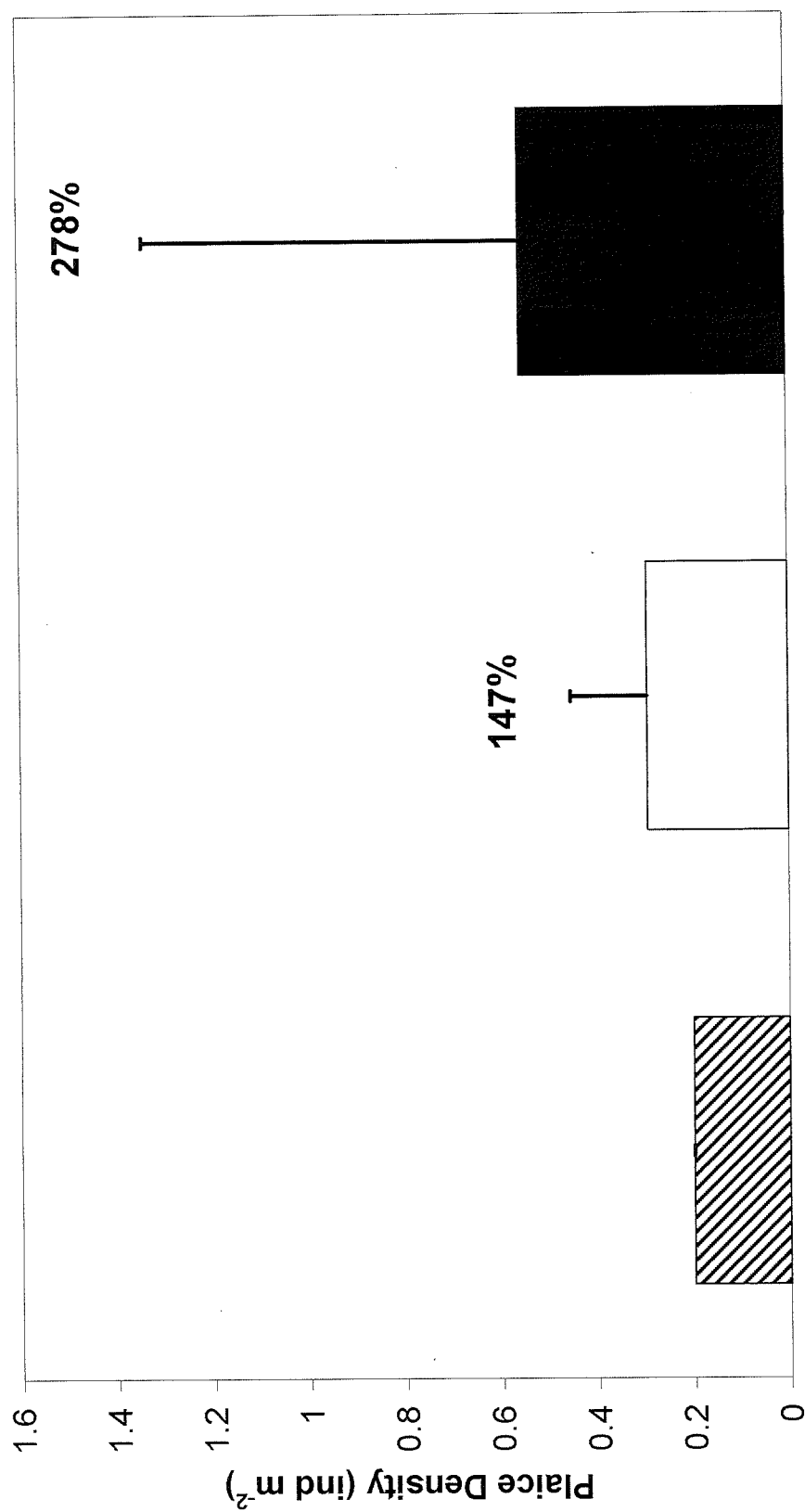


Figure 13: Densities of plaice caught at Blackness in June 2006. Striped bar is mean drop trap density ($n = 5$), clear bar is mean push net density ($n = 3$) and solid bar is lift net density ($n = 2$). Number above the bar is % efficiency relative to the drop trap. Error bars are $\pm S.E.$

Blackness July 2006

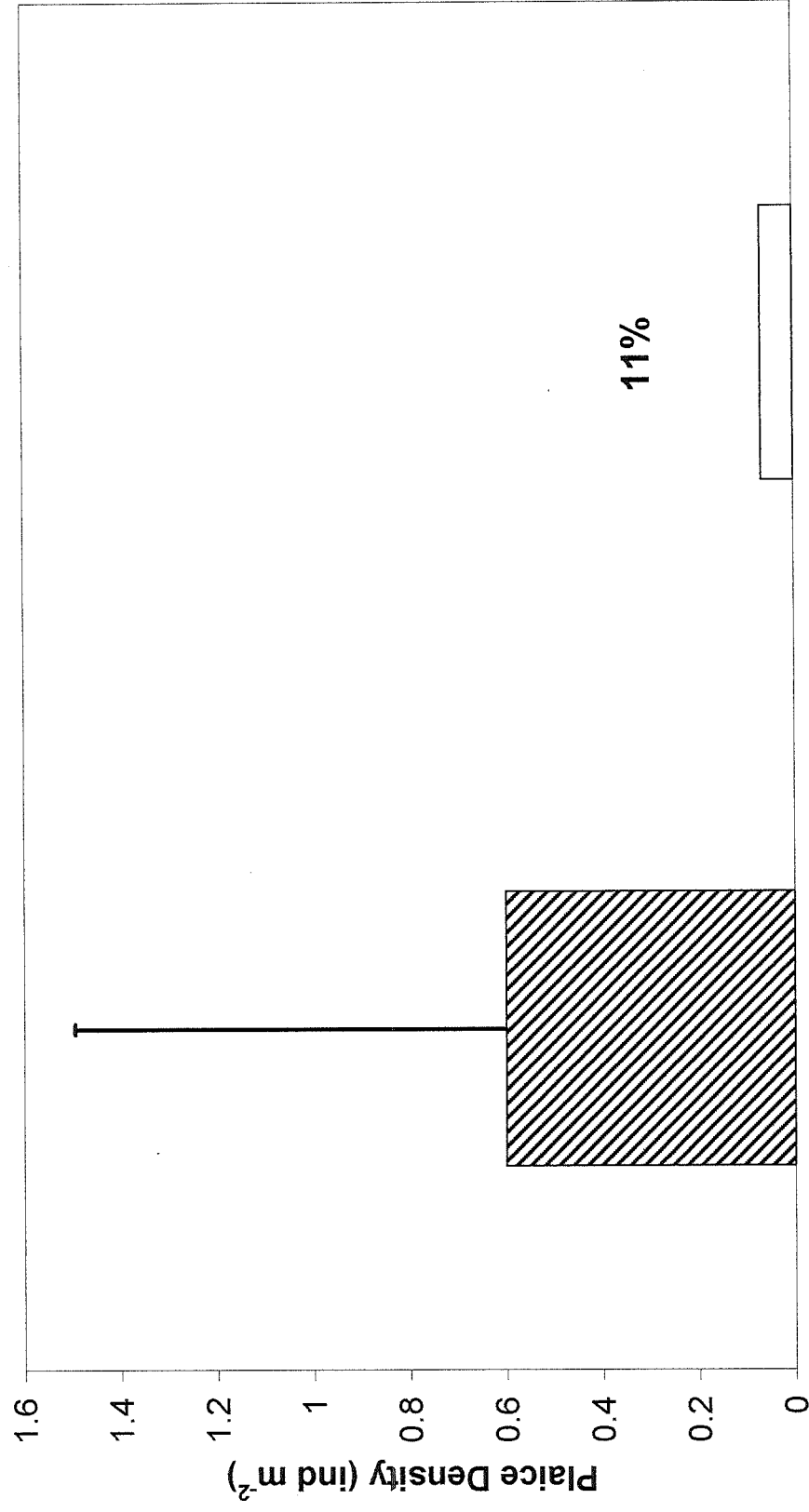


Figure 14: Densities of plaice caught at Blackness in July 2006. Striped bar is mean drop trap density ($n = 5$), clear bar is mean push net density ($n = 1$). Number above the bar is % efficiency relative to the drop trap. Error bars are $\pm$ S.E.

Silver Sands June 2006

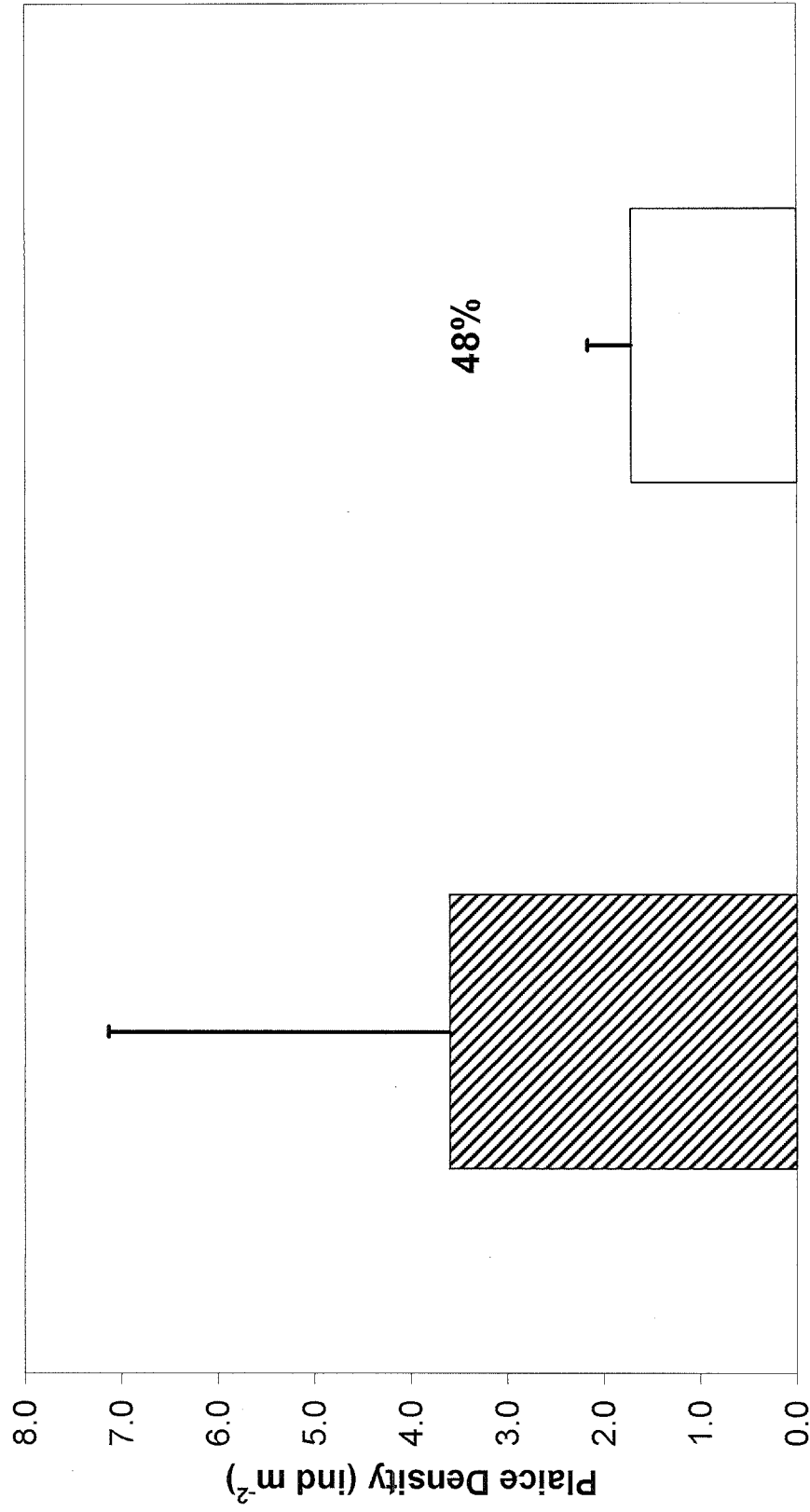


Figure 15: Densities of plaice caught at Silver Sands in June 2006. Striped bar is mean drop trap density ($n = 10$), clear bar is mean push net density ($n = 4$). Number above the bar is % efficiency relative to the drop trap. Error bars are $\pm$ S.E.

Silver Sands June 2006

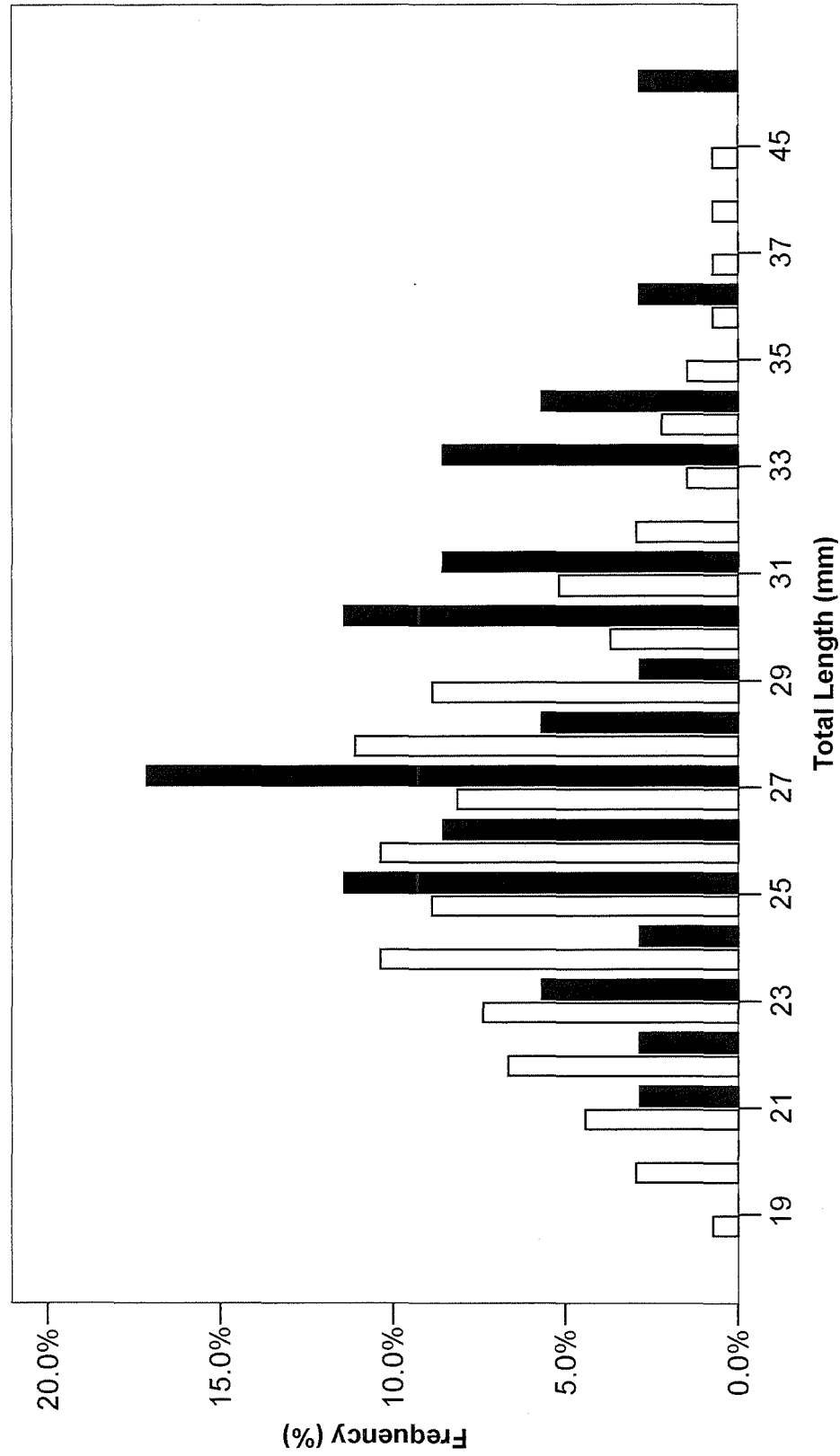


Figure 16: Length – frequencies of plaice caught at Silver Sands in June 2006. Clear bars are push net total lengths (mm; $n = 135$), solid bars are drop trap total lengths (mm; $n = 35$).

Blackness May 2005

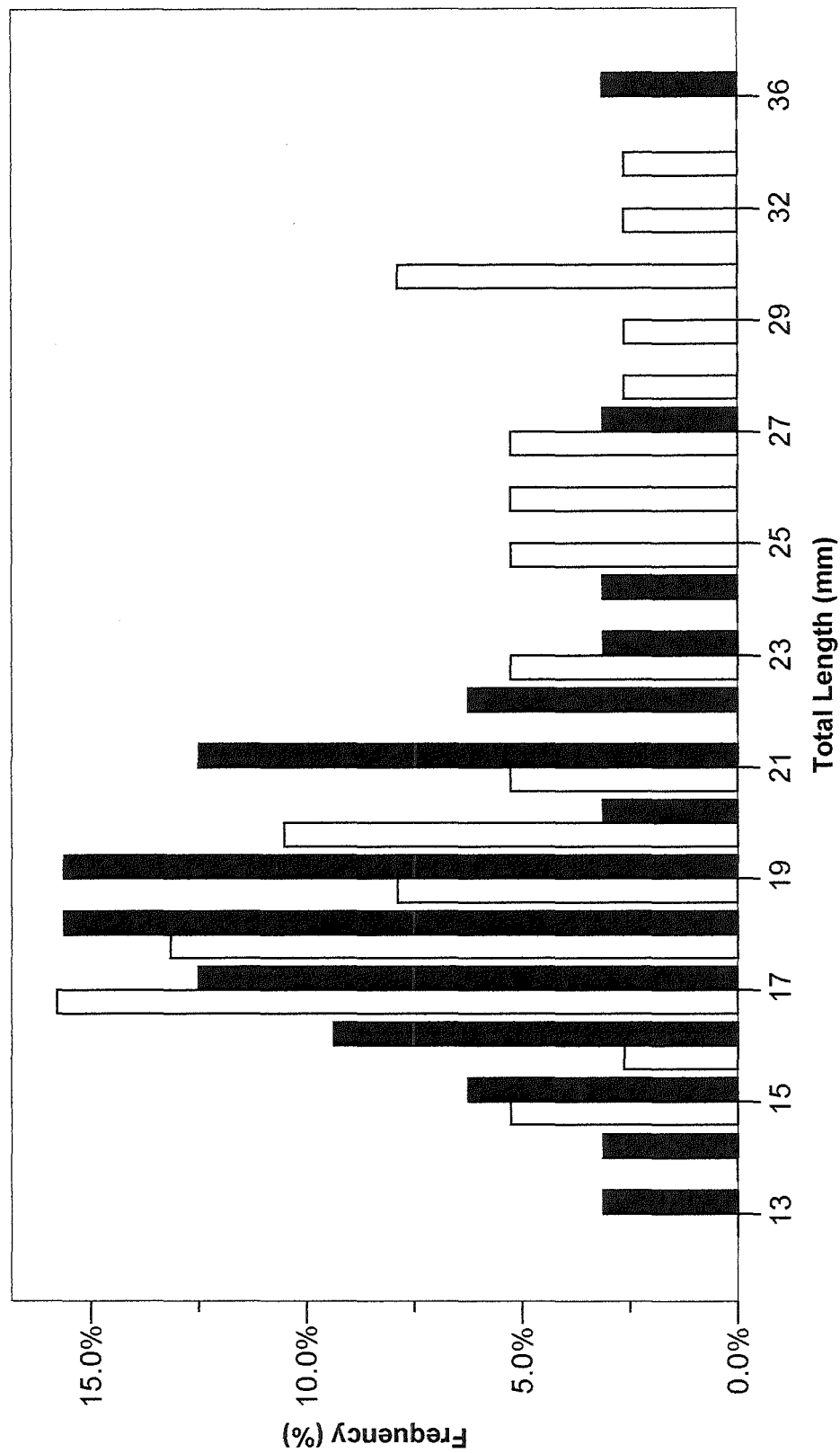


Figure 17: Length – frequencies of plaice caught at Blackness in May 2005. Clear bars are push net total lengths (mm; $n = 38$), solid bars are drop trap total lengths (mm; $n = 32$).

Blackness June 2006

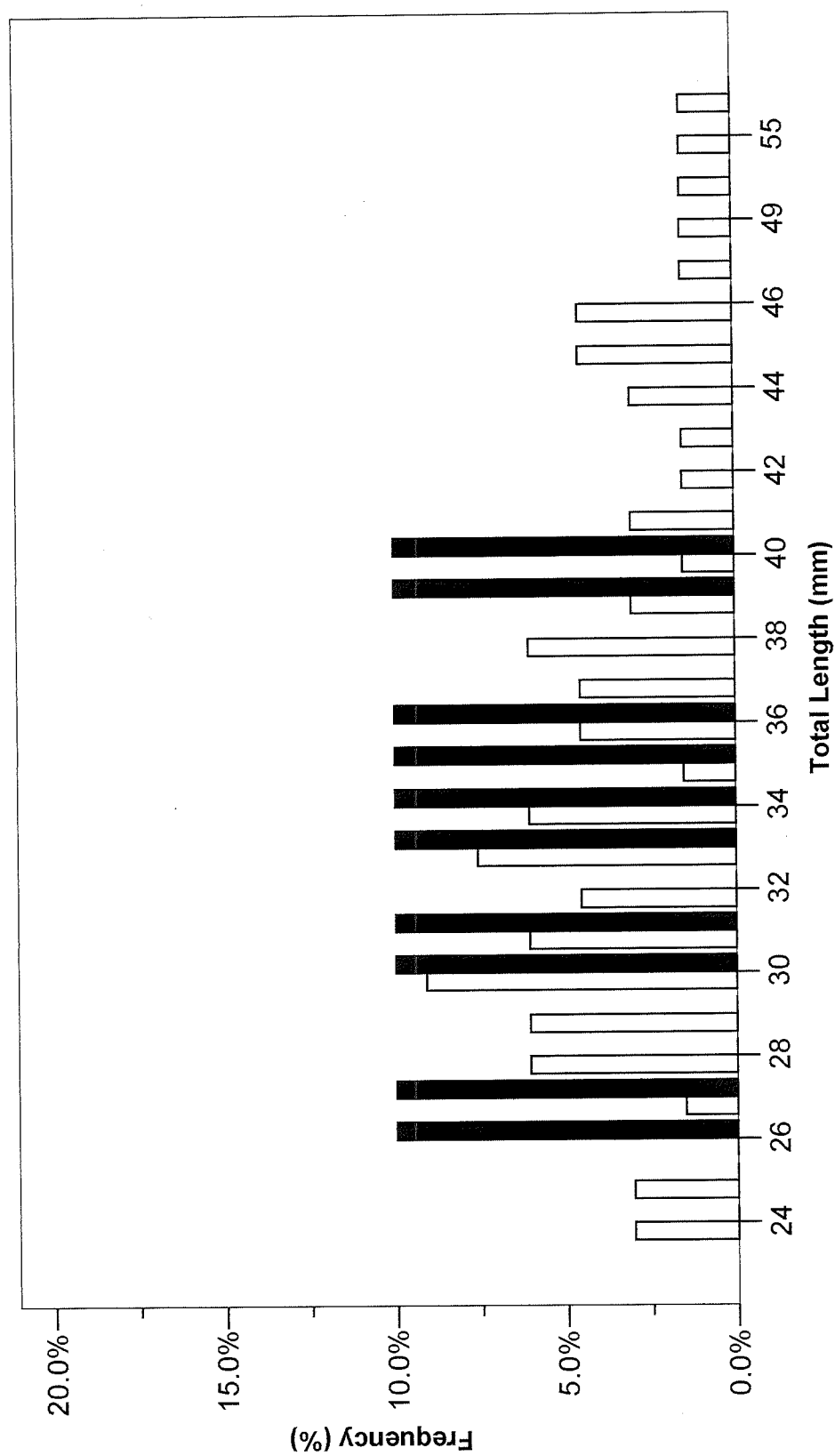


Figure 18: Length – frequencies of plaice caught at Blackness in June 2006. Clear bars are push net total lengths (mm; $n = 66$), solid bars are lift net total lengths (mm; $n = 10$).

2.3.2. Dispersion Analysis

Estimates of plaice dispersion measured using drop trap densities on each occasion, shown in Table 6; indicate that early samples showed a more clumped dispersion than the samples later in the season, at Blackness at least. As these samples were taken using the drop trap, this indicates small scale clumping (on the order of 1 m²). As samples were taken on an incoming tide this clumping may indicate that, as well as concentrating at the waterline, the youngest plaice may also exhibit schooling behaviour.

Table 6: Analysis of plaice dispersion during calibration trials. $\bar{x}$ is sample mean, s^2 is sample variance, v is degrees of freedom (number of samples – 1), D is dispersion coefficient, *Dispersion* is a descriptive from Fowler *et al.* (2004).

Sample (Site & Date)	$\bar{x}$	s^2	v	D	X^2	Dispersion
Blackness May 2005	2.710	4.374	13	1.611	20.947	Clumped
Blackness July 2006	0.600	0.800	4	0.750	3.000	Random
Silver Sands June 2006	3.600	12.489	9	3.469	31.222	Clumped

2.4. Discussion

Despite the differences in area sampled by each gear type, no evidence for size – selectivity for the target plaice was found. Thus, the 1.5 m push net can be used to give relatively unbiased estimates of plaice mean total lengths, and therefore, unbiased estimates of growth rates of plaice in the shallow intertidal habitats that are the focus of the present study. However, this result is only applicable to the size classes of plaice measured here (up to 55 mm total length). Other workers have shown that the efficiency of beam trawls is lower for larger fish (Kuipers, 1975); therefore it is possible that the push net becomes size – selective for individuals larger than 55 mm. However, there was only a single individual larger than 55 mm TL caught during both years, therefore this is not a consideration or even testable using data collected for the current study. The mesh size of the push net used in this study was 7 mm across the widest point at stretch. This mesh would allow smaller animals through (i.e. is size selective), however, plaice that are small enough to escape through this mesh are unlikely to have fully metamorphosed (Modin *et al.*, 1996), and, as such, are not in the age group the current study is focused upon.

Efficiency, defined by Rozas & Minello (1997) is the result of an inability of the gear to capture all of the animals in the sampled area (capture efficiency), combined with the ability to recover animals from the gear after they have been captured (recovery efficiency). Despite there being no differences in efficiency between the muddy and sandy habitats in the present study it was expected that the net would be less efficient in the muddy habitat due to differences in recovery

and capture efficiency. In the muddy habitats, the push net was often clogged with very fine silt which had to be removed, by washing through with seawater, and sifted through manually before fish could be recovered from the gear. This was not usually the case in the sandy habitats, although the net sometimes became clogged with vegetation that may have affected the nets efficiency. It is entirely feasible, due the ability of plaice to change their pigmentation to match their habitats colour (Heal  y, 1999), that more plaice were missed during the recovery phase of sampling in the muddy habitats, because of the greater effort required to find them amongst the silt. Recovery effort was greater in the estuarine habitats as the net, which is coloured differently from the fish, became covered in a fine coating of silt in the muddy habitat, reducing the contrast between the fish and the net which makes them easier to see against the net in coarser sediment that washes through the net. This could contribute to the reduced densities of the push net compared with simultaneous drop traps during two of the sample dates in the estuarine habitats.

Again, although the push net was not found to be less efficient than the drop trap in the muddy habitats, the net was sometimes observed to create a 'bow – wave' of silt, immediately in front of the tickler chains when pushed out of the water. If this wave of silt was present when the net was operated submerged, this moving sediment may reduce the nets efficiency in muddy habitats. Firstly, by allowing the fish more time to evade the net, as they may have been disturbed earlier than in sandy habitats. Alternatively, suggested by Gibson & Robb (2000), plaice tend to bury themselves in the sediment as a predation avoidance response,

thus, the buried fish could be pushed away from the mouth of the push net, by remaining buried in the moving 'wave' of sediment, thereby avoiding capture.

Efficiency also appears to be variable in the muddy habitat; with mean push net density ranging from 5% up to 17% of mean densities estimated using drop traps, which could be a serious problem when assessing population densities in these habitat types (Rozas & Minello, 1997). Butcher *et al.* (2005) suggested that multiple gear types be used to assess density distributions of target species. The current study has shown that the 1.5 m push net is suitable for estimates of lengths of small plaice in the muddy habitats, but density estimates are likely to produce high variability in these muddy areas, which may either obscure statistical differences, or increase the minimum number of samples to a logistically unfeasible number (Miller *et al.*, 1992). Thus, the drop trap, or passive gears such as fyke nets are more suitable for density estimates in the estuarine sites (e.g. van der Veer *et al.*, 1992; Rozas & Minello, 1997; Butcher *et al.*, 2005). However, the time it takes to obtain the drop trap samples would mean that the tidal state has changed by the time enough samples had been collected, resulting in a possible change in juvenile distribution and, hence, density.

The densities measured during the calibration trials did not show any clear, statistical differences between gear types. Thus, the hypothesis stated in the introduction cannot be accepted; there is no evidence to show the push net estimates lower densities and different length distributions than the drop trap. However, as the drop trap and push net densities were highly variable, it is possible that the replication used in the current study was of insufficient statistical

power to detect any differences in gear efficiency; only a larger number of replicates could answer this question.

Patchiness of dispersion in space may also play a role in variable efficiency. Dispersion analysis indicated that at least the earliest settlers in the current study, have a contagious dispersion on the intertidal flats in the estuary and the Outer Firth, which may become less clumped later in the season. Dispersion of plaice indicated that they were clumped together in June at both sites but randomly dispersed later in the season at least at one of the sites. This may have been a result of lower densities later in the season, or due to the fact that a small number of trap samples were taken in July 2006 at Blackness, compared to the number of trap samples taken in May 2005 at Blackness and in June 2006 at Silver Sands. However, the dispersion analysis could indicate that plaice exhibit schooling behaviour when they are very small, but this behaviour changes as they grow, perhaps as a result of competitive interactions or lower predation risk to larger plaice.

The patchy dispersion indicated by the drop trap samples may have been responsible for the apparently low efficiency of the drop trap at Blackness in June 2006. The trap was used in very shallow water (<5 cm) on several samples during that sampling period. The plaice may have been concentrated in slightly deeper water than this, which, when combined with the relatively low area of habitat sampled by the drop trap, would result in the apparent increase in plaice density estimates of the push net compared to the drop trap.

Chapter 3

Growth Rates of Juvenile Plaice in the Forth Estuary and Firth of Forth

3.1. Introduction

In temperate regions photosynthesis shows distinct seasonal patterns, determined by nutrient availability and changes in day length and intensity of sunlight; this climatic predictability results in predictable patterns of reproduction and growth of phytoplankton and phytobenthos, as well as marine macroalgae. These seasonal patterns of growth are reflected in the reproductive and growth patterns of temperate primary, secondary and tertiary consumers. Timing of reproduction to coincide with maximum food availability was first postulated, for Norwegian fish species, by Hjörts' (1914) 'critical period', and later, for temperate species in general, by Cushing (1969). This hypothesis is now known as the Cushing (occasionally the Cushing – Hjört) Match-Mismatch hypothesis. Cushing (1990) extended this hypothesis to species equator – ward of 40° latitude, some of which time their reproduction to coincide with blooms occurring as a result of seasonal upwelling.

Cushing (1990) points out that in seasonal tidal waters (coasts), there is a continuous primary production cycle that peaks in June or July and lists *Oikopleura* (an appendicularian) as the main food source for larval plaice, and that plaice time their reproduction in the southern North Sea to coincide with *Oikopleura*'s production cycle. These primary consumers depend on the timing of primary production, i.e. the plankton blooms in summer and autumn. An example of larval growth affecting later stages in life, and being affected by food availability, occurs in Arcto-Norwegian cod, where strong year – classes were generated when there was a delay in the production of *Calanus*, and the greater the delay, the larger the

weight of 6 mm larvae (Cushing, 1990). Similarly, year – classes of plaice in the southern North Sea appear to vary with water temperature, with very strong year classes appearing after cold winters; this has been attributed to a mismatch between egg – stage specific predation pressure, due to the incoming cohort appearing later than the main predators (*Crangon crangon*) on the nurseries, and length of development of eggs at lower temperatures (van der Veer *et al.*, 2000b).

It has been suggested that the greatest influence on fish recruitment variability occurs during the egg, larval and immediate post – larval stages, whereas during the late post-larval and early juvenile stages, density – dependent mortality has been shown to occur, which results in a fine regulation or dampening of between – year variability in recruitment (Zijlstra *et al.*, 1982; Zijlstra & Witte, 1985; van der Veer, 1986; van der Veer & Bergman, 1987; Beverton & Iles, 1992; Leggett & Deblois, 1994; van der Veer *et al.* 2000a;). However, Nash & Geffen (2000) suggest that this may not be the case for Irish Sea plaice, where year – class strength is generated during the nursery phase. Regardless of when recruitment variability and/or variability dampening occur, the nursery or juvenile phase processes play an important role in sustaining and regulating populations, and can have effects on later ontogenetic stages of many marine fishes (Rijnsdorp *et al.*, 1992; Leggett and Deblois, 1994).

Growth of juvenile fish is affected by many factors: water temperature, food availability, predation pressure, dissolved oxygen, salinity, wave exposure, inter- and intraspecific competition, anthropogenic disturbance (e.g. Blaber & Blaber, 1980; MacCall, 1990; Sogard, 1992; Gibson, 1994, 1997; Phelan *et al.*, 2000;

Beyst *et al.*, 2001; Meng *et al.*, 2001; Attrill and Power, 2002; Gilliers *et al.*, 2006; Jana *et al.*, 2006). It is the combination of these factors that results in use, by juvenile fishes, of habitats with different characteristics compared to habitats occupied by adult stages, and subsequent migrations between them; this has been visualised by the 'Migration Triangle' (Harden Jones, 1968; Cushing, 1982; Secor, 2002). Previous investigations of marine fish and invertebrate nurseries have stated that growth is important for very young fish, as fish that grow quickly move rapidly through the size-classes at the greatest risk of predation, thereby reducing the time they are susceptible to predation (e.g. Sogard, 1992; van der Veer *et al.*, 1994; Beck *et al.*, 2001; Heck *et al.*, 2003; Kamenos *et al.* 2004b; Andersen *et al.*, 2005). This is known as the 'bigger is better' hypothesis (Litvak & Leggett, 1992; Leggett & Deblois, 1994).

Juvenile plaice have different habitat requirements from adult plaice, and therefore are found concentrated onto shallow coastal habitats (Kuipers, 1977; Beverton, 1995). Predation pressure on juvenile plaice is thought mainly to come from *C. crangon* (only on plaice $TL \leq 30$ mm), but *Carcinus maenas* also frequently preys on juvenile plaice (only on plaice ≤ 51 mm TL) on many of the plaice juvenile habitats (van der Veer & Bergman, 1987). Because of these size refugia for 0+ plaice, it may be expected that growth does indeed play a role in determining any given juvenile habitats contribution to recruitment, thus determining whether it can be considered a nursery or not. Although fast growth does not automatically translate to survival to spawning age, it may be expected that habitats with similar growth rates may have the same nursery value for juveniles (e.g. MacCall, 1990; Gibson, 1994; Le Pape *et al.*, 2003; Gilliers *et al.*, 2006; Wennhage *et al.*, 2007).

Lamont (1964) summarised the findings of the Fishery Board of Scotland's research work from the beginning of the 20th Century until 1952, based on several research vessels trawl catches. Although these research hauls were conducted at depths greater than 4 m (2 fathoms in Lamont (1964)), the hauls show that plaice were present in fairly large numbers around the Outer Firth for the duration of the research. This indicates that the population that uses the Outer Firth as nurseries has been present for over a century. However, it is still unknown whether the juveniles that inhabit the Firth and estuary are spawned at Fife Ness and the eggs and larvae migrate into the estuary, and similarly, whether the juveniles migrate back to Fife Ness to spawn. Therefore, it cannot be said with any certainty that the population at Fife Ness is self-sustaining or not. It is unlikely that the firth juveniles have been spawned elsewhere, however, no firm connections have yet been made.

One of the reasons for examining growth of 0+ plaice in the Forth Estuary, is because newly – settled plaice populations there have been overlooked, mainly because only sandy bays are considered plaice nurseries. Rae (1970) stated that plaice nursery grounds are, without exception, sandy bottoms, without defining a sandy bottom. It must also be noted that Rae (1970) also stated that plaice nursery grounds for 3 to 4 year olds are sandy bottoms, not just referring to 0+ group plaice. It could be argued that that is the case, but this could mean that the term nursery ground was poorly defined, even in these early investigations (Beck *et al.*, 2001; Dahlgren *et al.*, 2006). Poxton & Nasir (1985) and Nasir (1985) stated that juvenile plaice were never found on muddy bays in the estuary. They based this assertion on a single trawl taken outside a muddy bay in the Outer Firth, and,

because they caught no fish in this trawl, stated that the estuary and muddy bays in the firth were not suitable nursery grounds. To explain the presence of juvenile plaice in the estuary, Poxton and Nasir (1985), Poxton (1987) and Elliott *et al.* (1990) stated this could be accounted for by the migration of 0+ and 1+ group individuals that had inhabited the sandy bays in the Outer Firth and then migrated west past the bridges in late summer, based upon the PhD of Ajayi (1983). Ajayi (1983) estimated 0.07×10^6 0+ group plaice move into the estuary after they migrate off the Outer Firth bays. These studies claim this in spite of the high numbers of 0+ plaice suggested to use the, in places, muddy Dutch Wadden Sea intertidal as a nursery (e.g. Kuipers, 1977; Zijlstra *et al.*, 1982; van der Veer & Witte, 1993). Berghahn *et al.* (1995) compared growth rates of plaice on a muddy site with growth of plaice on a nearby sandy site in the Wadden Sea, and concluded that the muddy site plaice grew faster than the sandy site juvenile plaice.

The current chapter is an attempt to determine whether the muddy bays in the Forth estuary can be considered candidate plaice nurseries, in contrast to previous work in the forth estuary (Poxton and Nasir, 1985; Poxton, 1987; Elliott *et al.*, 1990). Comparing growth rates of the estuarine sites with the 'classic' sandy Outer Firth sites gives an indication of the suitability of the estuary to support populations of 0+ juvenile plaice. Fast growth of juveniles does not indicate whether a site is a nursery according to Beck *et al.* (2001) definition, but fast growth of juveniles has been suggested to influence survival rates (Gibson, 1994) and, thus, habitats that support fastest growth of juveniles may be expected to contribute most to recruitment. In addition, once nursery sites (habitats with

greatest contribution to recruitment) have been identified, it is then possible to retrospectively determine whether growth is indeed important for ultimate recruitment. It is also desirable to compare the growth of plaice in the Forth estuary and Outer Firth to other plaice juvenile habitats, to give a further indication of habitat quality. Based upon observations from the Irish Sea, Dutch Wadden Sea and the Swedish West Coast, plaice always seem to grow at maximum possible rates (e.g. Zijlstra *et al.*, 1982; van der Veer & Witte, 1993). However, this is not the case for growth rates estimated by Amara (2004), who found growth limited at a level below that estimated by Fonds *et al.* (1992) model.

There are three main null hypotheses tested in the current work: 1) H_0 : Juvenile plaice are not capable of settling on and growing at Forth Estuary sites. 2) H_0 : Growth rates at estuarine sites are the same as growth rates in the Outer Firth. 3) H_0 : There are no differences in estimates of growth rates of juvenile plaice from the same site derived from two methods: measuring the change in median length over time, and measuring total length vs. age derived from otolith increments. In addition, a subsidiary null hypothesis is tested 1) H_0 : There are no differences between growth rates of juvenile plaice measured at field sites and growth rates predicted for those sites by a model assuming growth is determined solely by prevailing water temperature.

3.2. Methods and Materials

3.2.1. Site Description

Four sites were chosen for growth rate measurements: Blackness and Limekilns in the Forth Estuary, and Silver Sands and Gosford Bay in the Outer Firth (Figures 19-23). Blackness and Limekilns, the estuarine sites, were chosen because fish were caught regularly in pilot studies and because other sites in the estuary were difficult or impossible to sample due to coastal development or because sampling was considered too dangerous. Silver Sands was chosen because plaice were caught at high densities in pilot studies. Gosford Bay was chosen as, again, plaice were caught at high densities and plaice have been sampled by previous workers at this site using similar gear (Nasir, 1981, 1985; Poxton & Nasir, 1985), providing a medium-scale temporal comparison. Sediment characteristics have been described in Chapter 1, however, a brief summary of sediment characteristics at each site is given in Table 6.

Table 7: Summary of sediment characteristics (taken from chapter 1) N/A = Median $\Phi < 62 \mu\text{m}$. * Limekilns sediments are highly variable, see text for description.

Site	Median Φ	% Fines by weight(< 62 μm)	% Organic matter by weight
Blackness	N/A	77.42	10.13
Limekilns	N/A*	31.00*	6.54*
Gosford Bay	2.2	0.91	0.94
Silver Sands	2.7	2.29	1.27

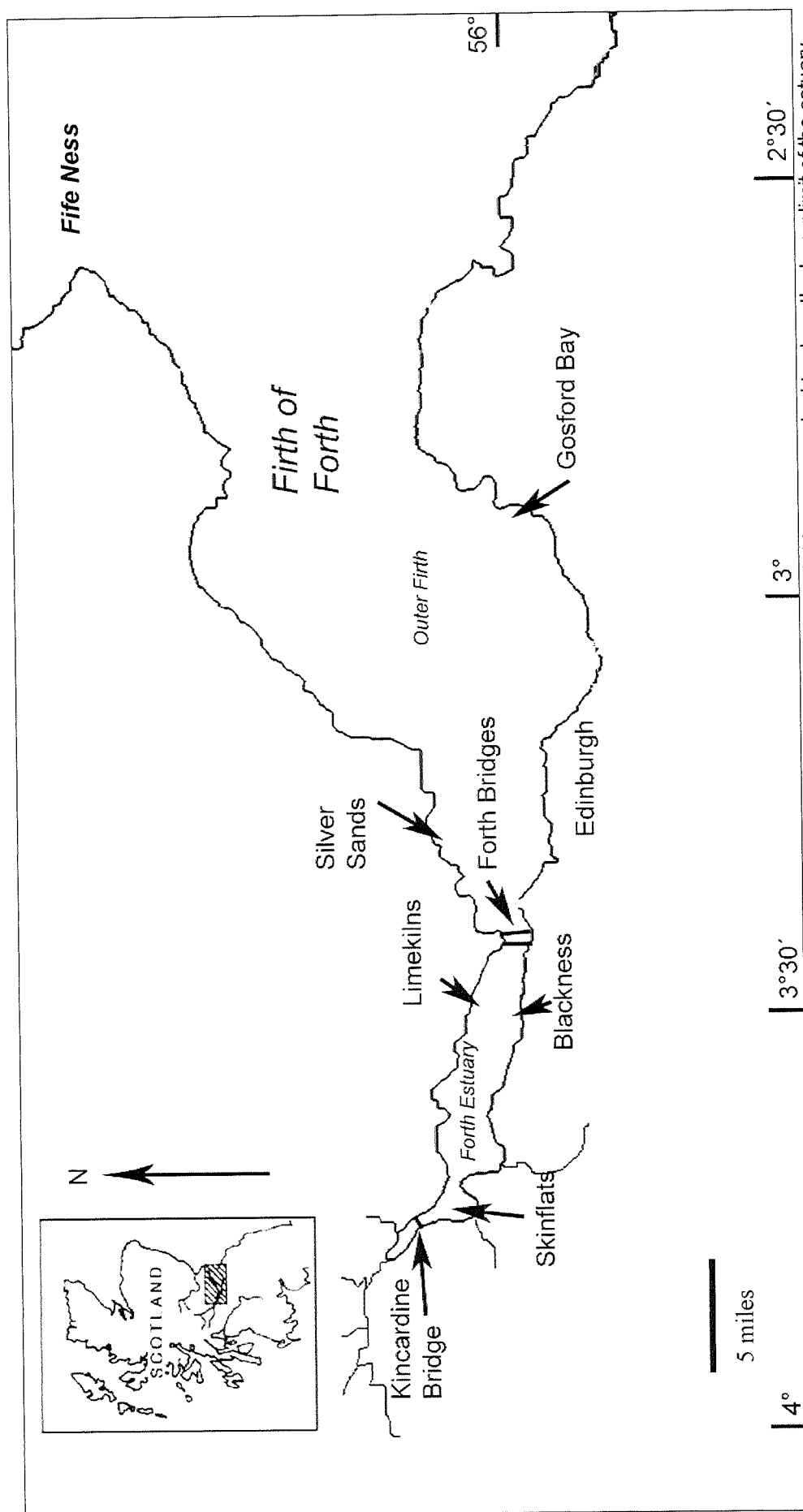


Figure 19: Map of the study area showing the sites sampled for fish in 2005 and 2006. The Forth bridges are marked to show the lower limit of the estuary.



Figure 20: Blackness sampling locations. Top: Low tide. Bottom: Flood tide



Figure 21: Silver Sands sampling locations. Top: Low tide. Bottom: Flood tide



Figure 22: Gosford Bay sampling locations. Top: High tide. Bottom: Low tide



Figure 23: Limekilns sampling locations. Top: Low water. Bottom: High water

3.2.2. Fish Sampling

The gear used to catch plaice at the four sites was the 1.5 m Riley push net, which is described in detail in *Chapter 2: Gear Calibration*. However, a short description is provided here. The push net operates in similar fashion to a towed beam trawl: tickler chains in front of the net disturb organisms in or on the sediment, these organisms then move into the water column and are captured by the trailing net. The push net is pushed from behind, minimising disturbance in front of the net from either boats or operators towing a net along the transect to be sampled. The net has two codends allowing the operator to walk between them, preventing fouling and entanglement if a single codend were used. The push net is shown in Figure 24.

Silver Sands was used as a 'reconnaissance' site to detect when juveniles began to settle out of the water column, and sampling began in the middle of February each year: the site was sampled once per week. Silver Sands was chosen as it was expected plaice would arrive at this site first. Poxton & Nasir (1985) and Bowman (1914) suggest that the spawning population for the Firth of Forth and Forth estuary, spawn at Fife Ness (Figure 19), with the eggs and larvae being transported along the northern (Fife) shore of the forth by the prevailing near shore current. Thus, it was expected that the first juveniles would be caught at Silver Sands before any of the other selected sites. Once fish were caught at Silver Sands, the other four sites were sampled at approximately four-week intervals. As each site took a whole flood tide to sample, one site was sampled per day per four-week period: hence it took four consecutive days to sample all four sites. The four sites (Blackness, Limekilns, Gosford Bay and Silver Sands) were sampled between

May and October 2005 and between May and December 2006. In 2006, a fifth site, at Skinflats in the lower middle estuary (Figure 19) was added to the sampling regime, however, no fish were caught here using the push net, despite I+ and II+ group plaice regularly caught on the cooling water intake for Longannet power station on the Fife shore directly opposite Skinflats (McLusky, pers. comm.; Lyndon, pers. comm.; Pers. obs.). Therefore, Skinflats was abandoned after June 2006.



Figure 24: Push net front (top), rear (middle) and in operation (bottom)

On each sampling occasion, a 50 m transect was measured out parallel to the shoreline, within the first hour after time of low water. Sampling was carried out at this time as it is expected that juvenile plaice density is at its highest on the flood tide (Gibson, 1973; Gibson *et al.*, 2002). Single 50 m transects were surveyed on each sampling date with one or two exceptions, these are noted in *Results*. 50m was chosen because this represented an appropriate compromise between maximising sample sizes, and ensuring that captured fish could be measured and returned to the sea rapidly and with minimum mortality.

A single push of the net along the 50 m transect usually provided sufficient individuals to measure the length frequency, however, on several occasions, less than 15 fish were caught and further transects were fished to gain sufficient individuals, these are noted in 3.3. *Results*. A minimum of 15 individuals was not always obtained, however this figure was the target in case of losses before lengths could be measured. The net was operated at a depth of between 0.10 and 0.50 m. After each transect was fished, the net was turned and pushed landwards, perpendicular to the shore so the net emerged from the water. The net was then examined and any plaice caught were placed into a bucket filled with seawater until ready to be measured. Once the net was emptied the total length (TL) of each individual was measured to the nearest mm. Total length is defined as the length measured from the anterior tip of the snout to the posterior tip of the caudal fin (Figure 25). After measurement, fish were returned to the sea; on each sampling date fish were out of the

sea for no longer than 4 hours, and on most dates for approximately 2 hours, in 2005 ten individuals were retained on each sampling date for otolith increment work.

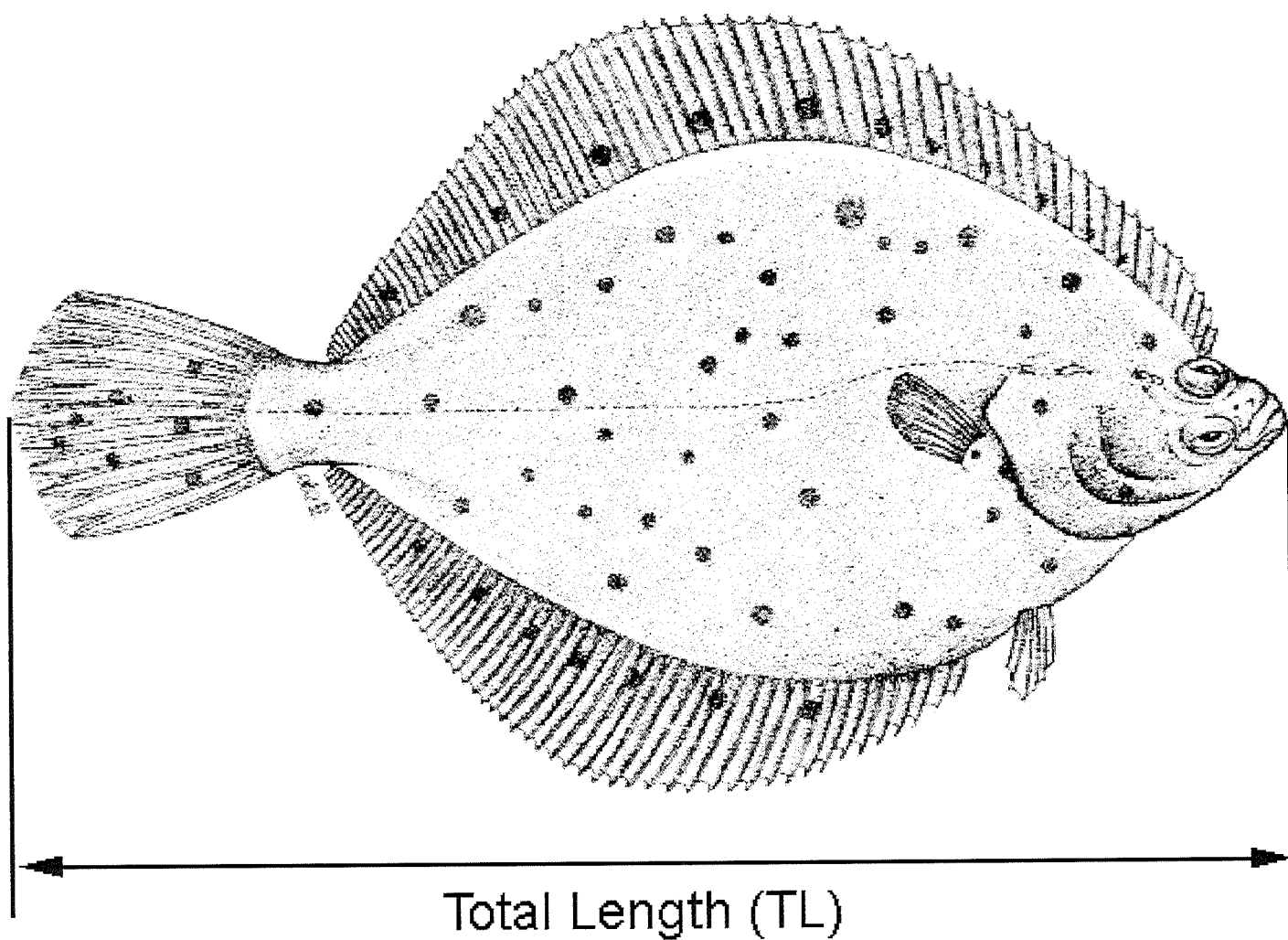


Figure 25: Length measurements of plaice used in current study (Total Length), from the extreme posterior tip to the extreme anterior tip. Copyright FAO.

3.2.3. Cohort Analysis

Growth rates were estimated for each site using two different methods: growth rates were first calculated by analysing length-frequency distributions recorded on each sampling date. Median and mean total lengths on each sampling date were calculated and the change in these averages over time was used as a proxy for growth rates. This is a commonly used technique for measuring growth rates of seasonal populations, and is valid provided migration, mortality and gear efficiency do not affect the length-frequency distributions (Amara, 2004). Gear efficiency is discussed in *Chapter 2: Gear Calibration*, migration and mortality are discussed in this chapter (see 3.4. *Discussion*). Overall and peak growth rates, in mm day⁻¹, were calculated for each site; Overall growth rates were calculated using the following formula:

$$G_o = \frac{(T_e - T_s)}{D_{e-s}} \quad (4)$$

where G_o is the overall growth rate, T_e is the highest median total length (mm) near the end of the sampling season, T_s is the median total length (mm) at the start of the sampling season and D_{e-s} is the number of days between the start and end of the sampling season. The highest median TL was used to calculate overall growth rates as migration is suspected to have occurred at several of the sites towards the end of the sampling periods in both years. Peak growth rates were calculated as the fastest increase in median TL between two consecutive sampling dates at each site.

In addition, growth rates measured using length-frequency distributions were compared with predicted maximum growth rates, estimated from the temperature-dependent model derived by Fonds *et al.* (1992):

$$dL = 0.0136T^{1.5} - 6 \times 10^{-9}T^6 \quad (5)$$

where dL is the predicted maximum growth rate (mm day^{-1}) and T is the mean water temperature ($^{\circ}\text{C}$). Mean water temperatures experienced by the individuals in this study were estimated using two different methods. In 2005 water temperatures were not recorded on each sampling date, instead, Sea Surface Temperatures (SST) were estimated using SeaWifs/MODIS-Aqua (SMA) satellite data. The SST data used in this study were acquired using the GES-DISC Interactive Online Visualization ANd aNalysis Infrastructure (Giovanni) as part of the NASA's Goddard Earth Sciences (GES) Data and Information Services Center (DISC) (NASA, 2007). The co-ordinates (latitude and longitude) used in NASA (2007) tool to delineate each site are shown in Table 8.

SSTs' extracted from MODIS-Aqua datasets are monthly averages; the spatial resolution of these processed data is 9 km^2 , therefore, the extracted data give only an approximation of temperatures experienced by the juveniles caught at each site. SSTs' from satellite data were then used as mean temperatures in Fonds *et al.* (1992) growth model (equation 5). In 2006 water temperatures were recorded directly, using a digital thermometer, on each sampling date (Figure 28). The mean temperature between two sampling dates was used as the mean

temperature (T) in the growth model. SST data were again used in 2006 to estimate maximum growth rates at each site. SST temperatures are shown in Figure 29.

There were no significant correlations (p values all greater than 0.05) between SSTs' and the water temperatures recorded in 2006 for each site. No retrospective estimates of water temperatures could be made for 2005 from satellite SSTs'.

Table 8: Latitudes and longitudes assigned to each site for SeaWiifs/MODIS-Aqua Sea Surface Temperature (SST) data extraction.

Site	Latitude	Longitude
Blackness	55.5 N - 56.0 N	3.3 W - 3.2 W
Limekilns	56.0 N - 56.0 N	3.3 W - 3.2 W
Gosford Bay	55.5 N - 56.0 N	3.0 W - 2.5 W
Silver Sands	56.0 N - 56.0 N	3.1 W - 3.1 W

3.2.3.1 Otolith Increments

Growth rates were also calculated using otolith microincrements taken from a sub – sample of the plaice caught in 2005 only. In order to provide a range of sizes of fish, the smallest and largest fish were used from each site: length increments were calculated for 30 lengths between the smallest and largest fish lengths, and individuals were selected for otolith analysis by the proximity to these lengths. I.e. individuals were selected if their TL was the closest to the calculated increment, whether higher or lower than that increment. Pairs of sagittal otoliths were

removed from captured individuals and the largest otolith of each pair (where both sagittae were successfully recovered) selected for microincrement analysis, using a scanning electron microscope (SEM). The selected sagittae were mounted, sulcus up, to 14 mm diameter, aluminium, pin-type SEM stubs with Araldite ® 2020 (a very slow setting, low viscosity, transparent epoxy adhesive). The stubs were left at room temperature for over a week to allow the epoxy to cure completely. The otoliths attached to the stubs were then prepared for analysis using the method of Karakiri & von Westernhagen (1988). The preparation consists of 3 steps: 1) grinding to remove otolith material up to the mid-plane of the otolith, 2) polishing to remove obscuring marks and ensure a flat surface, and 3) etching to reveal the microincrements. The apparatus used is shown in Figure 26, and consists of a turntable usually used for reproduction of vinyl-records. A glass disc with silicon-sealant around the circumference was used for the initial grinding step. A mixture of 9 µm carborundum particles and water was placed into the glass disc as an abrasive. The stubs with mounted otoliths were attached to the stylus arm and allowed to rest on the glass disc while the turntable rotated. This process abraded material from the otolith, which had to be checked frequently under a dissecting microscope to ensure sufficient material was removed, as well as ensuring grinding was not continued past the mid – plane of the otolith. After the grinding step, the glass disc was replaced by a plastic disc, again with silicon – sealant around the circumference. The otoliths were cleaned using distilled water and re – attached to the stylus arm before polishing. A 1 µm diamond slurry was

introduced to the surface of the plastic disc as the polishing medium and polishing was carried out until a smooth otolith surface was achieved. After the polishing step, a final etching step was carried out. This was done using a 0.1 M solution of ethylenediaminetetraacetic acid (EDTA), buffered to pH 7.2. EDTA is a chelating agent that sequesters di- and tri-valent metal ions. In the case of otoliths, the solution removes calcium ions from the otolith, whilst leaving the proteinaceous organic matrix relatively intact (Campana & Neilson, 1985). This results in the characteristic concentric rings found in otoliths, due to differences in elevation between the organic matrix and the inorganic component (calcium carbonate). An example of an otolith with rings and various other features imaged using a variable pressure SEM is shown in Figure 27.

A variable pressure SEM (Topcon SM – 300) was used to image the otolith increments, in order to ensure sufficient resolution to separate out all microstructural features; light microscopes can resolve objects of approximately 1 μm or slightly less (Campana, 1992) and some otolith increments may be smaller than this. In addition, the use of a variable pressure SEM, as opposed to a 'traditional' SEM that requires a high pressure vacuum in the sample chamber, allowed re – etching of otoliths if microincrements were not readily apparent on images. This is because a variable pressure SEM allows a small, regulated amount of gas into the sample chamber to carry charge away from the sample surface. A high pressure vacuum SEM requires samples to be coated with a conducting material, usually a thin layer of gold, which would not allow re – etching of

the sample if microincrements were not apparent. Re – etching with EDTA was carried out on around a third of the otolith samples. The microincrements were counted using the UTHSCA ImageTools count and tag tool. A transect from the start of the accessory primordia to the very edge of the otolith was selected for microincrement counting. Some otoliths required discontinuous transects as often a continuous line of apparent microincrements from the accessory primordia to the edge of the otolith, was not available. Growth rates for each site were calculated by plotting the age in days of each individual fish against the total length in mm of each individual. A regression line was fitted by the least – squares method, using 12 as the intercept on the y – axis, and the slope of the line gave a growth rate in mm day⁻¹. 12 mm is accepted as the mean size at which larvae metamorphose and settle onto the benthos (Ryland, 1966; Amara & Paul, 2003); however, length at settlement may be as high as 15 mm (Modin *et al.*, 1996). The exact stage of metamorphosis at which accessory primordia begin to form in plaice otoliths is unknown (Modin *et al.*, 1996); therefore both lengths have been used to indicate the range of growth rates at the four sites in 2005. The use of the same length at settlement for growth calculations for all beaches may not be accurate: fish may be larger or smaller than this at time of settlement on different beaches, or, indeed, different times on the same beach. Thus, the growth rates calculated here may not be directly comparable to other studies, but can be compared provided the assumed length at settlement is taken into account for calculations.

Settlement dates were calculated for each individual by subtracting the number of increments from the accessory primordia to the outermost edge of the otolith, from the date of capture of each individual. The formation of accessory primordia coincides with metamorphosis in plaice (Modin *et al.*, 1996), and metamorphosis occurs when the individual has settled onto its benthic habitat (Ellis & Gibson, 1995). Further, 0+ plaice are limited by depth (Gibson, 1973) and also exhibit strong alongshore site – fidelity (Burrows *et al.*, 2004). Thus, the exact period of time the individual fish has occupied the beach it was captured on can be calculated, as the four sites in the present study are separated by sufficiently deep water (Blackness from Limekilns and Silver Sands from Gosford Bay) and alongshore distance (Silver Sands from Limekilns and Gosford Bay from Blackness) to ensure that 0+ fish could not have migrated between them.

The various microstructural features of plaice otoliths have been validated by several workers: Al – Hossaini & Pitcher (1988), Al – Hossaini *et al.* (1989), Karakiri & von Westernhagen (1989) and Modin & Pihl (1994) validated the periodicity (daily) of plaice microincrements; Modin *et al.* (1996) validated the timing of formation of accessory primordia. Therefore, validation of otolith microstructures was considered an unnecessary duplication of effort in a species that has relatively well studied otolith structures. These validations are necessary before using microstructural features of otoliths as aging and life history markers (Campana & Neilson, 1985; Geffen, 1992).

In order to minimise bias during increment counts, counts were made using image codes that referred only to the stub and position of the stub in storage boxes, and the site of capture. No reference was made to sample date or length of the fish during the imaging and counting processes. Only after counting was complete were the lengths of the fish and date of capture revealed.

3.2.4. Plaice Densities

Densities of plaice on each sampling occasion were estimated by measuring linear transects parallel to shore, and calculating the area swept by the net. The number of fish caught was divided by the area swept to estimate the number of fish per square metre (ind. m⁻²). It was assumed that some fish evaded capture (see *Chapter 2*).



Figure 26: Apparatus used to prepare otoliths for microincrement analysis. The top Figure shows the turntable with the glass disc and carborundum used for the initial grinding step. The bottom Figure is a close up of a stub attached to the stylus arm in grinding position. The white material is silicon sealant used to keep the water/carborundum mixture on the glass disc.

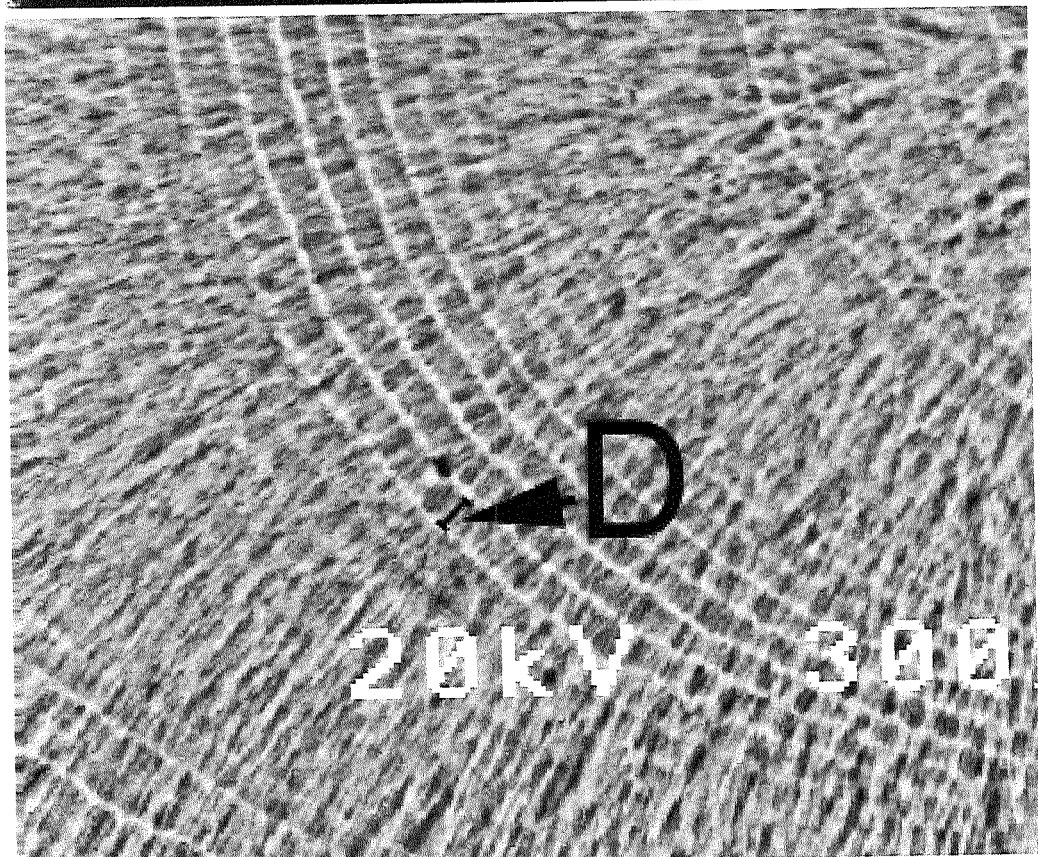


Figure 27: Top Figure is a SEM image of a prepared otolith. *N* is the Nuclear Primordium, which forms just before hatching, *L* corresponds to the larval phase of the individual, and *A* are Accessory Primordia, which form as the individual metamorphoses. The bottom Figure is a close up of the same otolith; *D* is a single microincrement which corresponds to a single day. The light rings in the bottom Figure are the protein organic matrix.

3.2.5. Caging Experiments

In June – July 2005, an experiment was carried out to determine growth rates of individual fish in cages placed at one of the field sites. The initial site chosen was Blackness, because the site is relatively sheltered and undisturbed by recreational users, thus minimising the risk of damage or removal caused by waves or vandalism. Each cage consisted of a wooden frame with a very fine mesh (<300 µm) net covering the top and four sides. The bottom of the cage was enclosed by a strong wire mesh (6 mm x 6 mm). Five cages were placed in the water at a low spring tide and pushed into the sediment such that the sediment was forced through the wire mesh to form a substrate inside the cage. Plaice were caught at the same time and a single individual was measured and placed into each cage; the cage tops were then sealed using metal staples.

The cages were left for approximately ten days before re-examination. Unfortunately, only a single individual was recovered from the cages, and predators (Green shore crab, *Carcinus maenas*) were found in three of the cages. It is likely the crabs were in the cages before they were sealed, however, the mode and timing of entry was unknown. A second trial with four new fish and the remaining original fish was begun immediately, however, no fish were recovered from the second trial and one of the cages was missing. Thus, it was decided to abandon the caging experiments, as a better design would likely prove too costly and time-consuming.

3.2.6. Statistical Analyses

Growth rate estimates were compared between sites using the analysis of covariance procedures for testing homogeneity of regression coefficients described in Zar (1999). Data were tested for normality and homogeneity of variances and ln-transformed where appropriate. Where significant differences were found, Tukeys procedure for multiple comparisons described by Zar (1999) was used to determine which of the estimates were different. ANCOVA tests were used to compare the growth rates estimated by the cohort analysis method in 2005 and 2006, between the four sites. Thus, one test was carried out for 2005 estimates and one test for 2006 estimates. Growth rates were compared between years at the same site, using a t-test equivalent (comparing two slopes) of the ANCOVA procedures (Zar, 1999) for more than 2 slopes, for a further four comparisons (Table 16). Growth rate estimates were also compared between sites using the ANCOVA procedure on otolith-estimated growth rates in 2005. A comparison was made between the estimates of growth rates derived from the cohort analysis and otolith increment methods (Table 17). Data from within sites collected in 2005 were used in ANCOVA analyses. The relationship between plaice density and growth was analysed by a fitted line plot (Figure 55).

3.3. Results

Limekilns had the highest temperature recorded in 2006, peaking in August at slightly over 28 °C (Figure 28). Silver Sands had the lowest temperature except for the start of June when Gosford Bay was lower, and the end of October when Blackness was lower. It must be noted that these temperatures were recorded around the time of low water, and represent a measurement of only a few minutes on each sample date. The average temperatures experienced by the fish over the course of the nursery ground phase may be different to those recorded here; however, the temperatures in Figure 28 give an indication of the range of temperatures at the sites.

Monthly average temperatures estimated using SeaWifs/MODIS-Aqua (SMA) SST data for 2005 and 2006 are shown in Figure 29. The SSTs extracted for all 4 sites are similar (Figure 29) and were used as water temperature proxies in the temperature – dependent growth model of Fonds *et al.* (1992). Water temperatures recorded at each site in 2006 are shown in Figure 28. These water temperatures were used to calculate maximum growth rates predicted by Fonds *et al.* (1992) growth model, in addition to the growth rates calculated using SMA SSTs, in 2006.

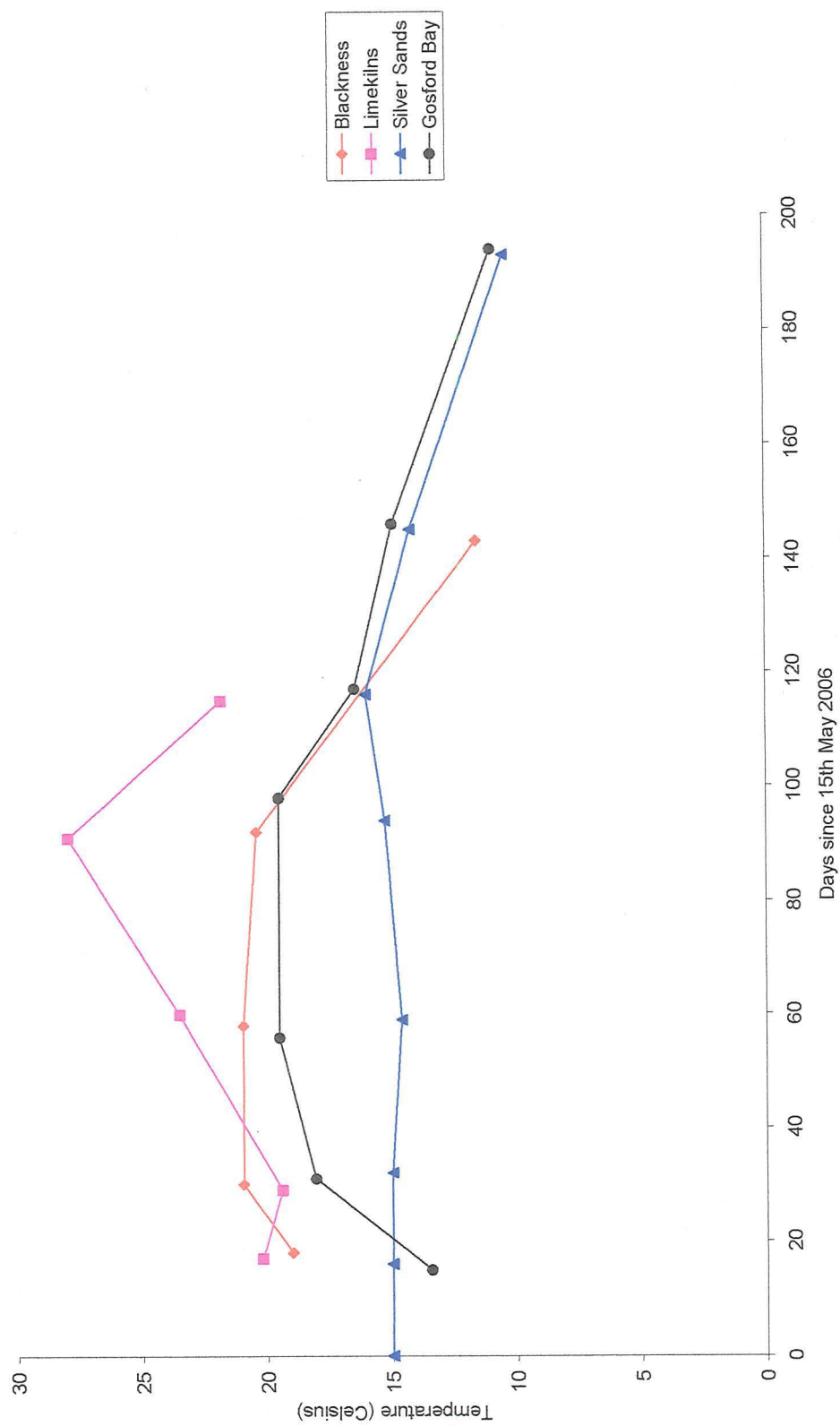


Figure 28: Plots of mean water temperature recorded at each site in 2006

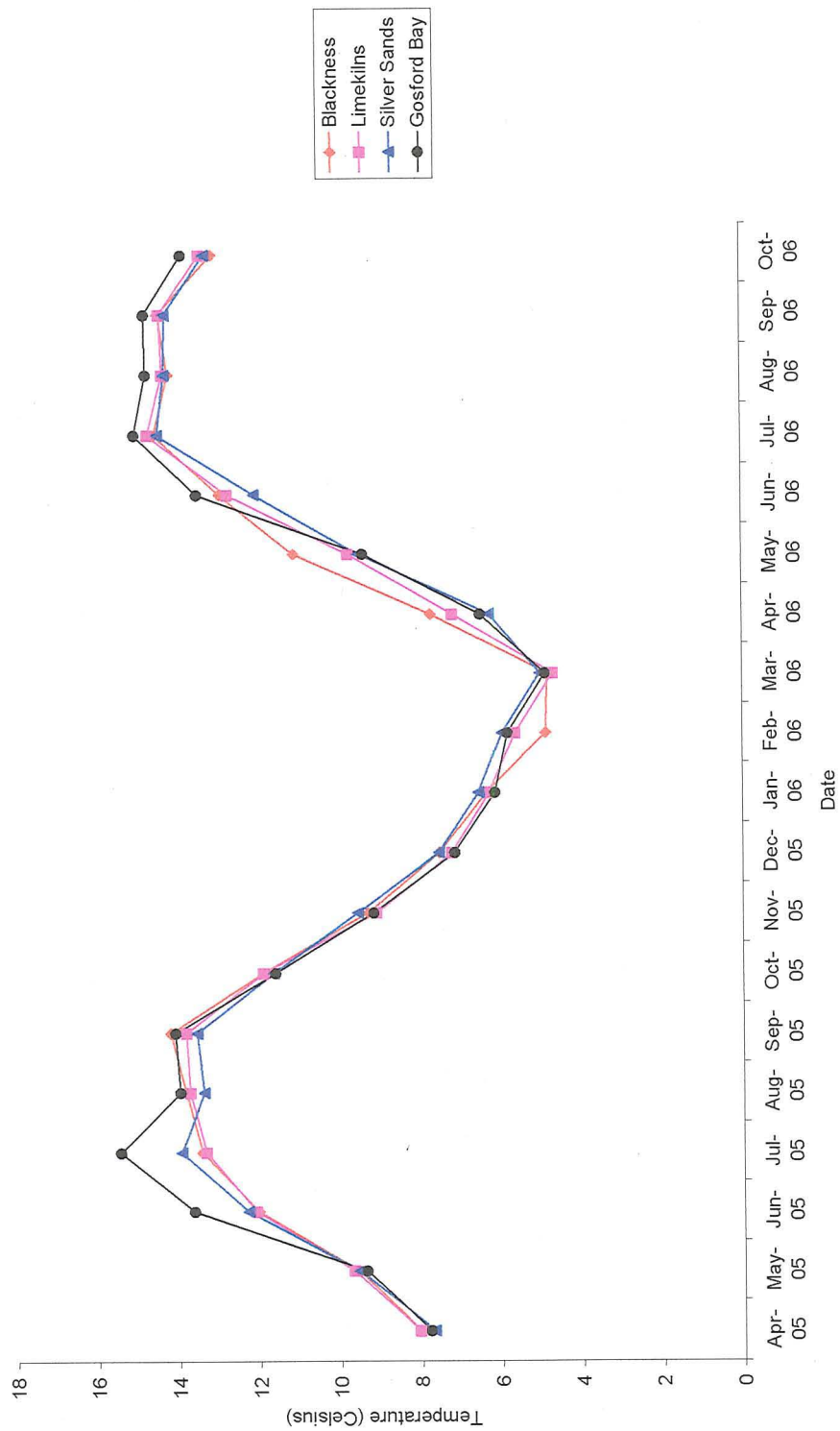


Figure 29: Plots of monthly average SST for each site extracted from SeaWifs/MODIS-Aqua satellite data. These data were used as mean temperatures in Fonds *et al.* (1992) growth model

3.3.1. Length – Frequencies of plaice total lengths.

Length – frequency distributions of plaice caught at each site on each sample date are shown in Figures 30 – 37. Median TLs are indicated by a vertical line with the corresponding value above the line. Silver Sands was used as a reconnaissance site in both years, as it was predicted that larval plaice would arrive here first, due to the circulation of water between the hypothesised spawning area (Fife Ness, Figure 19) and the Firth of Forth (Poxton and Nasir, 1985). In 2005 the first individuals were caught at Silver Sands on 27th April; in 2006 the first individual was caught on 28th April. However, no fish were caught again at Silver Sands in 2005 until 31st May; at Blackness, fish were caught on 30th May 2005. In 2006, no fish were caught at any of the sites until 15th May at Silver Sands. Thus, for length measurements, Silver Sands has the earliest sample date in 2006, while Blackness has the earliest sample date in 2005.

The length – frequency distributions on each sample date (Figures 30 – 37) are based, in some cases, upon combined catches; the push net swept variable areas because low catch rates on some sample dates meant additional transects were required, therefore the numbers of individuals presented are not comparable between dates. Densities of plaice caught and area swept by the net on each sample date are presented in Tables 9 and 10. Silver Sands had the highest density of plaice in both years.

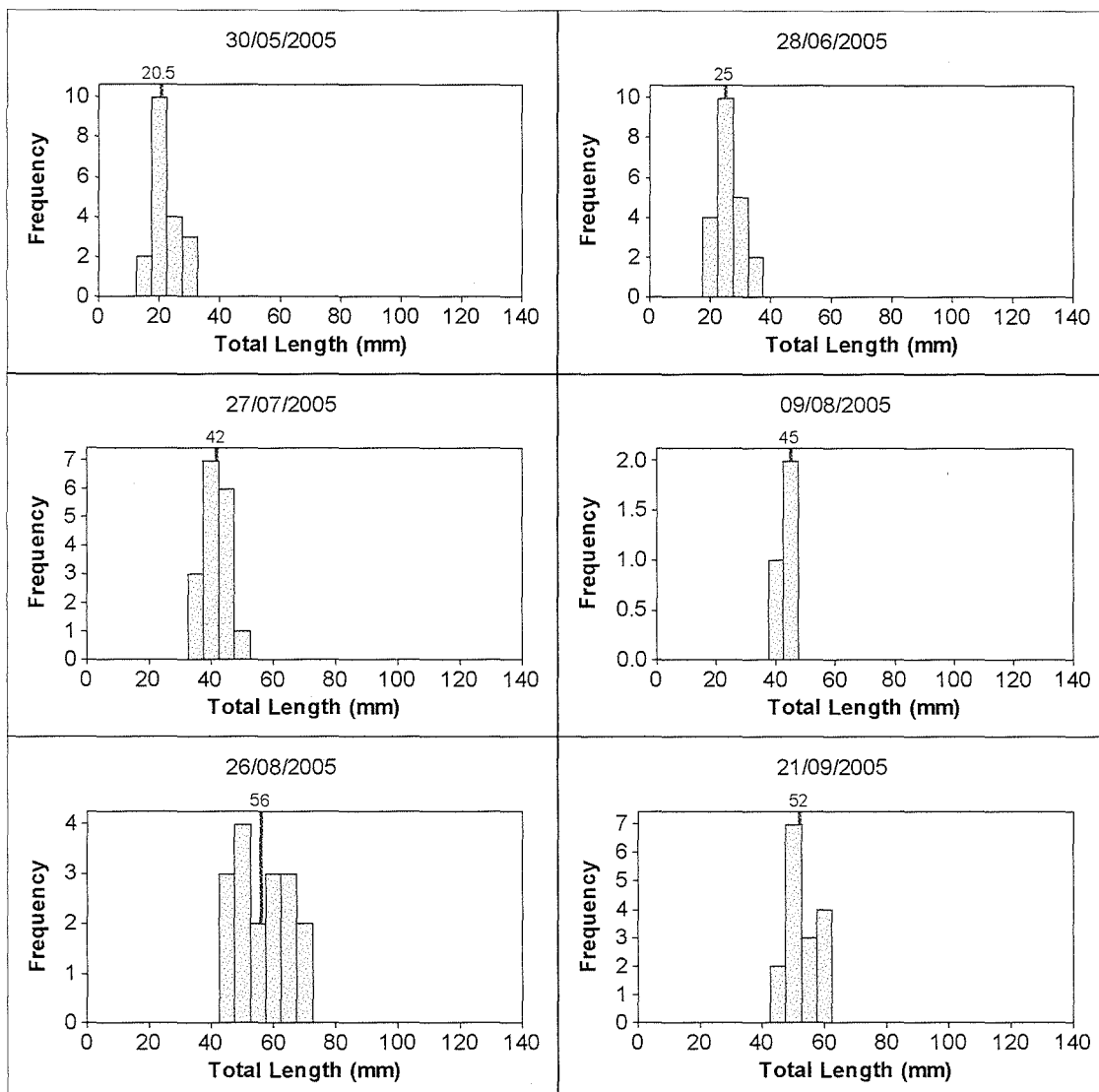


Figure 30: Blackness 2005 length – frequency histograms. Numbered line is median total length.

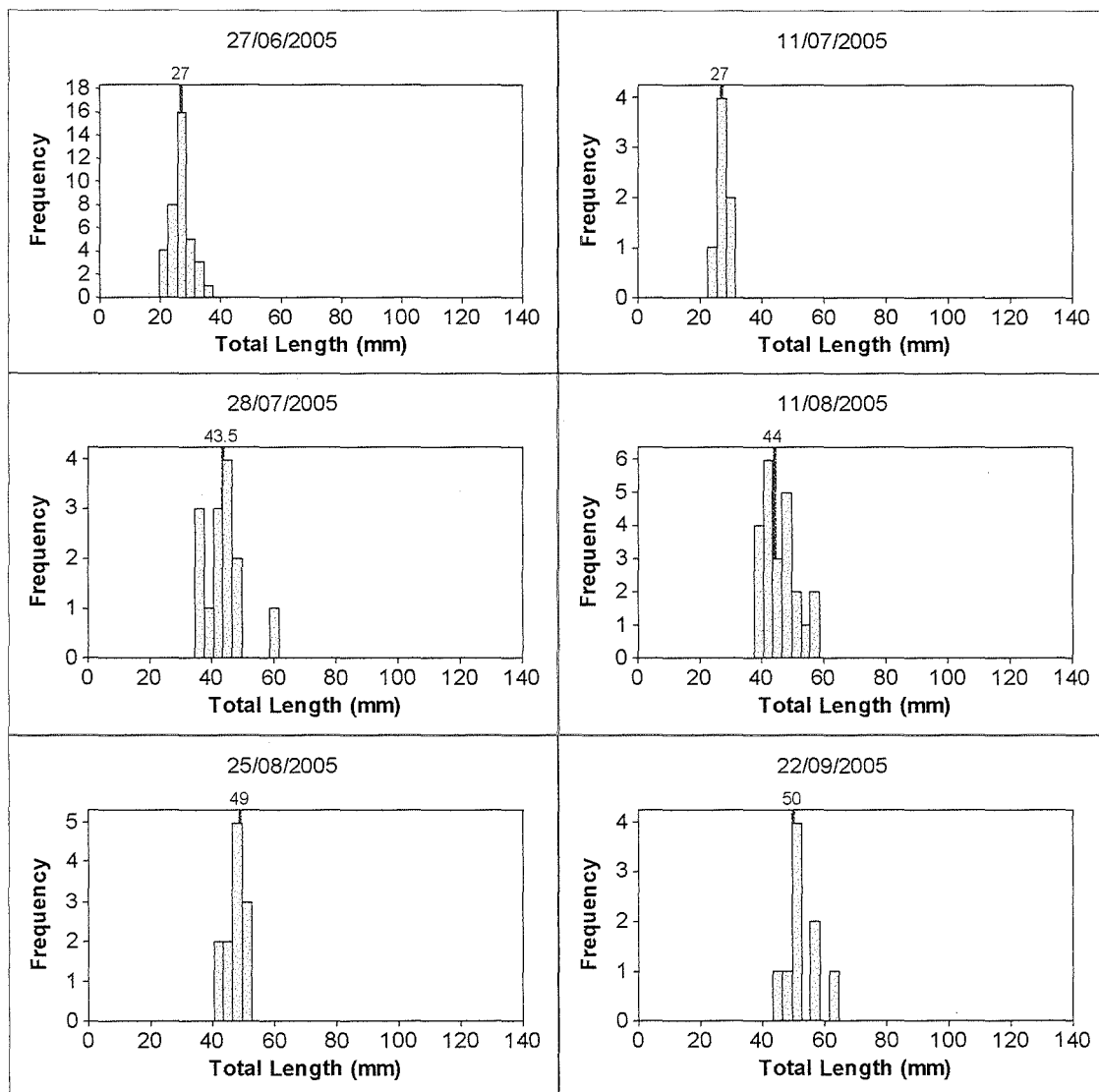


Figure 31: Limekilns 2005 length – frequency histograms. Numbered line is median total length.

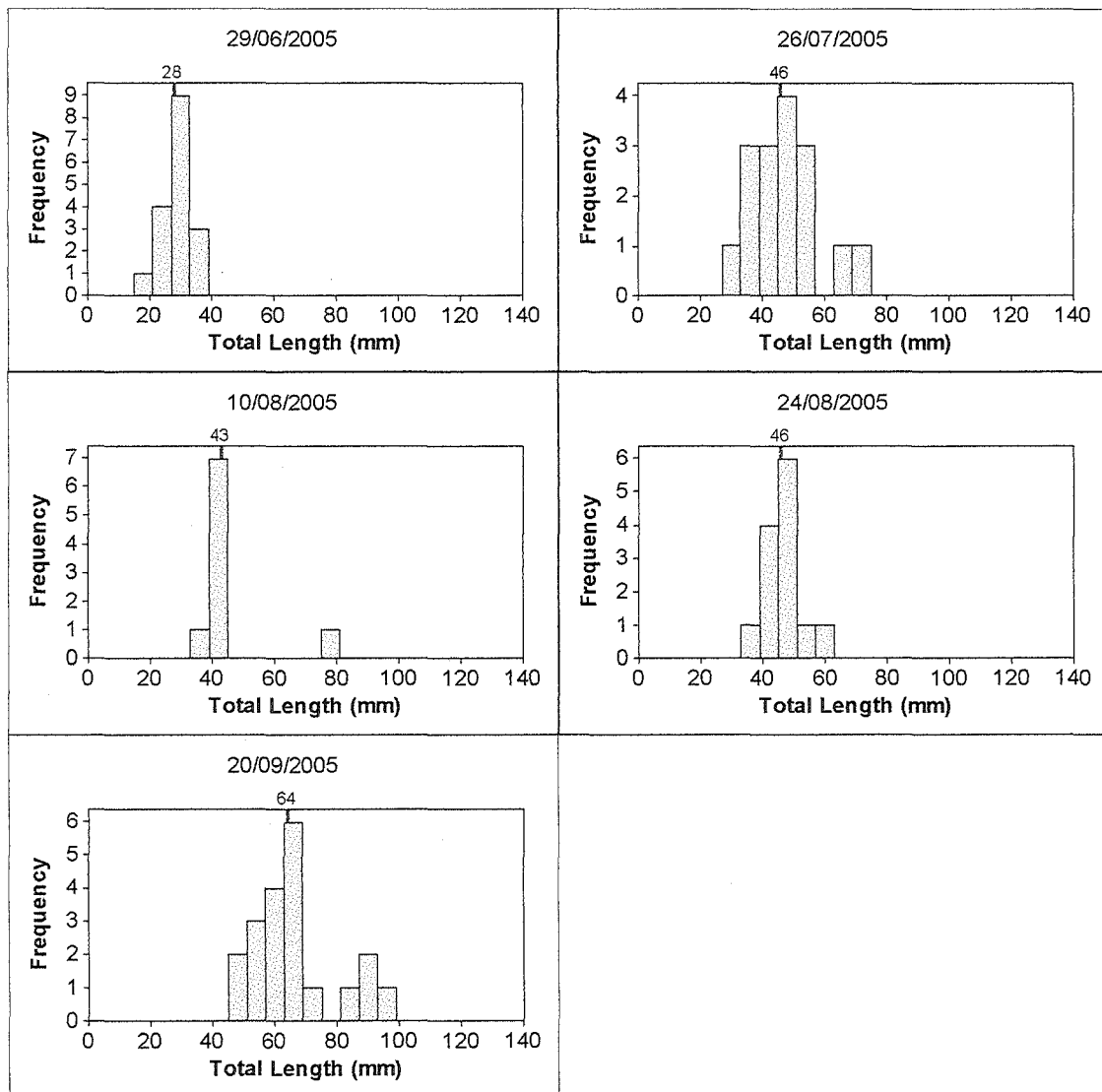


Figure 32: Gosford Bay 2005 length – frequency histograms. Numbered line is median total length.

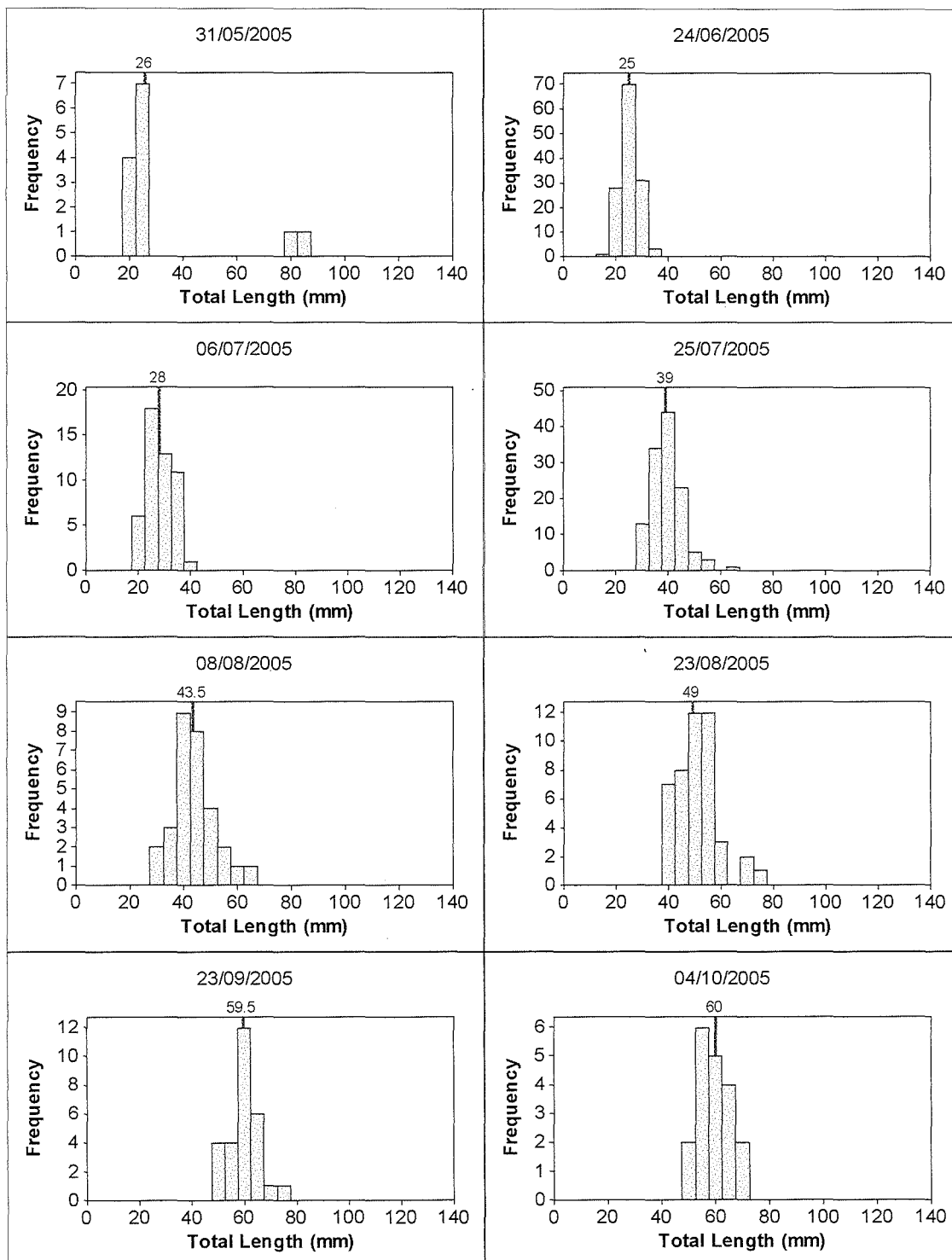


Figure 33: Silver Sands 2005 length – frequency histograms. Numbered line is median total length.

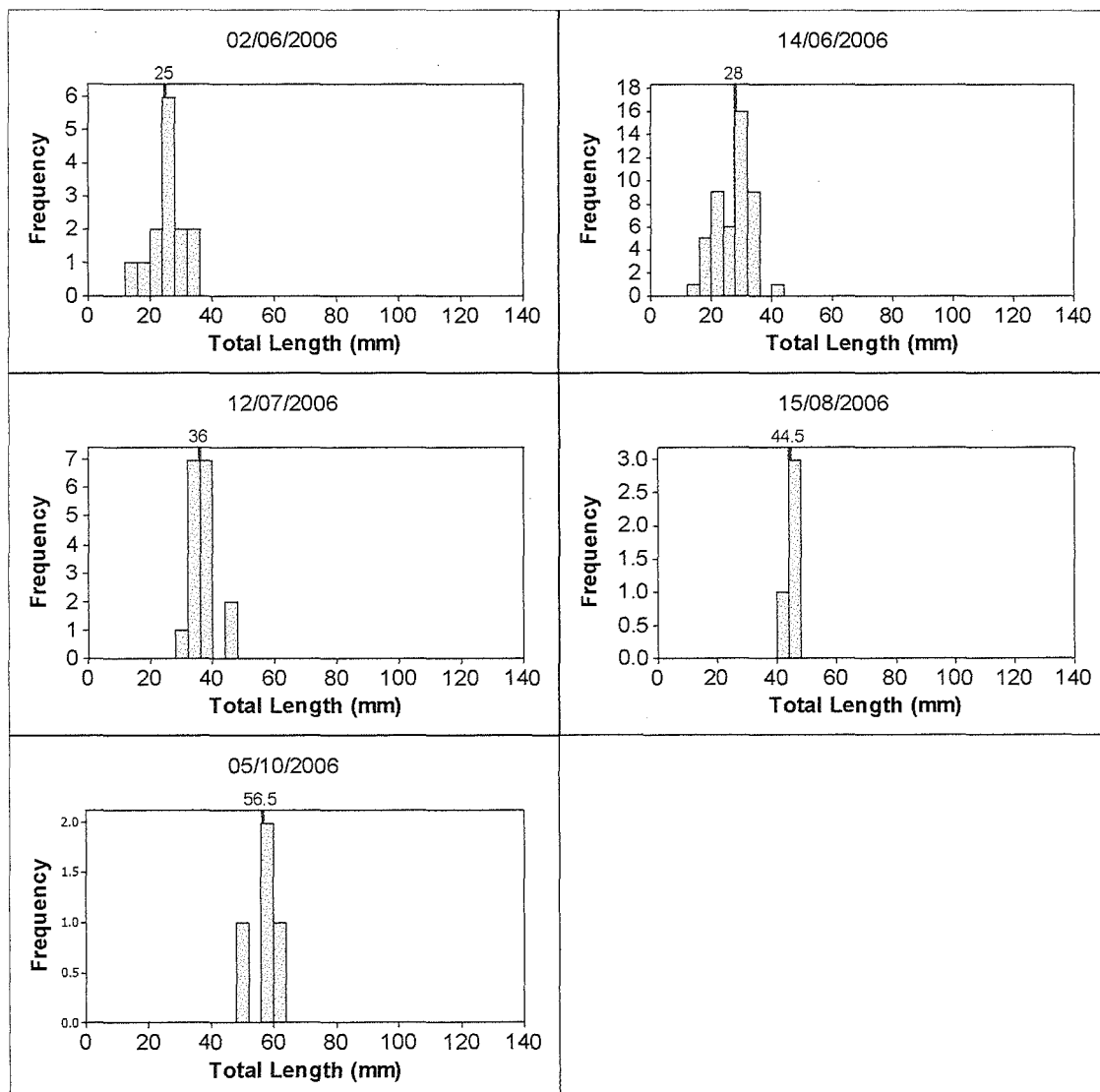


Figure 34: Blackness 2006 length – frequency histograms. Numbered line is median total length.

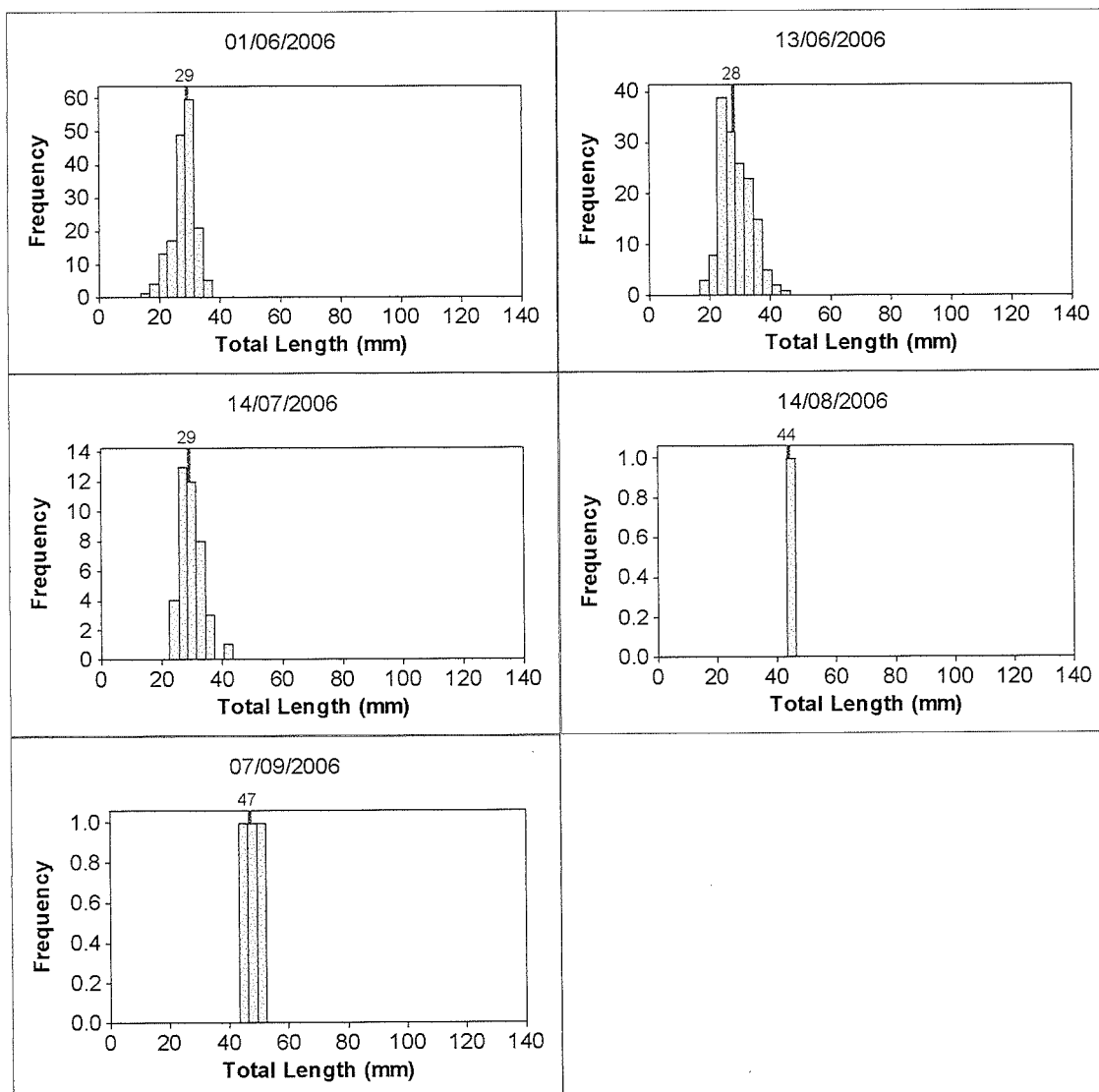


Figure 35: Limekilns 2006 length – frequency histograms. Numbered line is median total length.

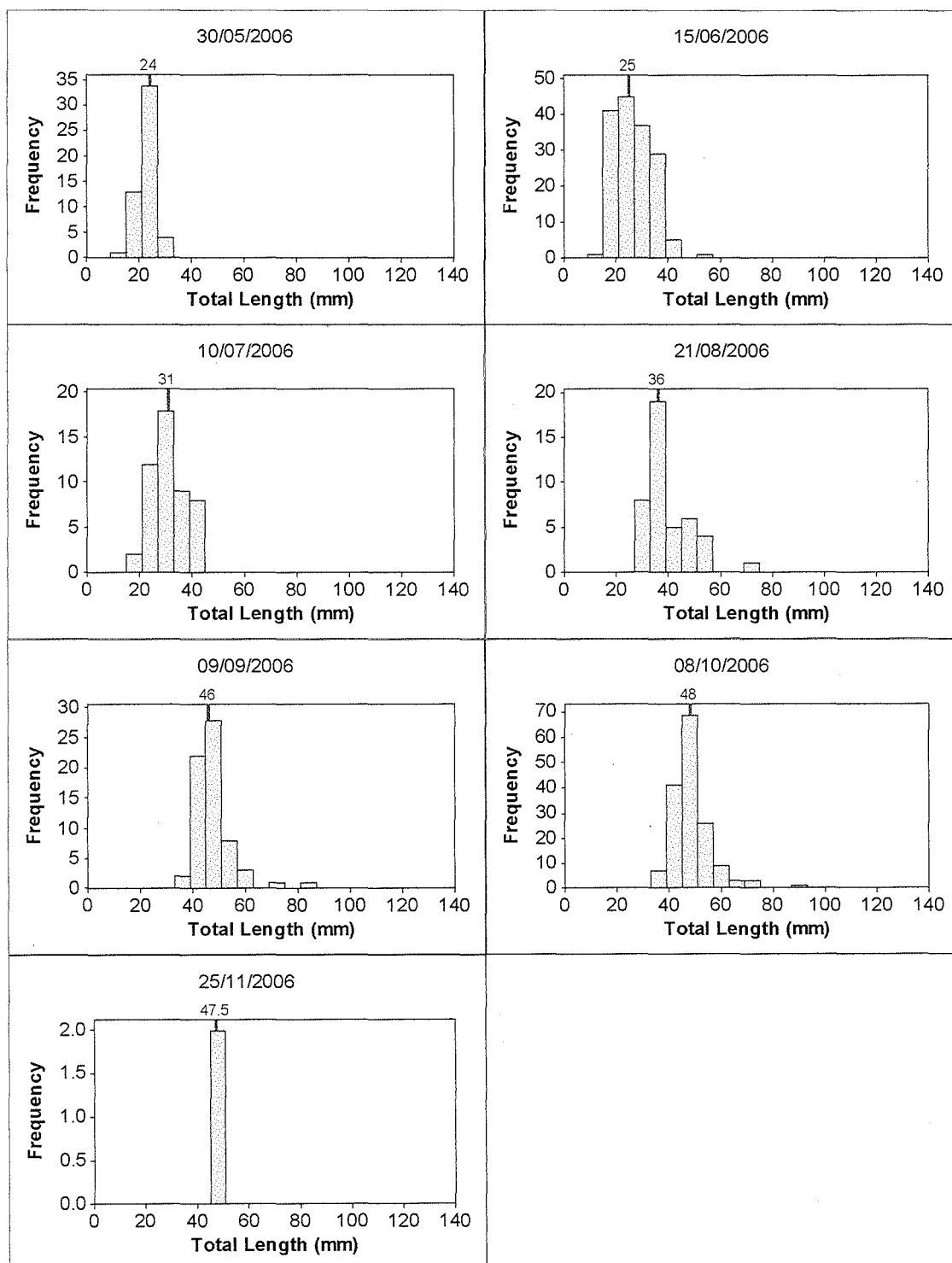


Figure 36: Gosford Bay 2006 length – frequency histograms. Numbered line is median total length.

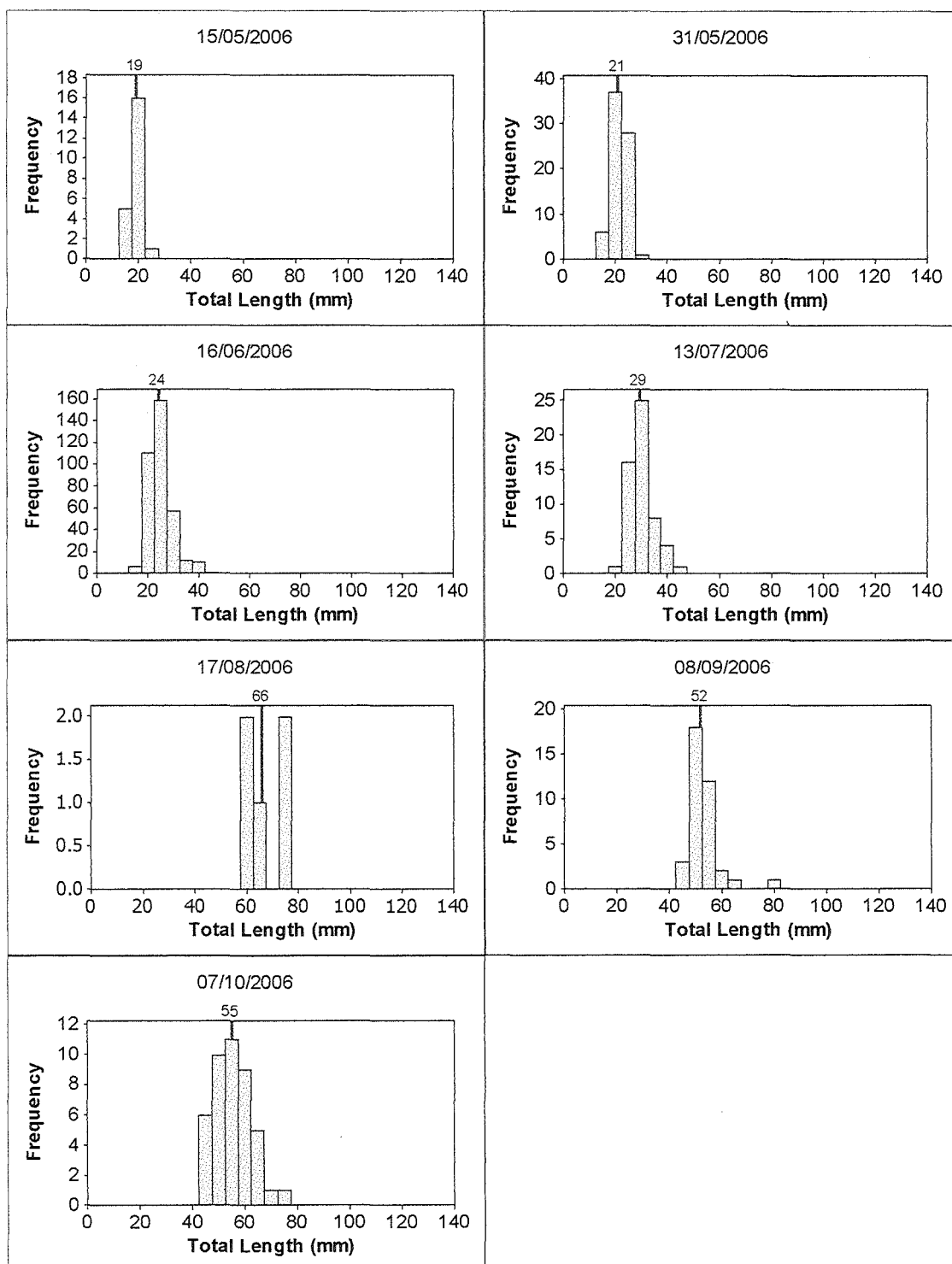


Figure 37: Silver Sands 2006 length – frequency histograms. Numbered line is median total length.

Median total lengths at all four sites increased over time in both years. The first sample at Silver Sands in 2005 (Figure 33) had a bimodal distribution: two individuals of approximately 85 mm TL were caught in this sample. These two fish were likely to have been I+ group that had returned to the intertidal zone. The median TLs of the first samples when all four sites had been sampled as close to each other as possible, during the last week of June 2005, were similar, but Gosford Bay was highest (28 mm, Figure 32), Limekilns was next highest (27 mm, Figure 31) and Silver Sands and Blackness were the lowest (25 mm, Figures 30 and 33). Fish caught in 2005 at the two estuarine sites, Blackness and Limekilns, showed a peak in median total length from August to September and no increase in median TL was observed after these peaks (Figures 30 – 31). At Blackness, the mean and median total lengths reached a peak in August 2005, and then declined in September. This reduction is probably due to length dependent depth selection (Gibson, 1973; Gibson *et al.*, 2002). At Limekilns, the median TL peaked in September 2005. In the Outer Firth, Silver Sands showed a cessation of increase in median TL between September and October 2005, and the peak median TLs of the fish between these months was approximately 4 mm higher than the peak median TL at Blackness 2005, and 10mm higher than the peak median TL at Limekilns 2005. At Silver Sands, the maximum TL of plaice caught was lower in October 2005 than September 2005: the apparent cessation of growth and reduction in maximum TL are again probably caused by migration to deeper water. The peak median TL at Gosford Bay in 2005 was higher than any of the other sites, and occurred at the end of the sampling season.

Samples were taken at Silver Sands and Gosford Bay on consecutive days at the end of May 2006; Gosford Bay had a higher median TL (24 mm, Figure 36) than Silver Sands (21 mm, Figure 37). At the start of June 2006, Limekilns had the highest median TL, Blackness had the next highest, Gosford Bay median TL was lower and Silver Sands again had the lowest. This is in contrast to 2005, where Gosford Bay had the highest median TL, Limekilns had the next highest and Blackness and Silver Sands had the lowest median TLs, (Figures 30 – 37). Again, the median TLs at all sites increased over time in 2006, however, the peak median TLs in 2006 were lower than the peak median TLs in 2005 at all sites, with the exception of Blackness. Silver Sands and Gosford Bay showed only a slight increase in median TL after September 2006. There was a peak in median TL at Silver Sands in August 2006 (Figure 37), however, only five fish were caught in the sample, which was a four times lower number of individuals than the next lowest catch in the middle of May (Figure 37, Table 10). Additionally, there was a large drop in median TL measured in the next sample at Silver Sands in September 2006. Hence, the August 2006 sample was excluded from growth rate calculations (Table 12).

Densities of plaice in 2005 (Table 9) indicate that Silver Sands in July had the highest peak density of any of the sample periods, at any of the sites in 2005. At Blackness over the whole sampling season, densities remained relatively constant. At Limekilns, there was a trend of a reduction in density from June to the end of July 2005, with an increase in August followed by another reduction at

the end of August and middle of September (Table 9). The density fluctuations probably have a localised cause e.g. temperature, wave energy, tidal height or turbidity. At Gosford Bay, there was an increase in density at the end of August 2005, however, the general trend was a reduction in density over time. At Silver Sands, the densities were much higher than the other three sites. The density fluctuations at Gosford Bay and Silver Sands are also likely to be caused by local conditions.

In 2006, the densities of 0+ group plaice caught on three of the four sites, Limekilns, Silver Sands and Gosford Bay, were much higher than those in 2005 (Figures 30 – 37, Tables 9 – 10). The overall trend at Blackness was of an increase in density at the start of the intertidal phase, followed by a reduction in density at the end of the intertidal phase, which were lower than densities at Blackness 2005 at the end of the intertidal phase. Densities at Limekilns in 2006 were highest in the first sample taken at the start of June, and decreased gradually until the middle of July, before decreasing steeply from the July sample onwards. At Gosford Bay and Silver Sands in 2006, densities fluctuated during the season before a final reduction in November.

Table 9: Summary of catch composition of push net hauls in 2005.

Site	Date	Days since 31st May 2005	Total area swept (m ²)	Total No. of Plaice	Density (ind. m ⁻²)
Blackness	30/05/2005	0	150	20	0.133
	28/06/2005	29	210	20	0.095
	27/07/2005	58	75	17	0.227
	09/08/2005	71	75	3	0.040
	26/08/2005	88	75	17	0.227
Limekilns	21/09/2005	114	75	16	0.213
	27/06/2005	28	60	37	0.617
	11/07/2005	42	120	7	0.058
	28/07/2005	59	135	7	0.052
	11/08/2005	73	75	23	0.307
Gosford Bay	25/08/2005	87	135	12	0.089
	22/09/2005	115	135	9	0.067
	29/06/2005	30	150	17	0.113
	26/07/2005	57	75	18	0.240
	10/08/2005	72	225	9	0.040
Silver Sands	24/08/2005	86	150	13	0.087
	20/09/2005	113	180	20	0.111
	31/05/2005	1	75	13	0.173
	24/06/2005	25	90	133	1.478
	06/07/2005	37	75	49	0.653
	25/07/2005	56	75	123	1.640
	08/08/2005	70	300	30	0.100
	23/08/2005	85	75	45	0.600
	23/09/2005	116	120	28	0.233
	04/10/2005	127	67.5	19	0.281

Table 10: Summary of catch composition of push net hauls in 2006.

Site	Date	Days Since 15th May 2006	Total area swept (m ²)	Total No. of Plaice	Density (ind. m ⁻²)
Blackness	02/06/2006	18	75	14	0.187
	14/06/2006	30	75	47	0.627
	12/07/2006	58	75	17	0.227
	15/08/2006	92	75	4	0.053
	06/09/2006	114	75	0	0.000
	05/10/2006	143	53.85	4	0.074
	22/11/2006	191	75	0	0.000
Limekilns	01/06/2006	17	75	170	2.267
	13/06/2006	29	75	154	2.053
	14/07/2006	60	75	42	0.560
	14/08/2006	91	75	1	0.013
	07/09/2006	115	75	3	0.040
	06/10/2006	144	75	0	0.000
	23/11/2006	192	75	0	0.000
Gosford Bay	30/05/2006	15	75	52	0.693
	15/06/2006	31	75	159	2.120
	10/07/2006	56	225	49	0.218
	21/08/2006	98	75	43	0.573
	09/09/2006	117	75	65	0.867
	08/10/2006	146	75	160	2.133
	25/11/2006	194	150	3	0.020
Silver Sands	15/05/2006	0	N/A	22	N/A
	31/05/2006	16	75	72	0.960
	16/06/2006	32	75	354	4.720
	13/07/2006	59	75	55	0.733
	17/08/2006	94	75	5	0.067
	08/09/2006	116	75	38	0.507
	07/10/2006	145	75	43	0.573
	24/11/2006	193	75	1	0.013

3.3.2. Cohort Analysis – Growth Rates

Changes in median total lengths for each site are shown in Figures 38 – 45. Plots of 2005 median TL (Figures 38 – 41) have predicted growth slopes calculated using Fonds *et al.* (1992) temperature-dependant model, using SMA SSTs as a proxy for average temperature (T in equation 5); 2006 Figures (42-45) have growth rates predicted by the model using temperatures measured on each occasion, in addition to the SMA – predicted growth slopes. The SMA SST predicted growth rates tended to underestimate slightly the growth rates compared to growth rates predicted using directly measured water temperatures, likely to be due to the relatively low spatial and temporal resolution of the satellite data (monthly averages over 9 km²). However, at Limekilns in 2006, growth rates predicted using Fonds *et al.* (1992) model with measured water temperatures showed a negative growth rate between 50 and 100 days after 15th May 2006. This is because the model was based upon the measurement of growth rates of captive laboratory-reared fish, up to a maximum of 22 °C, with the assumption that growth rate of juvenile plaice peak at 18 °C, and temperatures of 26 °C and above are lethal (Waede, 1954 cited in Fonds *et al.*, 1992), with a corresponding drop in growth rate above 22 °C and negative growth rates above 26 °C. The temperature recorded at Limekilns that caused the negative growth in the model was over 28 °C, which is considered lethal (Fonds *et al.*, 1992). However, the growth rate measured

at Limekilns ended up in close agreement with the model after the drop in predicted growth (Figure 43).

The close agreement of the SMA SST – predicted growth rates and the growth rates predicted by directly measured water temperatures in 2006, suggest that the SMA SST predicted growth rates in 2005 are likely to be very close to growth rates predicted using water temperatures, had they been measured, thus are useful indicators of maximum growth at these sites.

The growth rates measured by tracking the change in median TL are lower than the growth rates predicted by Fonds *et al.* (1992) model at all sites and both years.

Table 11: Growth rates calculated by change in median total lengths of push net hauls in 2005.

2005		
Site	Overall Rate (mm day ⁻¹)	Peak Rate (mm day ⁻¹)
Blackness	0.40	0.65
Limekilns	0.37	0.97
Gosford Bay	0.43	0.67
Silver Sands	0.29	0.58

Table 12: Growth rates calculated by change in median total lengths of push net hauls in 2006.

2006		
Site	Overall Rate (mm day ⁻¹)	Peak Rate (mm day ⁻¹)
Blackness	0.25	0.29
Limekilns	0.18	0.33
Gosford Bay	0.18	0.53
Silver Sands	0.25	0.40

Overall and peak growth rates for both years are shown in Tables 11 and 12. Overall growth rates calculated using the change in median total lengths in 2005 (Table 11), were similar at three sites, Gosford Bay (highest), Blackness and Limekilns, with Silver Sands the lowest overall growth rate. Peak growth rates (maximum change in median TL between two consecutive sampling dates) were different: these showed that Limekilns had the highest growth rate, Gosford Bay and Blackness were similar and Silver Sands was again the lowest.

In 2006, overall growth rates were much lower than 2005 for Blackness, Limekilns and Gosford Bay, while Silver Sands 2006 had a slightly lower overall growth rate than Silver Sands in 2005. Blackness and Silver Sands had the highest overall growth rates in 2006 and were identical, while Limekilns and Gosford Bay had the lowest overall growth rates and were also identical. Peak growth rates in 2006 were also lower than peak growth rates recorded in 2005. The peak growth rates for Gosford Bay were the highest, while the other Firth site, Silver Sands, had the next highest; the two estuarine sites had the lowest peak growth rates, with Limekilns slightly higher than Blackness.

Regression parameters for changes in length-frequencies and results of ANCOVA testing for homogeneity of regression are shown in Table 13. These parameters are for ln-transformed total lengths (mm). Results of

Tukeys multiple comparisons are shown in Tables 14 and 15. Testing for homogeneity of regression coefficients showed a significant difference between sites in 2005 ($F = 4.31$; $df = 3, \infty$; $p < 0.01$) and 2006 ($F = 36.00$; $df = 3, \infty$; $p < 0.001$). Tukeys multiple comparisons for 2005 and 2006 regression coefficients indicated that, in 2005, the two estuarine sites were significantly different from the two Outer Firth sites only and the two Outer Firth sites were not significantly different from each other. The regression parameters suggest the estuarine sites showed higher growth rates than the Outer Firth sites in 2005 (Table 14). In 2006, Limekilns was significantly different from all of the other sites, and Gosford Bay was significantly different from Silver Sands. Blackness was not significantly different from any of the sites in 2006 (Table 15). Table 16 shows between year comparisons of each sites growth rates; all sites were significantly different from the same site between years. This highlights the greater between-year variability of growth rates, and that the estuary and the Outer Firth sites can have similar growth patterns.

Table 13: Regression parameters for ln-transformed TLs' (y), measured from push net samples. * indicates significant r^2 . Values of x are days (no of days since 15th May 2005 and 30th May 2006, respectively).

Year	Site	b	a	r^2
2005	Blackness	0.011	3.025	0.851*
	Limekilns	0.011	2.984	0.778*
	Silver Sands	0.009	3.054	0.769*
	Gosford Bay	0.009	3.150	0.650*
2006	Blackness	0.007	3.069	0.542*
	Limekilns	0.003	3.265	0.070*
	Silver Sands	0.008	2.944	0.750*
	Gosford Bay	0.006	3.058	0.680*

Table 14: Parameters from Tukeys multiple comparisons test of 2005 ln-transformed TLs' from push-net samples. SE is standard error; q is the test statistic (calculated using SE and b coefficients' of the relevant site in Table 13). * indicates significant differences at $\alpha = 0.05$, ** indicates significant differences at $\alpha = 0.001$ ($k = 4$, $DF_p = \infty$ (actual number is 675)).

Comparison	$(S^2_{Y.X})_p$	SE	q
Blackness 2005 - Limekilns 2005	0.087	0.00058	-0.24
Blackness 2005 - Silver Sands 2005	0.092	0.00048	-3.79*
Blackness 2005 - Gosford Bay 2005	0.106	0.00059	-3.70*
Limekilns 2005 - Silver Sands 2005	0.086	0.00041	-4.14*
Limekilns 2005 - Gosford Bay 2005	0.087	0.00049	-4.25*
Silver Sands 2005 - Gosford Bay 2005	0.091	0.00035	-1.05

Table 15: Parameters from Tukeys multiple comparisons test of 2006 ln-transformed TLs' from push-net samples. SE is standard error; q is the test statistic (calculated using SE and b coefficients' of the relevant site in Table 13). * indicates significant differences at $\alpha = 0.05$, ** indicates significant differences at $\alpha = 0.001$ ($k = 4$, $DF_p = \infty$ (actual number is 1565)).

Comparison	$(S^2_{Y.X})_p$	SE	q
Blackness 2006 - Limekilns 2006	0.023	0.00060	-7.78**
Blackness 2006 - Silver Sands 2006	0.030	0.00049	0.89
Blackness 2006 - Gosford Bay 2006	0.042	0.00056	-3.00
Limekilns 2006 - Silver Sands 2006	0.028	0.00042	12.23**
Limekilns 2006 - Gosford Bay 2006	0.036	0.00046	6.41**
Silver Sands 2006 - Gosford Bay 2006	0.036	0.00018	11.50**

Table 16: Parameters from between years comparisons of growth estimates at each site, from t-tests of homogeneity of regression coefficients using ln-transformed TLs'. t is the test statistic, v is degrees of freedom, and p is the significance level of the test.

Site	t	v	p
Blackness	3.248	159	<0.005
Limekilns	11.636	458	<0.001
Silver Sands	4.856	1023	<0.001
Gosford Bay	8.602	600	<0.001

Blackness 2005

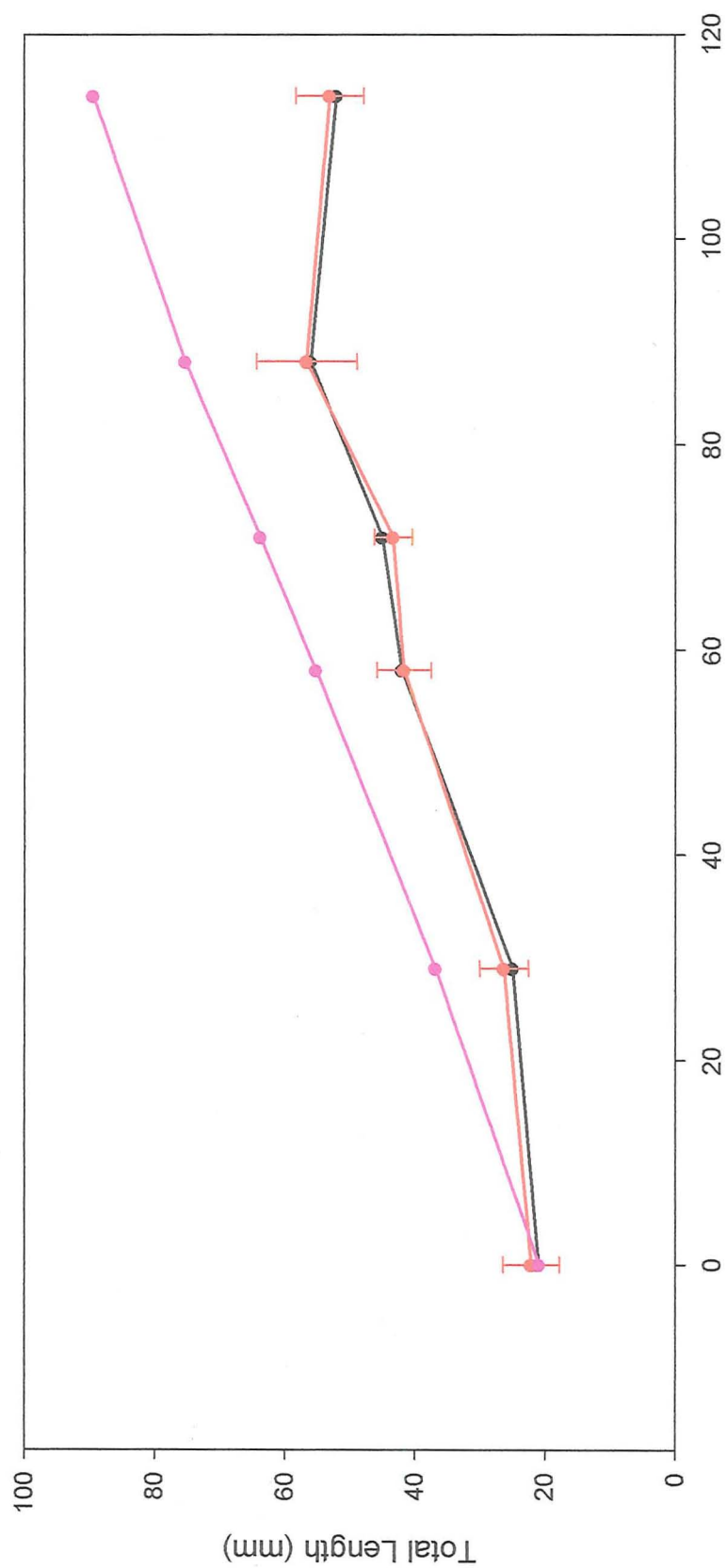


Figure 38: Change in median total length (mm) at Blackness in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates. Error bars are $\pm$ S.E.

Limekilns 2005

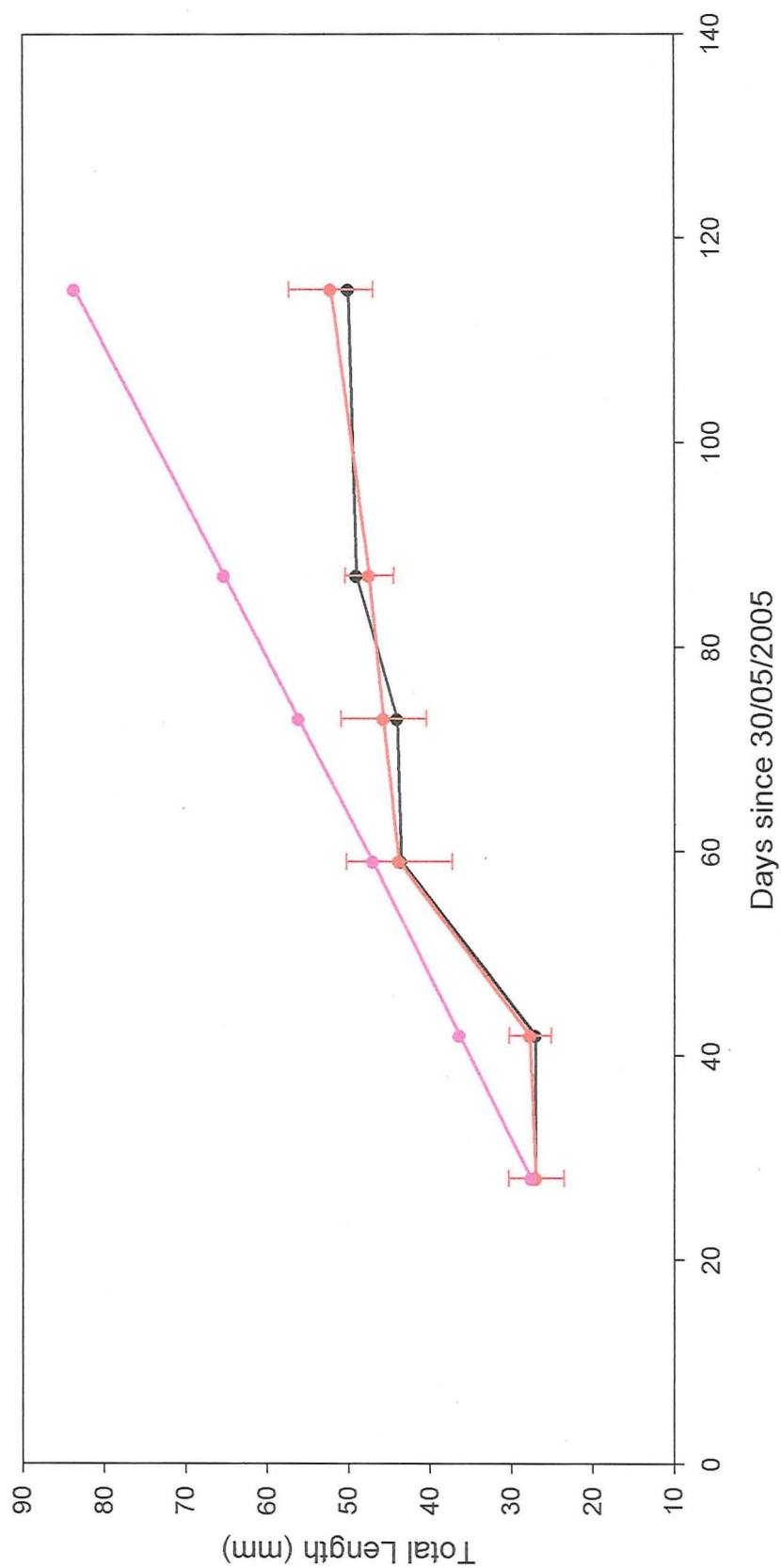


Figure 39: Change in median total length (mm) at Limekilns in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates. Error bars are $\pm$ S.E.

Gosford Bay 2005

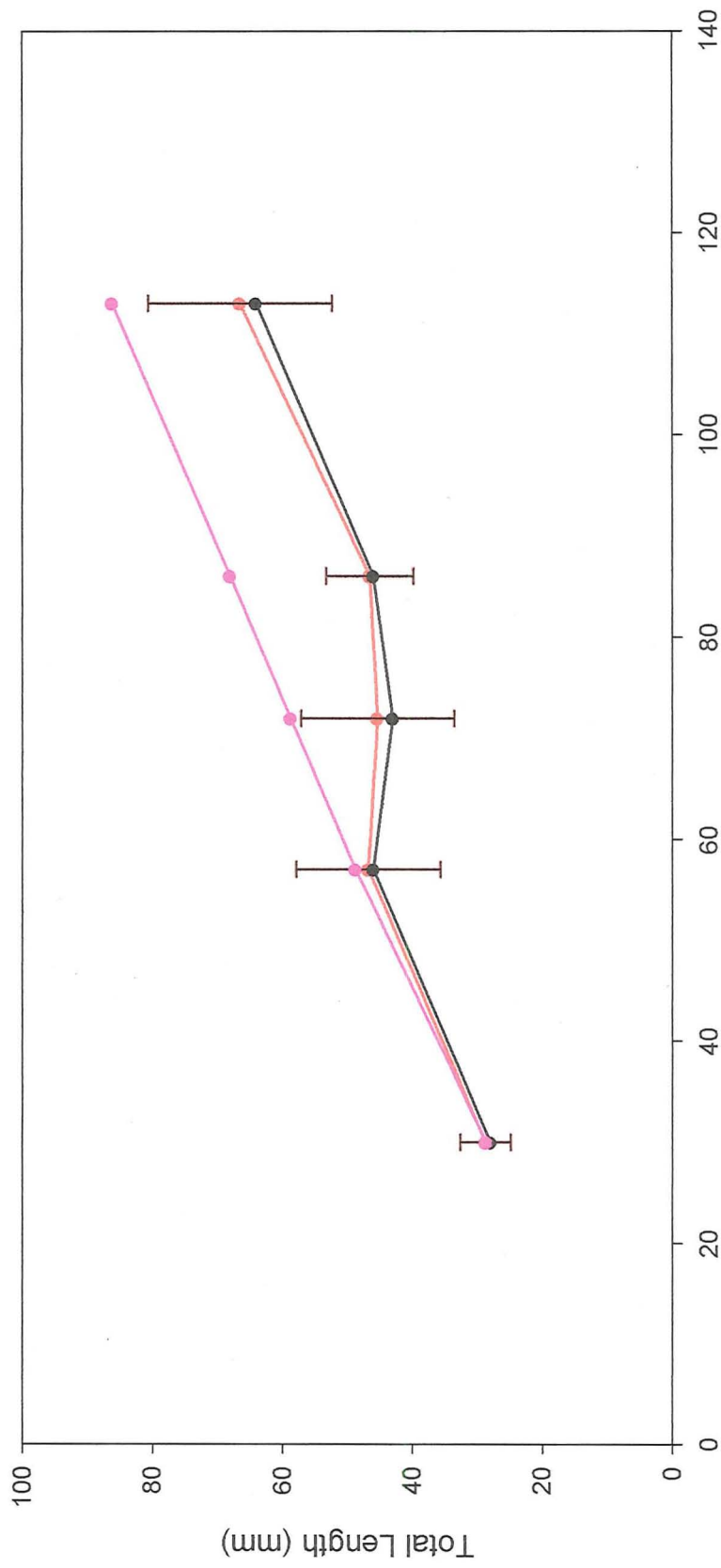


Figure 40: Change in median total length (mm) at Gosford Bay in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates. Error bars are $\pm$ S.E.

Silver Sands 2005

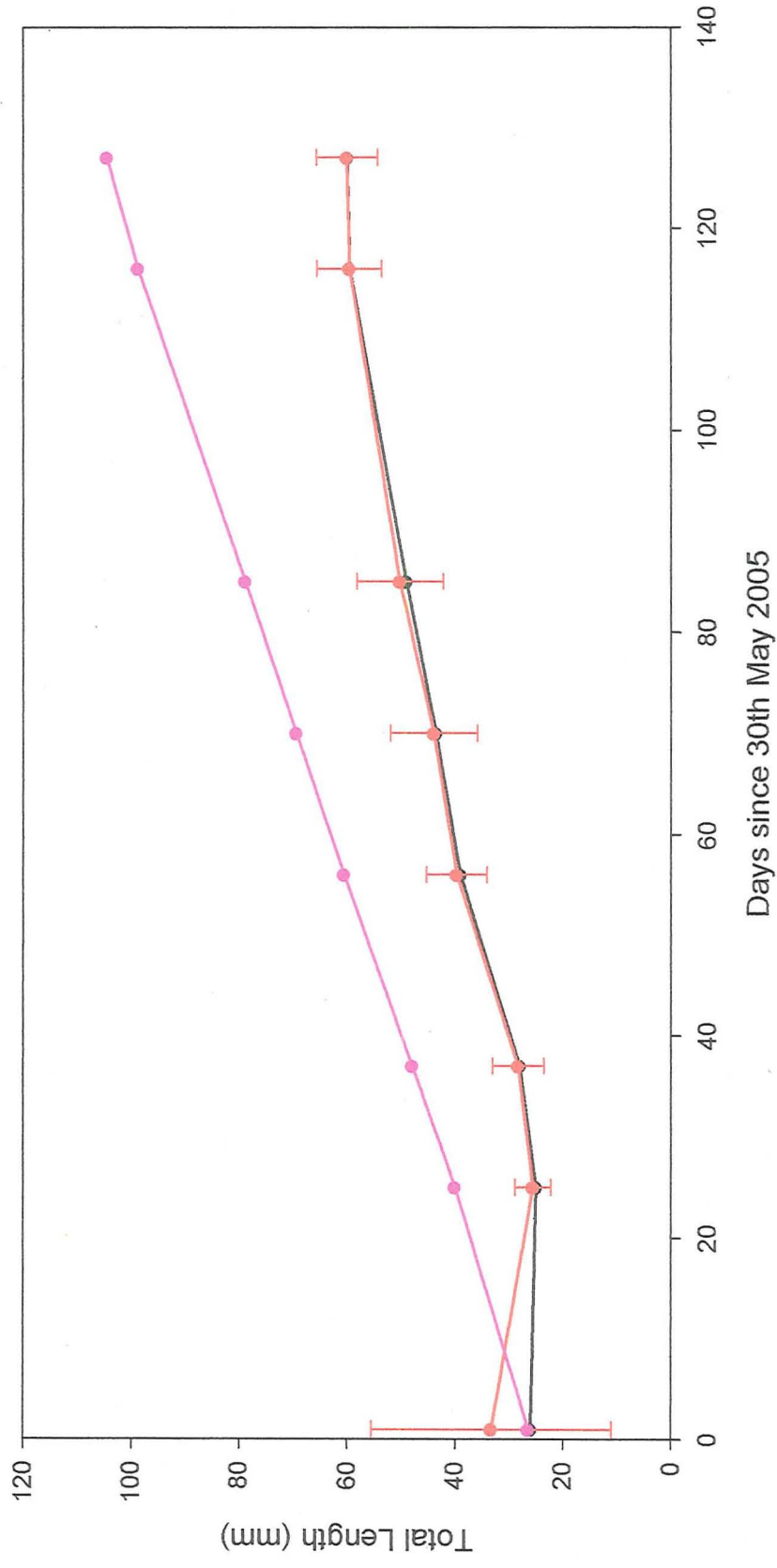


Figure 41: Change in median total length (mm) at Silver Sands in 2005. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates. Error bars are $\pm$ S.E.

Blackness 2006

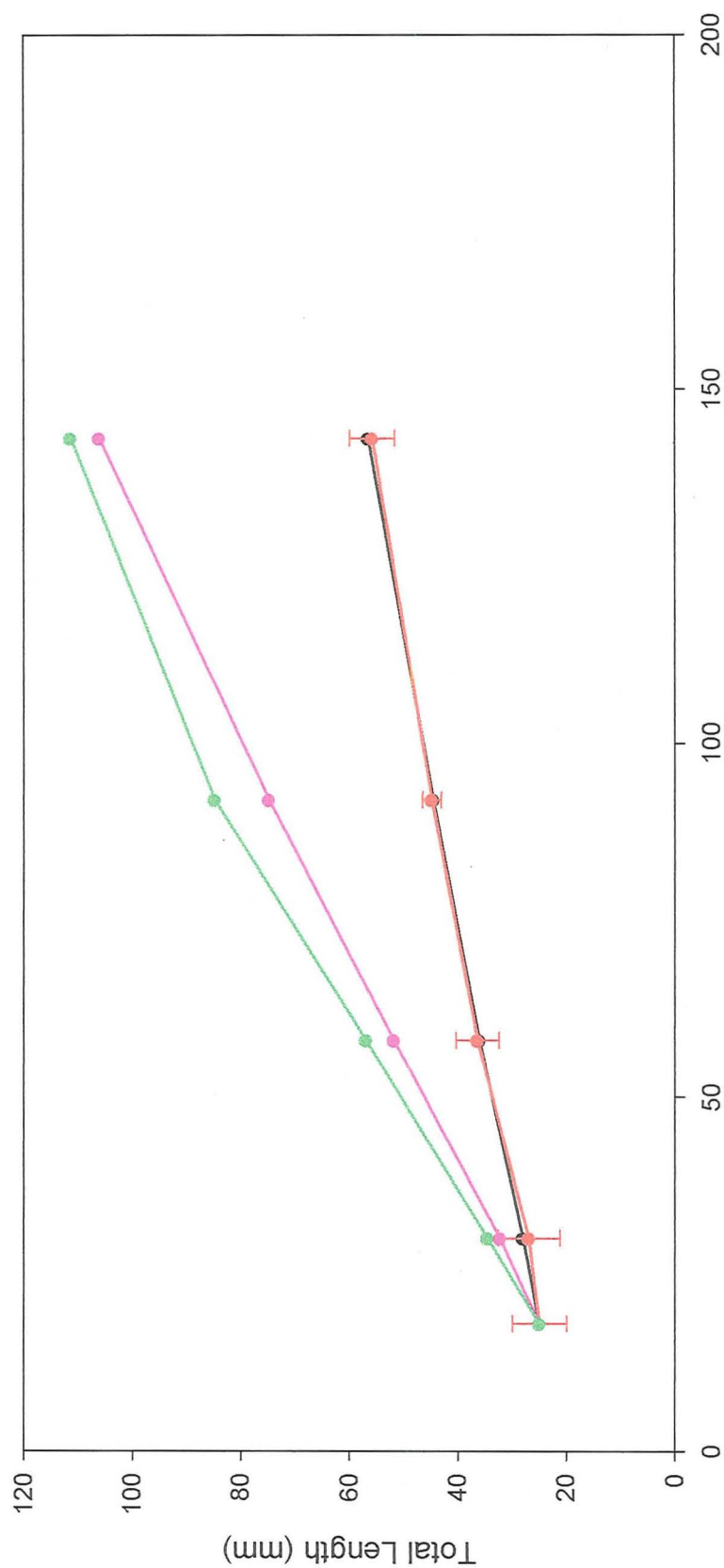
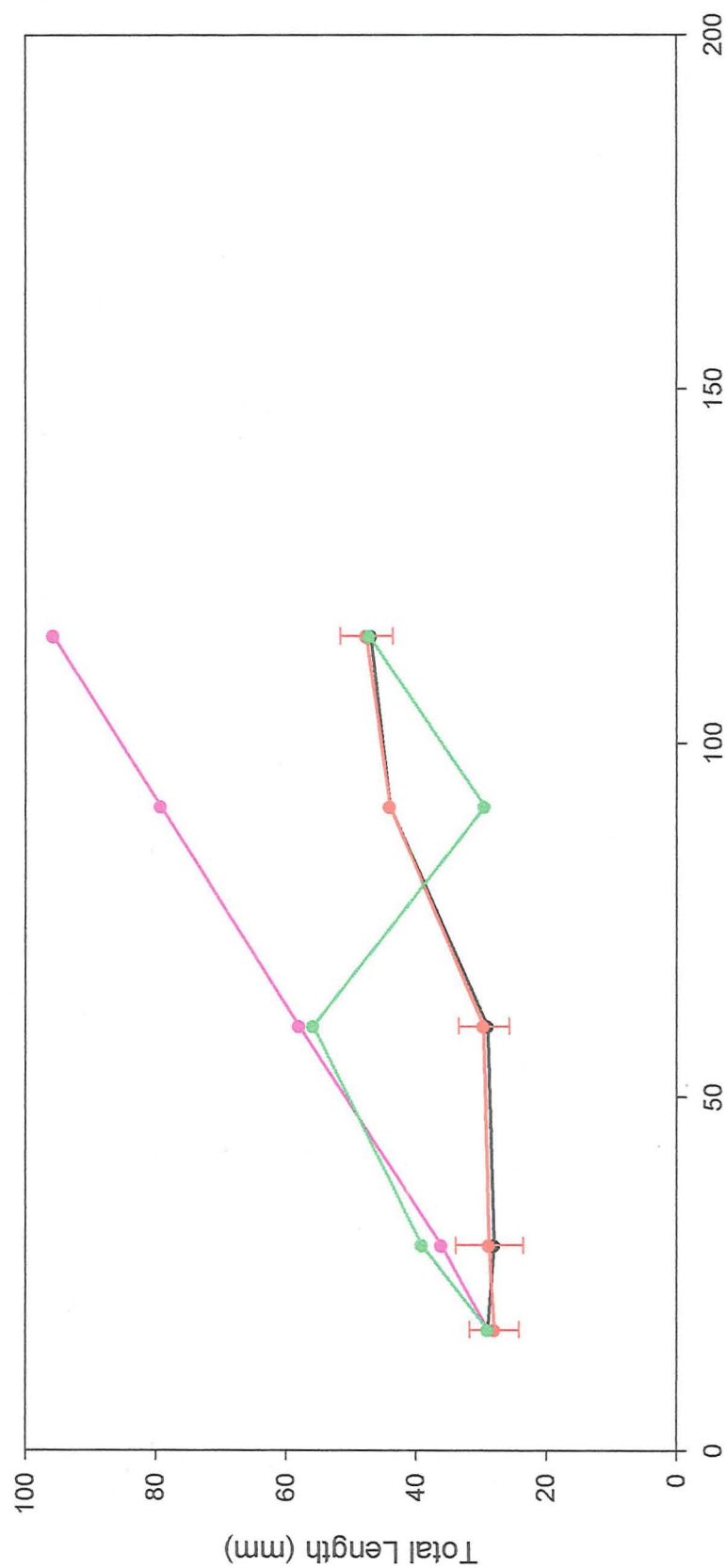


Figure 42: Change in median total length (mm) at Blackness in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWiifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates Error bars are $\pm$ S.E.

Limekilns 2006



Days since 15th May 2006

Figure 43: Change in median total length (mm) at Limekilns in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates. Error bars are $\pm$ S.E.

Gosford Bay 2006

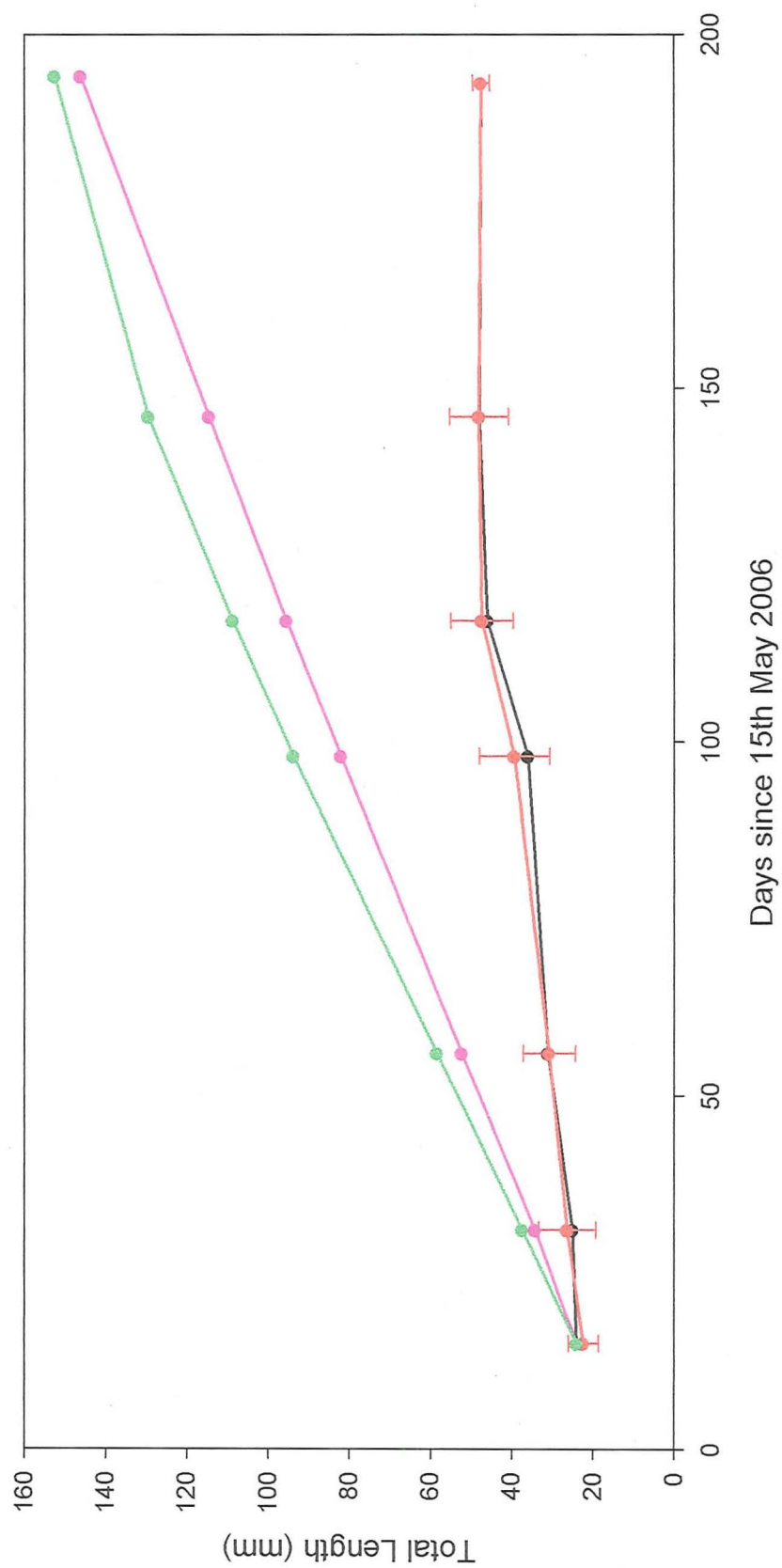


Figure 44: Change in median total length (mm) at Gosford Bay in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWiFS/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates. Error bars are $\pm$ S.E.

Silver Sands 2006

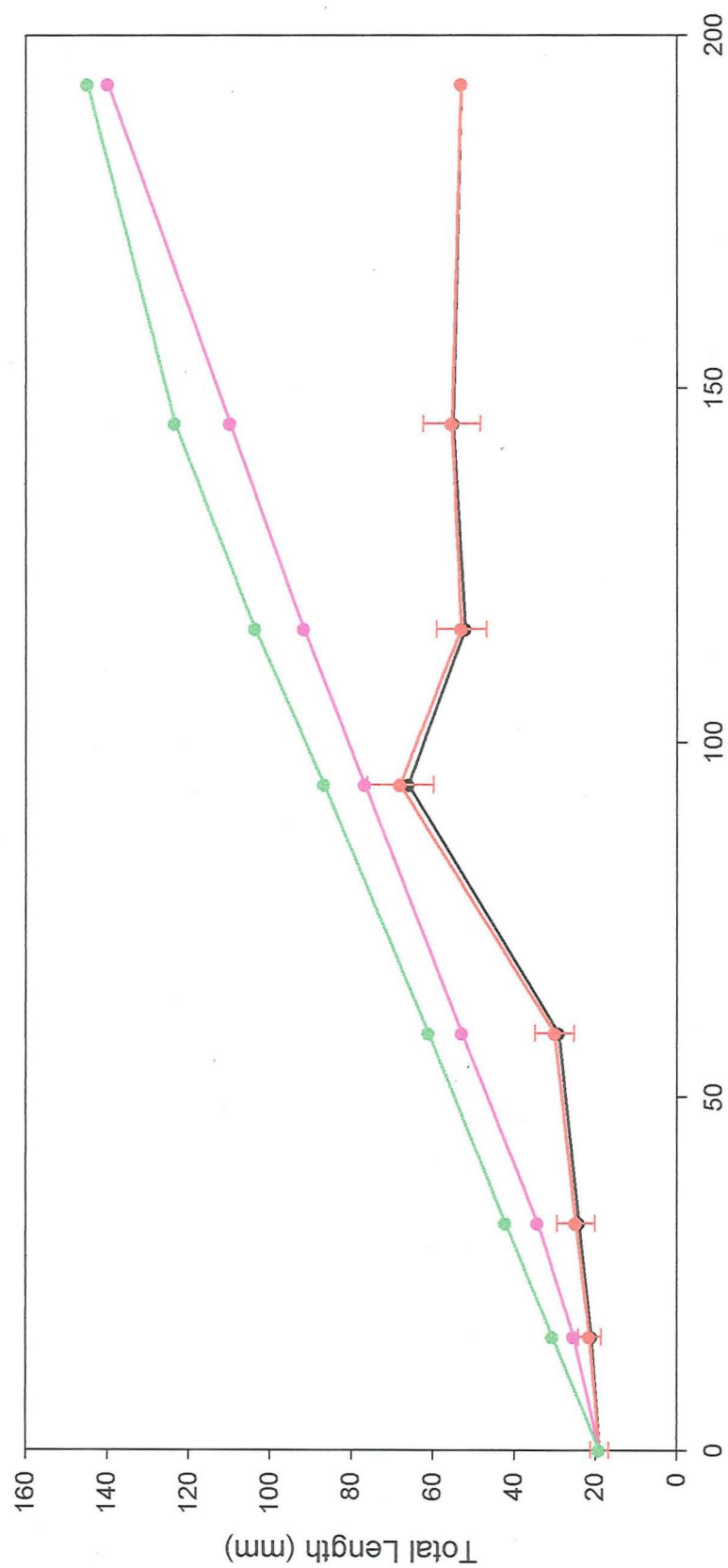


Figure 45: Change in median total length (mm) at Silver Sands in 2006. Red symbols are means, black symbols are medians, pink symbols are SeaWiifs/MODIS-Aqua predicted growth rates, green symbols are mean temperature predicted growth rates. Error bars are $\pm$ S.E.

3.3.3. Otolith Increment Analysis

Otolith microincrement counts, TL (mm) of each individual, capture dates, settlement dates and individual growth rates are shown in Tables 18 – 21. There are two different growth rates calculated for each individual plaice in Tables 18 – 21. These are calculated for settlement lengths of 12 and 15 mm. 12 mm is usually acknowledged as the length of plaice at metamorphosis, corresponding to settlement onto the benthic habitat (Amara and Paul, 2003), while Modin *et al.* (1996) found complete metamorphosis at lengths up to 15 mm. Scatterplots of TL (mm) vs. age in days (otolith microincrements since settlement), with fitted line plots calculated by least – squares and corresponding regression equations with the y-intercept set at 12 mm, are shown in Figures 46 – 49. The calculated slopes indicate the increase in total length over time for plaice caught at each site in mm day^{-1} . Regression slopes indicate a slightly different pattern of growth rates compared to growth rates estimated by tracking changes in length – frequency distributions over time. Growth rates calculated using otolith increments indicate that Gosford Bay and Blackness had the highest growth rates in 2005, however Silver Sands was the second lowest with Limekilns the lowest growth rate in 2005. Growth rates calculated by length – frequency distributions indicated that Silver Sands was the lowest and Limekilns the second lowest, a reversal of the otolith increment – calculated growth rates.

Regression parameters are shown on each scatterplot. ANCOVA testing for homogeneity of regression coefficients indicated no difference

between growth estimates for each site in 2005 ($F = 1.178$, $df = 3, 67$, $p > 0.25$).

In addition, scatterplots of growth rate vs. settlement date, with lines fitted by least – squares regression, coefficients of determination (r^2) and correlation coefficients, shown in Figures 50 – 53, indicate that growth rates of individual plaice were higher if the plaice settled later in the year; this was true at all sites. It must be noted that lines fitted by regression in Figures 50 – 53 are used only to indicate the trend of the growth rates over time, no conclusions can be drawn regarding the dependence of growth rates on settlement date.

Table 18: Increment analysis of Blackness 2005 otoliths. Growth rate_{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.

TL (mm)	Increment Count	Capture Date	Settlement date	Growth Rate ₁₂ (mm day ⁻¹)	Growth Rate ₁₅ (mm day ⁻¹)
69	103	26/08/2005	15/05/2005	0.55	0.52
47	59	27/07/2005	29/05/2005	0.59	0.54
35	56	27/07/2005	01/06/2005	0.41	0.36
45	66	09/08/2005	04/06/2005	0.50	0.45
46	52	27/07/2005	05/06/2005	0.65	0.60
37	52	27/07/2005	05/06/2005	0.48	0.42
39	51	27/07/2005	06/06/2005	0.53	0.47
42	50	27/07/2005	07/06/2005	0.60	0.54
59	79	26/08/2005	08/06/2005	0.59	0.56
48	46	27/07/2005	11/06/2005	0.78	0.72
42	39	27/07/2005	18/06/2005	0.77	0.69
56	68	26/08/2005	19/06/2005	0.65	0.60
40	50	09/08/2005	20/06/2005	0.56	0.50
51	67	26/08/2005	20/06/2005	0.58	0.54
50	67	26/08/2005	20/06/2005	0.57	0.52
33	36	27/07/2005	21/06/2005	0.58	0.50
69	63	26/08/2005	24/06/2005	0.90	0.86
56	61	26/08/2005	26/06/2005	0.72	0.67
53	78	21/09/2005	05/07/2005	0.53	0.49
52	69	21/09/2005	14/07/2005	0.58	0.54
59	68	21/09/2005	15/07/2005	0.69	0.65
62	61	21/09/2005	22/07/2005	0.82	0.77
55	61	21/09/2005	22/07/2005	0.70	0.66

Table 19: Increment analysis of Limekilns 2005 otoliths. Growth rate_{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.

TL (mm)	Increment Count	Capture Date	Settlement date	Growth Rate ₁₂ (mm day ⁻¹)	Growth Rate ₁₅ (mm day ⁻¹)
26	49	27/06/2005	09/05/2005	0.29	0.22
30	43	27/06/2005	15/05/2005	0.42	0.35
26	43	27/06/2005	15/05/2005	0.33	0.26
49	68	28/07/2005	21/05/2005	0.54	0.50
25	33	27/06/2005	25/05/2005	0.39	0.30
24	40	11/07/2005	01/06/2005	0.30	0.23
37	56	28/07/2005	02/06/2005	0.45	0.39
42	55	28/07/2005	03/06/2005	0.55	0.49
37	55	28/07/2005	03/06/2005	0.45	0.40
42	53	28/07/2005	05/06/2005	0.57	0.51
61	52	28/07/2005	06/06/2005	0.94	0.88
49	48	28/07/2005	10/06/2005	0.77	0.71
55	62	11/08/2005	10/06/2005	0.69	0.65
41	47	28/07/2005	11/06/2005	0.62	0.55
45	51	25/08/2005	05/07/2005	0.65	0.59
56	77	22/09/2005	07/07/2005	0.57	0.53
62	74	22/09/2005	10/07/2005	0.68	0.64
50	71	22/09/2005	13/07/2005	0.54	0.49
57	69	22/09/2005	15/07/2005	0.65	0.61

Table 20: Increment analysis of Silver Sands 2005 otoliths. Growth rate_{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.

TL (mm)	Increment Count	Capture Date	Settlement date	Growth Rate ₁₂ (mm day ⁻¹)	Growth Rate ₁₅ (mm day ⁻¹)
33	66	06/07/2005	01/05/2005	0.32	0.27
31	46	06/07/2005	21/05/2005	0.41	0.35
24	33	24/06/2005	22/05/2005	0.36	0.27
28	43	06/07/2005	24/05/2005	0.37	0.30
20	43	06/07/2005	24/05/2005	0.19	0.12
35	37	06/07/2005	30/05/2005	0.62	0.54
46	68	08/08/2005	01/06/2005	0.50	0.46
50	67	08/08/2005	02/06/2005	0.57	0.52
46	64	08/08/2005	05/06/2005	0.53	0.48
26	27	06/07/2005	09/06/2005	0.52	0.41
48	65	23/08/2005	19/06/2005	0.55	0.51
41	35	25/07/2005	20/06/2005	0.83	0.74
54	63	23/08/2005	21/06/2005	0.67	0.62
71	101	04/10/2005	25/06/2005	0.53	0.55
65	78	23/09/2005	07/07/2005	0.68	0.64
42	46	23/08/2005	08/07/2005	0.65	0.59
67	68	23/09/2005	17/07/2005	0.81	0.76
73	68	23/09/2005	17/07/2005	0.90	0.85
62	66	23/09/2005	19/07/2005	0.76	0.71
56	55	23/09/2005	30/07/2005	0.80	0.75

Table 21: Increment analysis of Gosford Bay 2005 otoliths. Growth rate_{12, 15} calculated using initial total length of plaice as 12 or 15 mm, respectively.

TL (mm)	Increment Count	Capture Date	Settlement date	Growth Rate ₁₂ (mm day ⁻¹)	Growth Rate ₁₅ (mm day ⁻¹)
35	59	29/06/2005	01/05/2005	0.39	0.34
28	49	29/06/2005	11/05/2005	0.33	0.27
26	42	29/06/2005	18/05/2005	0.33	0.26
32	42	29/06/2005	18/05/2005	0.48	0.40
76	78	10/08/2005	24/05/2005	0.82	0.78
96	110	20/09/2005	02/06/2005	0.76	0.74
50	51	26/07/2005	05/06/2005	0.75	0.69
41	62	10/08/2005	09/06/2005	0.47	0.42
46	73	24/08/2005	12/06/2005	0.47	0.42
62	68	24/08/2005	17/06/2005	0.74	0.69
67	89	20/09/2005	23/06/2005	0.62	0.58
56	84	20/09/2005	28/06/2005	0.52	0.49

Blackness 2005

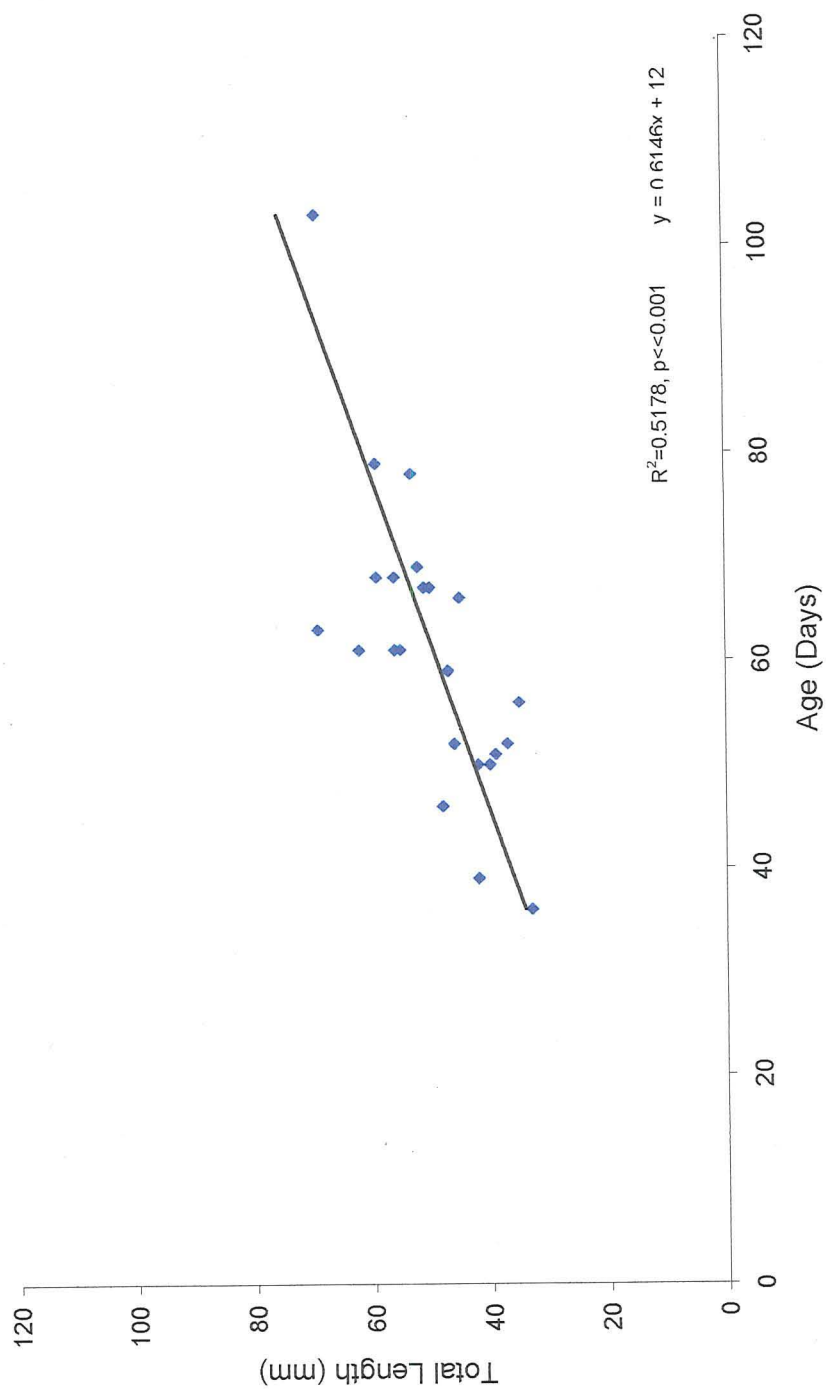


Figure 46: Scatterplot of individual fish age in days against total length in mm from Blackness in 2005. The co-efficient of regression is an estimate of growth rate on this site, $p < 0.001$. $n = 23$.

Limekilns 2005

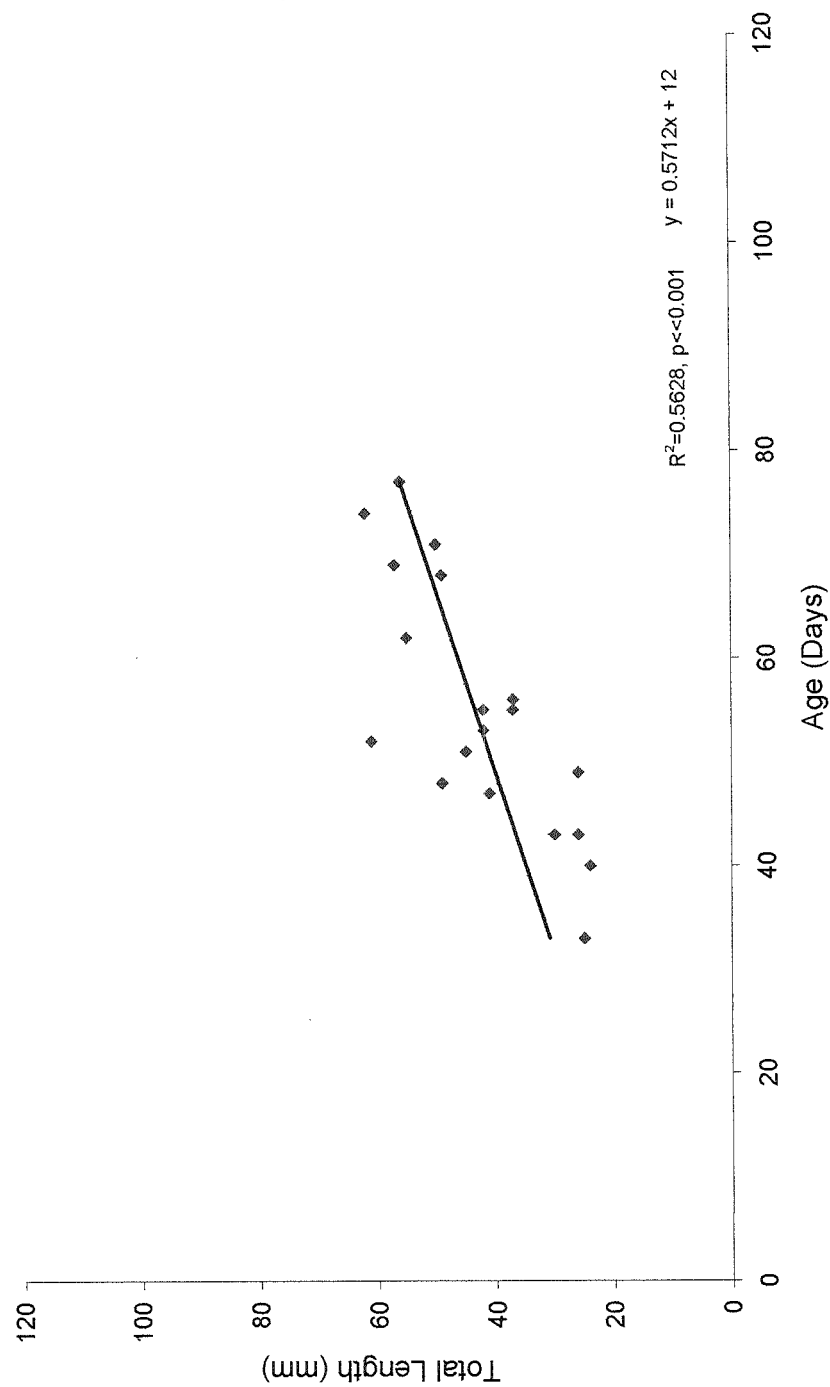


Figure 47: Scatterplot of individual fish age in days against total length in mm from Limekilns in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 19$.

Silver Sands 2005

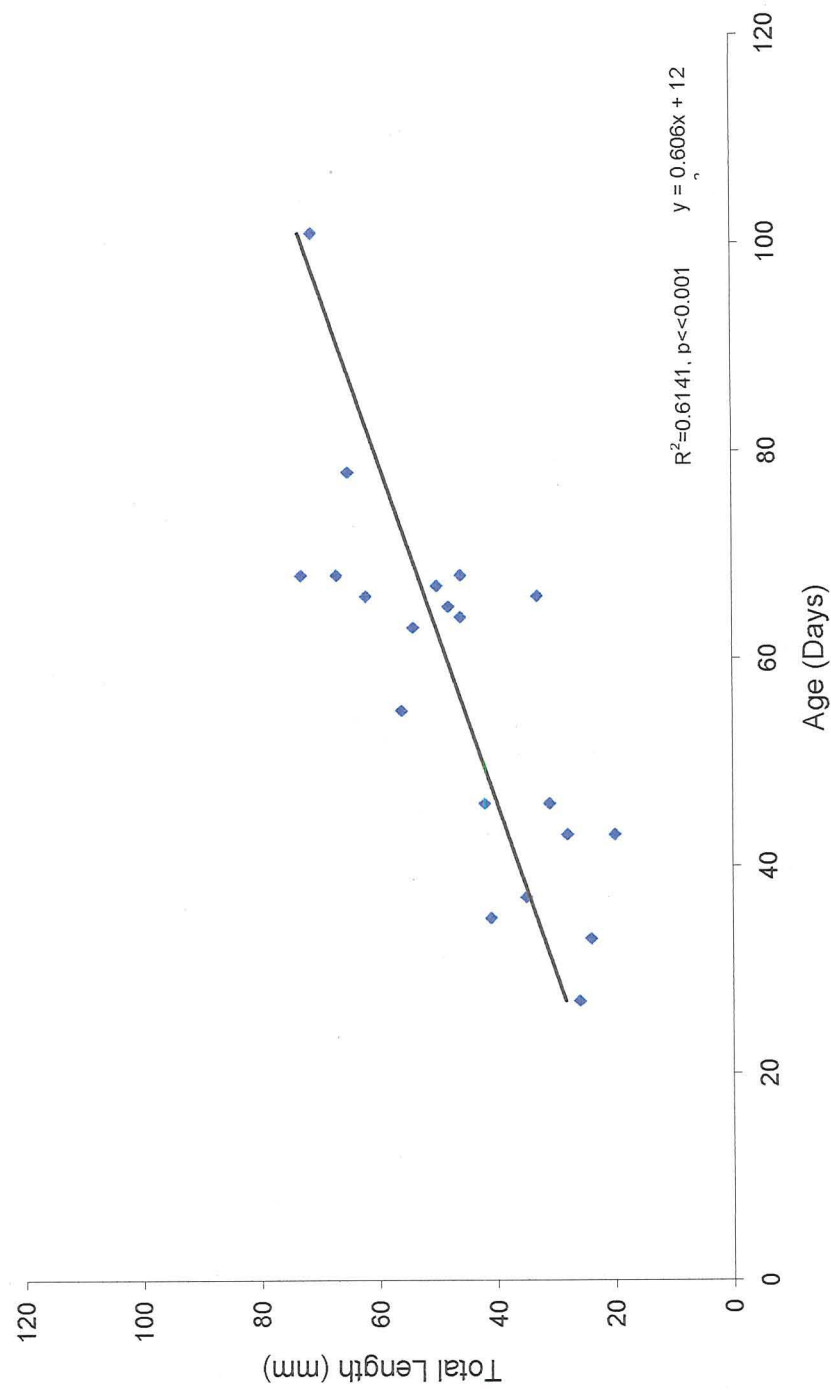


Figure 48: Scatterplot of individual fish age in days against total length in mm from Silver Sands in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 20$.

Gosford Bay 2005

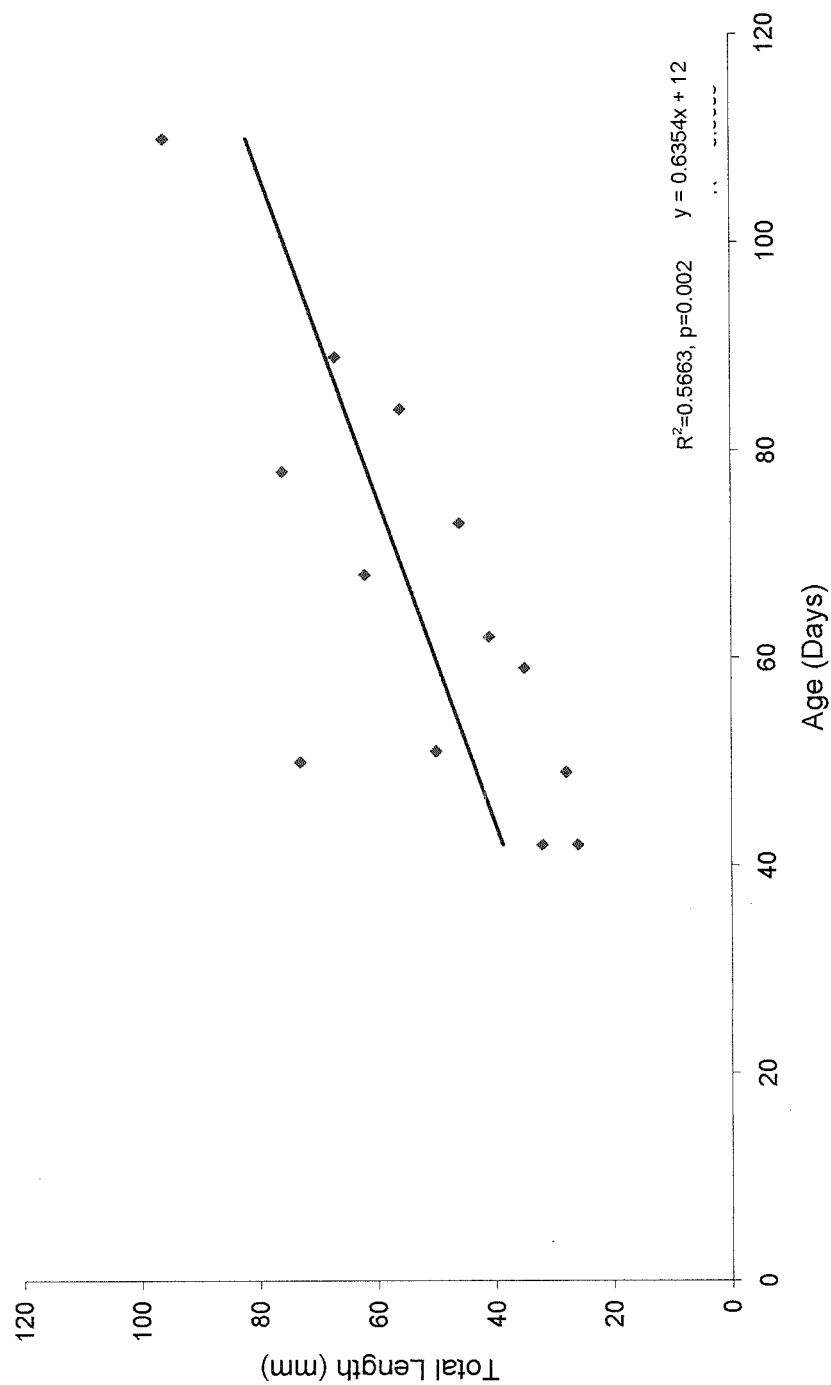


Figure 49: Scatterplot of individual fish age in days against total length in mm from Gosford Bay in 2005. The co-efficient of regression is an estimate of growth rate on this site. $n = 13$.

Blackness 2005

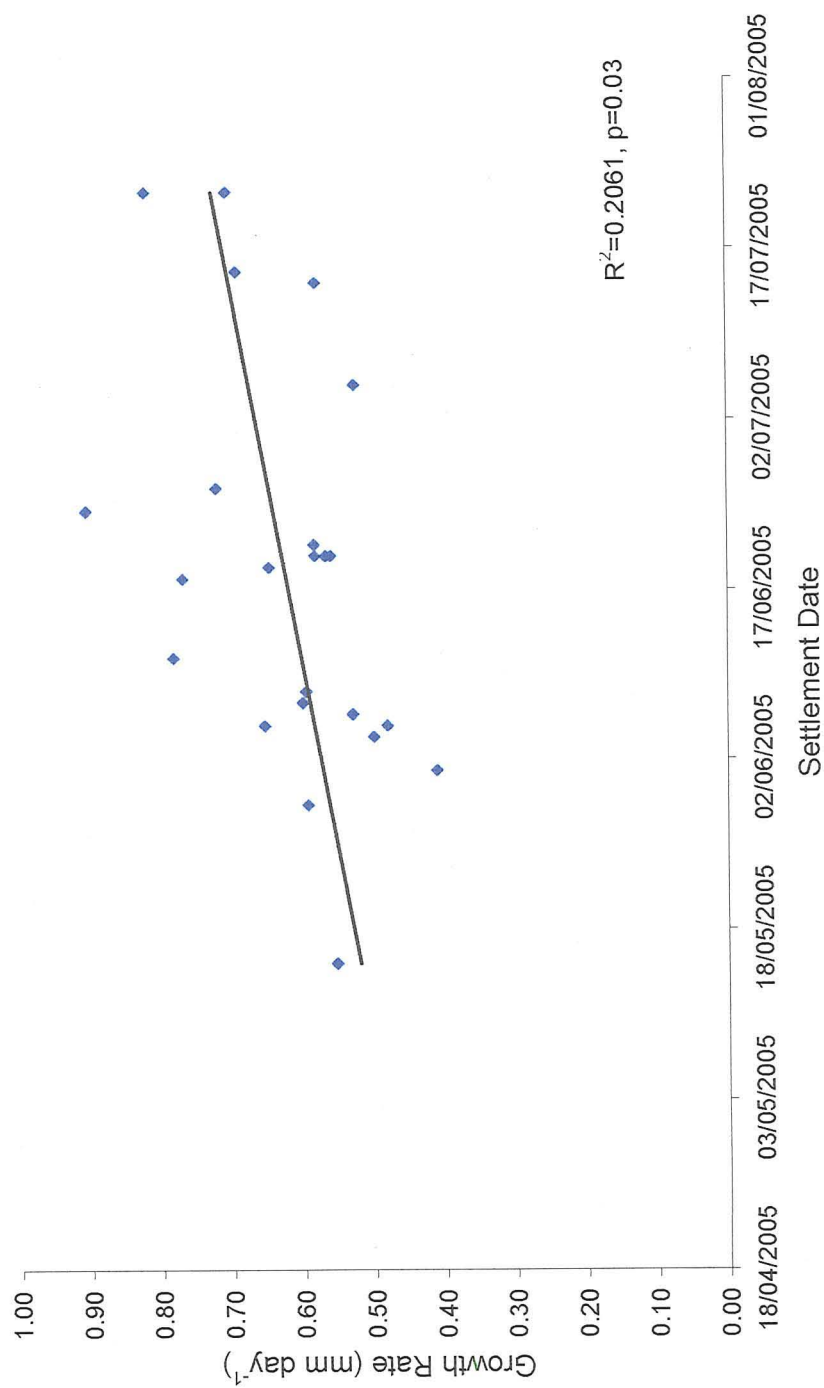


Figure 50: Scatterplot of individual fish settlement date against otolith-derived growth rate from Blackness in 2005. $n = 23$.

Limekilns 2005

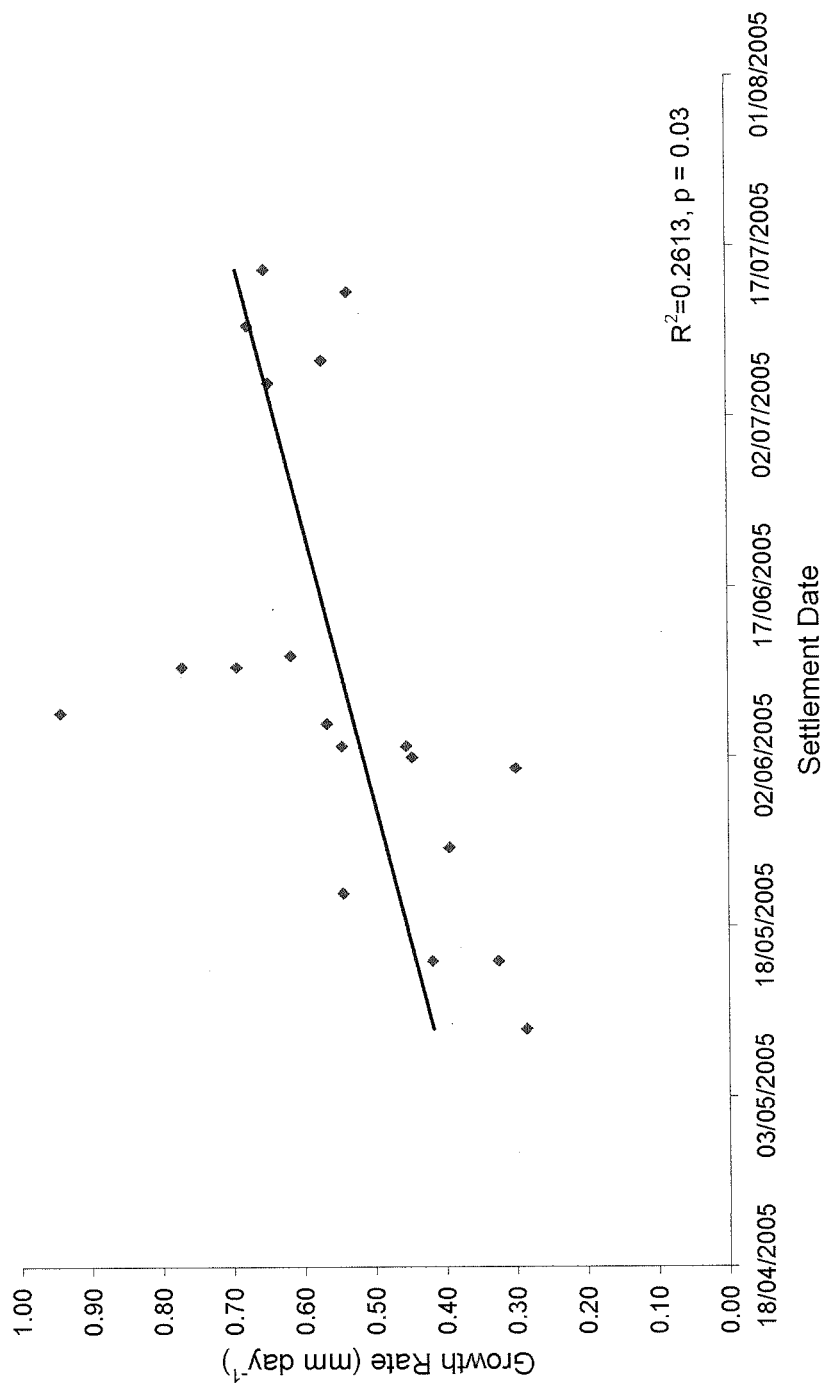


Figure 51: Scatterplot of individual fish settlement date against otolith-derived growth rate from Limekilns in 2005. $n = 19$.

Silver Sands 2005

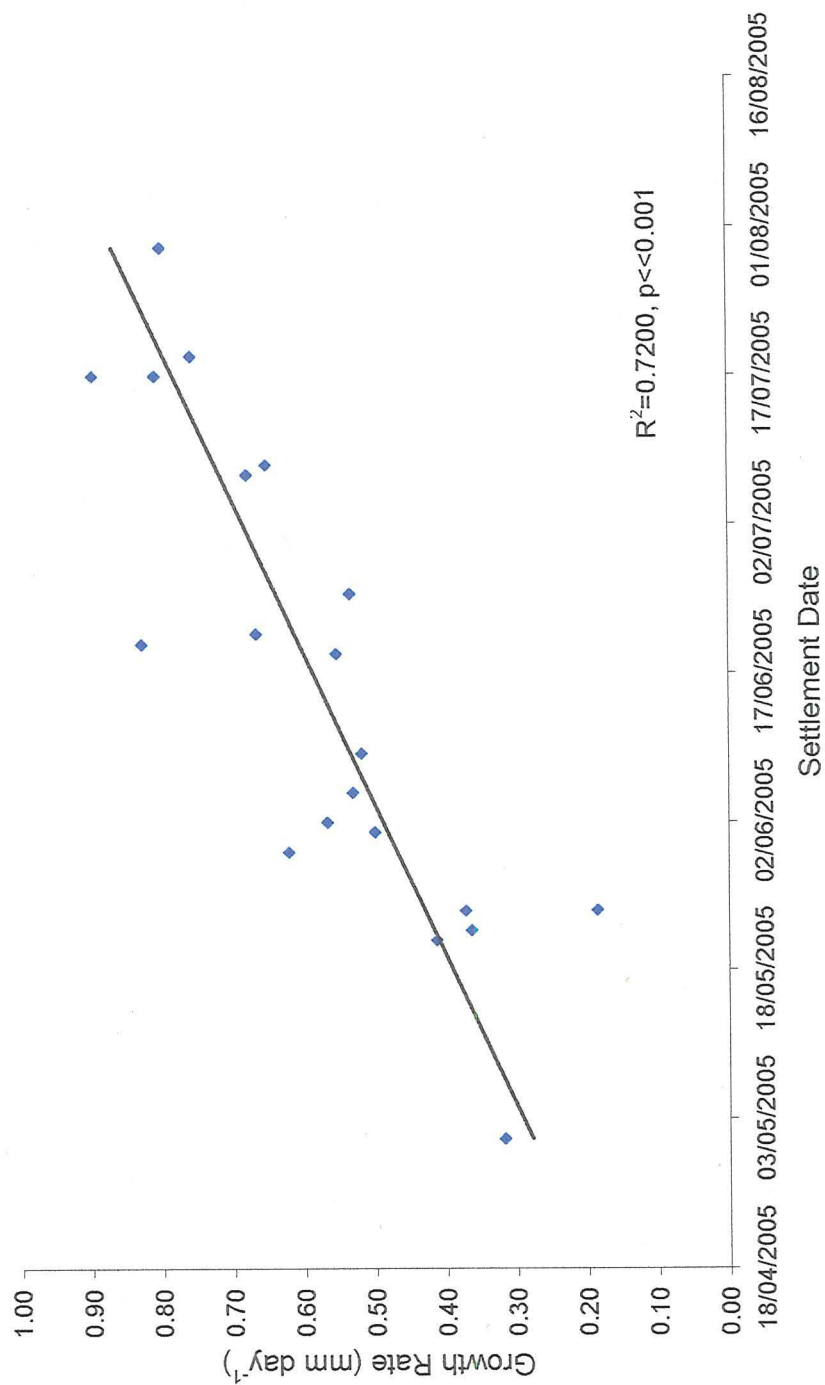


Figure 52: Scatterplot of individual fish settlement date against otolith-derived growth rate from Silver Sands in 2005. $n = 20$.

Gosford Bay 2005

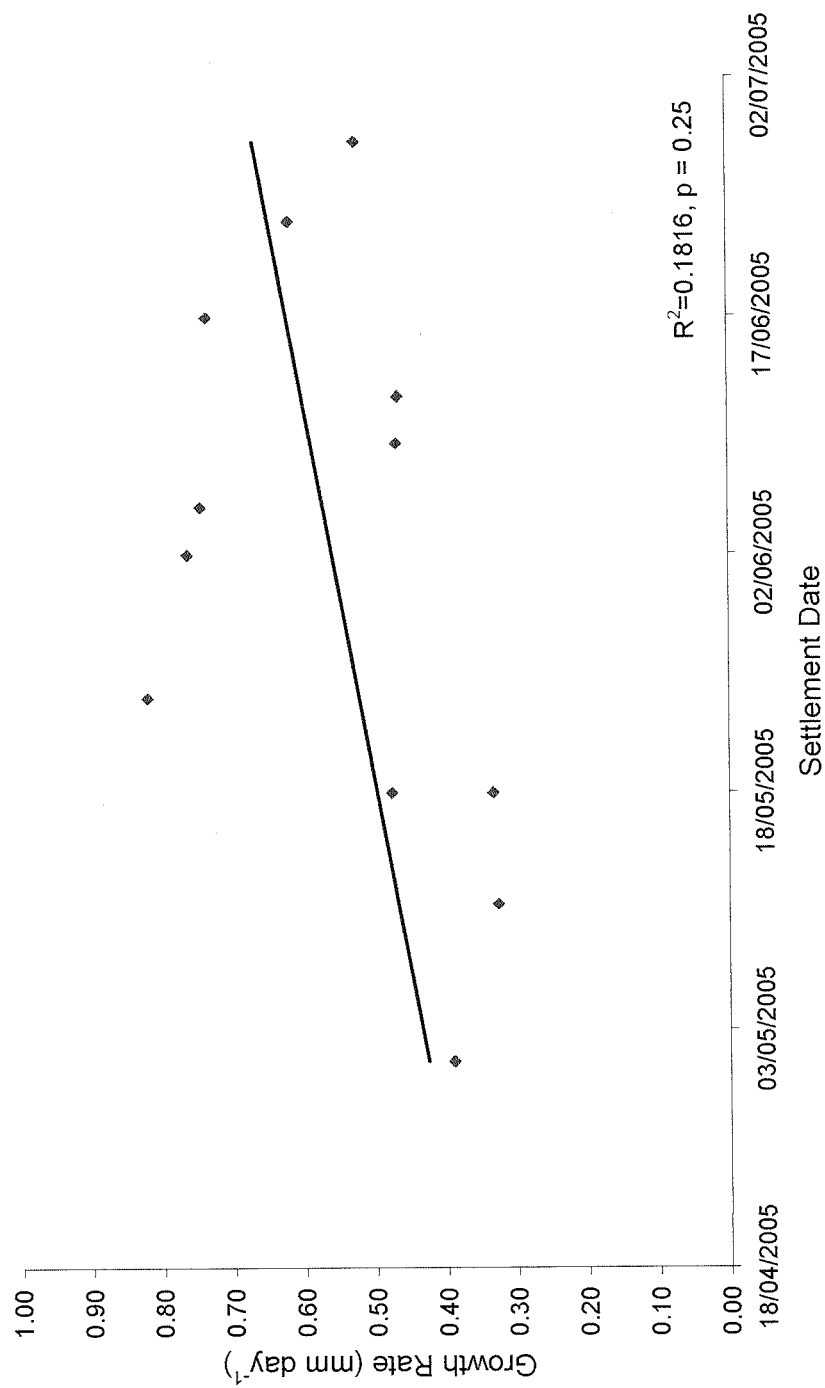


Figure 53: Scatterplot of individual fish settlement date against otolith-derived growth rate from Gosford Bay in 2005. $n = 13$.

Table 17: Site comparisons of growth rates estimated by push net lengths with growth rates estimated by otolith increment analysis. All TLs were ln-transformed and regression coefficients compared for differences using t-tests. No significant differences were found between slopes of regression estimated using different methods.

Site	<i>t</i>	<i>v</i>	<i>p</i>
Blackness	0.839	96	>0.05
Limekilns	1.648	108	>0.05
Silver Sands	0.393	454	>0.05
Gosford Bay	0.705	83	>0.05

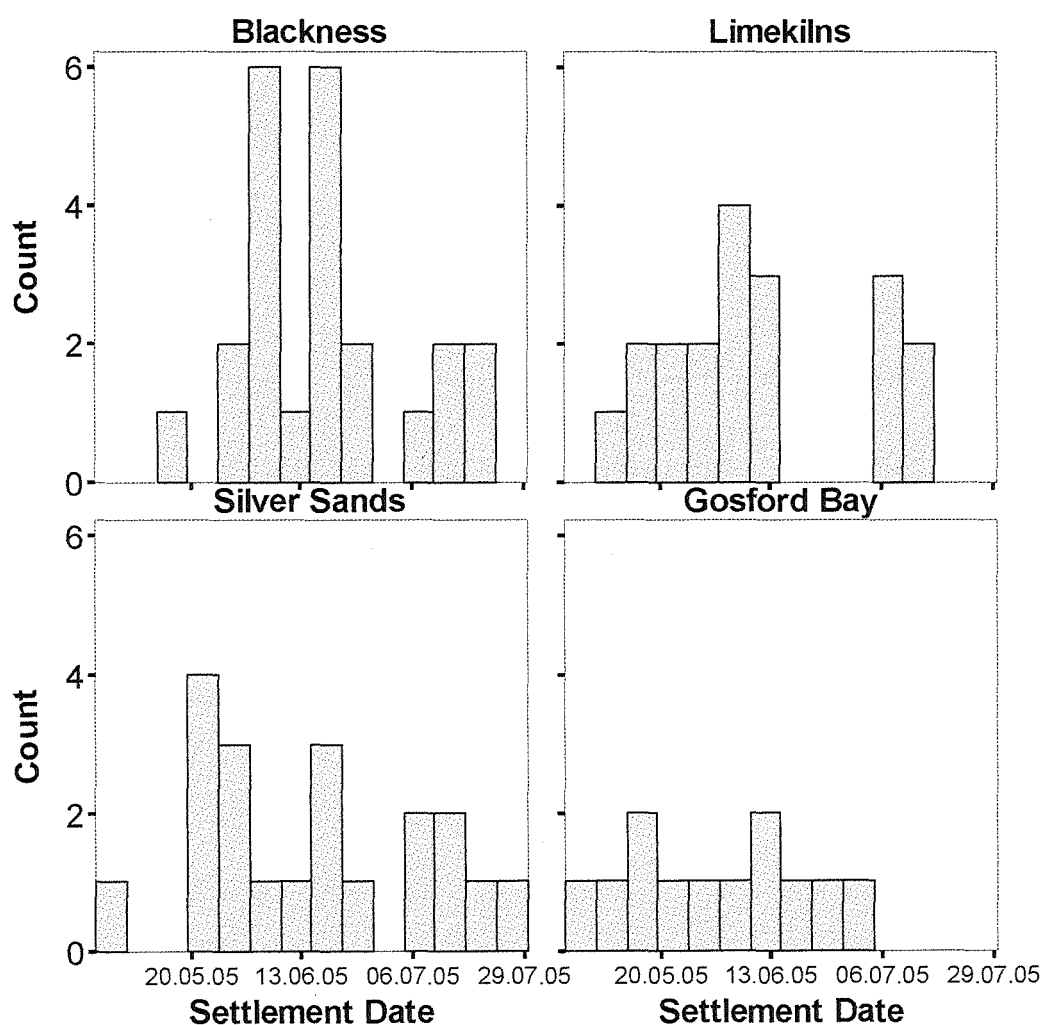


Figure 54: Histogram of settlement date frequency distributions at each site calculated using otolith increment analysis from fish caught in 2005

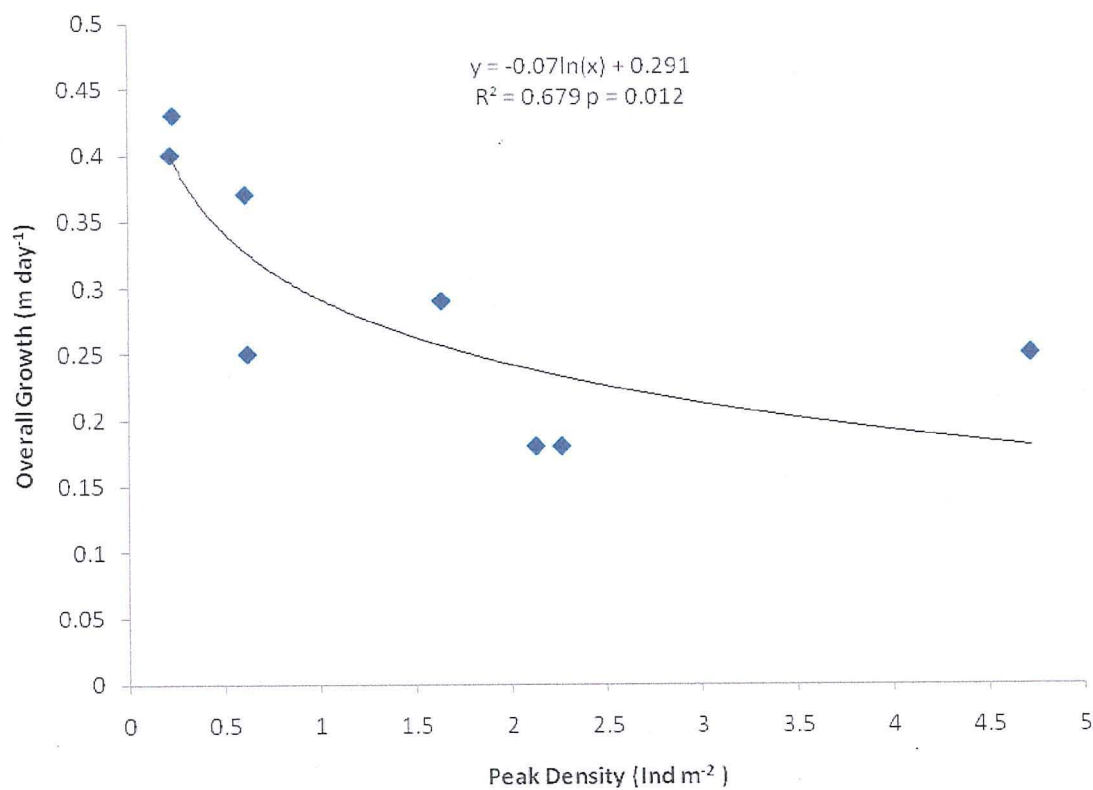


Figure 55: Scatterplot of density vs. growth rates estimated by push net samples. The fitted line plot shows a significant logarithmic relationship.

3.4. Discussion

Several questions need to be addressed about the limitations of the methods used to estimate growth rates in the current study, before any conclusions can be drawn. Firstly, the question of why the mean total length (TL) of fish caught by the net when fish first began to be caught in May of both years, was larger than the TL at which plaice are known to metamorphose, coincident with settlement onto nursery habitats (Wimpenny, 1953; Lockwood, 1974; Alhossaini *et al.*, 1989; Modin *et al.*, 1996; Amara & Paul, 2003; Nash & Geffen, 2005;). The size (TL only) at metamorphosis and immediately post – metamorphosis suggested by Amara and Paul (2003), is approximately 12 mm, but this is variable, with a suggested maximum of 15 mm (Modin *et al.*, 1996), and a possible minimum of 9 mm standard length (Alhossaini *et al.* 1989). However, the smallest fish caught for the present study were usually 12 mm TL, with the exception of a single individual of 11 mm TL, caught at Silver Sands in 2005. The mean and median TL of fish caught in the earliest samples in both years for the present study were usually between 18 – 25 mm TL (Figures 30 – 37). This is explained by the findings of Gibson (1973), Kuipers (1973), Zijlstra *et al.* (1982) van der Veer & Bergman (1986) and Gibson *et al.* (2002): these authors found two types of settlement behaviour in different juvenile habitats. Gibson (1973) found that newly – metamorphosed plaice settled onto the sandy bays in the west of Scotland at depths of 3 m or so, before migrating into much shallower water and developing tidally-rhythmed migrations on and off the intertidal. However, in contrast to this, van der Veer & Bergman (1986) found that settlers on the muddy Wadden Sea

nurseries of the Dutch coast settle into intertidal pools for up to a week, and possibly as long as a month for late settlers, before beginning tidally – rhythmised migrations. This self – imposed ‘stranding’ and subsequent migration into deeper tidal waters may be explained by changing temperature tolerances as the fish grow, and is assumed to be an escape response to evade potentially dangerous high temperatures (van der Veer & Bergman, 1986). These two settlement behaviours would explain the large average size of the fish (>12 mm) caught earliest in the present study: it is not possible to deploy the push net subtidally, which would miss newly – transformed individuals at Silver Sands and Gosford Bay (Gibson, 1973). In addition, during 2004 and 2005, very small plaice were observed in small pools at Blackness during March (pers. obs.), which, unfortunately were not sampled; this phenomenon was not observed at Silver Sands, the ‘reconnaissance’ site. The capture of an 11 mm individual (less than the accepted length at metamorphosis), suggests that newly settled individuals were at a depth either greater (sandy Outer Firth) or less (muddy estuarine) than the depth usually sampled by the push – net, and was not due to length – dependent selection by the push nets mesh. While this means the very earliest growth rates were not measured, the same depths were sampled at all four sites, and are comparable with each other. For the purposes of growth measurements in the current study, i.e. to determine whether muddy estuarine sites are also putative nurseries, the capture of individuals in the same depths of water is sufficient. The push net technique, while sufficient to compare early growth rates, is not capable of sampling in deeper waters where the largest 0+ individuals can, and do (Gibson, 1973; Lockwood, 1974), migrate to.

The hypothesised differences in settlement behaviour between the estuarine and sandy sites in the present study, suggests that the size – dependent depth selection observed in previous studies may be related to the habitat type: with settlers onto muddy habitats exhibiting intertidal pool 'stranding' and settlers onto sandy habitats exhibiting deeper water selection. Further, as the plaice observed in the current study are assumed to have spawned from a single spawning aggregation at Fife Ness (Bowman, 1914; Poxton & Nasir, 1985), the differences in settlement behaviour between the estuarine and sandy habitats suggests plaice settlement behaviour and early post – larval habitat selection is highly variable and changes according to habitat type.

A second explanation for possible differences in settlement behaviour between the estuary and the Outer Firth is that larvae were spawned from a different spawning aggregation that has adapted to estuarine sites, manifested as different settling behaviour. This would require a spawning population either within the estuary, or from a spawning aggregation outside the estuary and whose eggs and larvae are somehow transported past the Outer Firth without settling there, although the latter would be even more difficult to imagine given the intimate proximity of the estuary and Outer Firth, and the limited ability of plaice larvae to control where currents transport them. Investigations of concentrations of plaice eggs in the east of Scotland have shown that a population in the estuary is unlikely. Bowman (1914), Bowman (1921), Lamont (1964), Poxton (1987), Elliott *et al.* (1990) and Greenwood *et al.* (2002) and references within these, have conducted surveys, or reported on historical surveys, of adults in the estuary and in the Firth and have found no spawning adults, although a small number of mature males and

females have been caught in the Firth. However, as no quantification of the exact location of newly settled plaice was attempted for the current study, the assessment of development of tidal migrations and possible reasons for the anecdotal differences between both habitat types in the estuary and Outer Firth is not possible here. The assessment of tidal migrations would require depth stratified sampling over the course of tidal cycles and the whole of the juvenile intertidal phase season.

The length – frequency distributions shown in Figures 30 – 37, show that at all sites and all years, with the exception of Gosford Bay in 2005, median lengths remained static until between four and six weeks after settlement had begun. This is likely to be due to continual settlement of juveniles during the first month after catching the earliest juveniles at each site. The continual settlement is likely to be caused by a protracted spawning period, and because plaice are batch spawners over a period between December and May at Fife Ness, although the peak spawning is thought to be later than more southerly spawning aggregations (Bowman, 1914; Wimpenny, 1953; Poxton & Nasir, 1985; Rijnsdorp *et al.*, 2005). Influx of new settlers between sampling dates would have the effect of flattening out growth curves calculated using the change in length – frequency distributions over time, thus underestimating growth rates, and is the main reason why otolith increment analysis was used in 2005 to determine growth rates. As long as the relative differences between each sites growth rates calculated by the two methods are the same, comparing length – frequency changes over time is representative of growth conditions on each of the four habitats surveyed for the current study, and is sufficient for testing of the hypotheses in 3.1. *Introduction*. Overall growth rates

measured by changes in length – frequencies over time in 2005 (Table 11) were, in order of highest – lowest: Gosford Bay, Blackness, Limekilns and Silver Sands. Peak growth rates measured by changes in length – frequencies over time in 2005 show a different order, again from highest – lowest: Limekilns, Gosford Bay, Blackness and Silver Sands. Growth rates measured by otolith increment analysis in 2005 were, in order of highest – lowest: Gosford Bay, Blackness, Silver Sands and Limekilns, a reversal of position for Silver Sands and Limekilns. However, comparison of regression slopes of otolith increments on age (Figures 46 – 49) by testing for homogeneity of regression coefficients (Zar, 1999) shows that the slopes are not significantly different ($F = 1.178$, $df = 3, 67$, $p > 0.25$). Means of individual growth rates estimated by otolith increment counts in 2005 showed a slightly different pattern, in order of highest – lowest: Blackness, Silver Sands, Gosford Bay and Limekilns. However, growth rates calculated by regression slopes of otolith increments (Figures 46 – 49) and from changes in length – frequency distributions (Figures 30 – 37 and 38 – 45) are more likely to accurately reflect growth conditions on each site over the whole season: peak growth rates between two consecutive sampling dates and individual growth rates estimated from otolith increments may be affected disproportionately by short – term variability in water temperatures, plaice density and food availability. Indeed, individual growth rates were higher for later settlers than for the earliest settlers at all four sites in 2005 (Figures 50 – 53).

The greater period of larval supply to Silver Sands suggests that larvae follow the prevailing currents from Fife Ness around the north shore of the Outer Firth, as suggested by Poxton & Nasir (1985). This larval supply has to be taken

into account when measuring growth rates using length – frequency distributions and, as such, the growth rates measured by otolith increment analysis are likely to be more accurate than growth rates estimated by length – frequency distributions. As no otoliths were analysed from 2006, larval influx cannot be assessed completely for this year, however, the growth rate at Silver Sands was the highest measured in 2006, albeit not statistically different from Blackness, so an upwards adjustment would not alter the relative positions of any of the sites in 2006. Future work must bear larval influx in mind and assess this using the otolith increment technique used here, combined with larval sampling if possible (e.g. van der Veer & Witte, 1993).

Plaice densities increased from initial settlement during May in both years, peaking in June - July in 2005 and in June 2006, before a general trend of reduction later in the year (Figures 30 – 37, Tables 9 and 10). The declines in density are due to a combination of migration into deeper water (Gibson, 1973; van der Veer & Bergman, 1986; Gibson *et al.*, 2002) and mortality. This resulted, in 2006, in low or no catches at all four sites by November. However, reductions in density occurred more rapidly at the two estuarine sites compared to the two Outer Firth sites. The water temperatures during October and November 2006 were lower than summer temperatures at all sites, suggesting that, if migration to deeper water and cessation of tidally – rhythmized migrations was responsible for the reduction in densities, then temperature is unlikely to be the cause of migrations at the two marine sites. In the two estuarine sites, densities had declined rapidly by the middle of August 2006 (Table 10). The mean TL of fish caught at Blackness during August 2006 was 44.5 mm; the water temperature recorded at the site on

this date was approximately 18 °C, and the mean temperature for the month from SMA SST data was approximately 14 °C. The temperatures recorded in August 2006 by both methods are similar to the temperatures recorded in June and July 2006. According to Fonds *et al.* (1992) temperature – dependent growth model for plaice, these temperatures are within the optimal range for plaice, again suggesting that temperature was not responsible for the cessation of tidally – rhythmmed migrations at the two estuarine sites. However, temperature tolerances of 0+ plaice >50 mm TL are unknown, and it is possible that these plaice require very low temperatures. In addition to unknown temperature tolerances, the temperatures used in the growth model have a relatively low temporal resolution, possibly underestimating the temperatures actually experienced by the individual fish. Another explanation for the cessation of tidally- rhythmmed migrations onto the intertidal, is that the fish are exploiting richer, but previously unavailable food resources. These new food sources may be available to larger plaice because of a lower predation risk to the plaice as a result of increasing length, or the plaice can handle larger food items and the food items may only be available in deeper waters, or previously exploited food resources have also migrated to deeper waters, meaning the drivers of food – related, tidally – rhythmmed migrations onto the intertidal are no longer present.

The apparent cessation of growth at Blackness and Limekilns, and the maximum TL of around 50 – 60 mm in both years at these two sites, is likely to be caused by offshore migration of juveniles, or the previously mentioned cessation of migration onto the intertidal. Gibson *et al.* (2002) show that plaice begin migration to deeper waters at TL > 50 mm, which would explain the apparent cessation of

growth at the two estuarine sites. However, the reason for the migration may be related to water temperature, as suggested by Gibson *et al.* (2002). Plaices' upper limit for temperature tolerance decreases with the size of the individual (negative correlation) (Fonds *et al.*, 1992); therefore, the warmer water at the estuarine sites may impose a depth limit for larger fish, such that the fish have a minimum depth limit that increases as the fish grow, causing the migration to deeper waters. However, Gibson *et al.* (2002) point out that optimal growth rates are temperature related. In the estuary, a more complete range of optimal growth conditions may be available for selection by individuals (e.g. Attrill & Power, 2004). To examine whether migrations were age-mediated, ANOVA ($p=0.186$) was conducted on otolith daily increment counts between sites in 2005. There was no suggestion that the average age of individuals was different between the estuarine and Outer Firth sites in 2005, although the oldest fish were caught at Gosford Bay, suggesting plaice may stay in intertidal or shallower waters for a longer period of the 0+ phase in the marine sites.

Sediment preferences of plaice may play a role in habitat selection of larger plaice: smaller fish have a much narrower range of sediment sizes that they can bury themselves in (Gibson & Robb, 2000). It may be that the subtidal sediments at the sites in the estuary and Outer Firth are unsuitable for smaller plaice, and as they grow they can exploit coarser sediments. This may offer an explanation for the apparent growth cessation recorded at the two estuarine sites: larger plaice may prefer coarser sediment than the sediments found on the intertidal at Blackness and Limekilns, moving to deeper water to find their preferred sediment type. However, sediment preference may also be a result of prey preferences: different

prey types may inhabit different sediment type, or larger prey items may inhabit coarser sediment types, which would be reflected in plaice sediment preference. All of these factors, i.e. water temperature, sediment particle – size distributions, predation risk and food availability are likely to influence plaice depth selection to some degree; the importance and dominance of each of these factors will also change as individuals grow, resulting in changing depth associations and/or preferences with changing size of the individual (e.g. Gibson, 1973; Gibson & Robb, 1992, 1996, 2000; Burrows, 1994, Burrows & Gibson, 1995; Ellis & Gibson, 1995; Gibson *et al.*, 1998; Burrows, 2001; Gibson *et al.*, 2002).

The temperatures used for Fonds *et al.* (1992) model of growth may not represent the exact temperatures experienced by individual plaice. The water temperatures measured in 2006 are only a 'snapshot' of daytime temperatures, night time temperatures would be lower than these. The satellite SST data are an integration of temperatures over a four week period, and hence, although the SSTs' are means over a relatively large area (9 km²), are more likely to represent the temperature conditions experienced by the population at each site. These estimates of water temperature were used as there are no detailed records available from any concerned agencies or institutions.

Notwithstanding the aforementioned limitations of the methodology used in the current study, growth rates of the earliest stages of 0+ group plaice can now be examined in order to determine whether the Forth estuary may also contain plaice nurseries. The definition of a nursery used for the current study is that of Beck *et al.* (2001), where a habitat is a nursery if it contributes more to recruitment than

other habitats occupied by conspecifics. As stated in the introduction, survival in early post – larval stages may directly influence survival to maturity, and survival of very youngest fish may depend on fast growth. Thus, if growth rates of the estuary and the Outer Firth sites are comparable, then the estuary potentially contains nurseries of a similar quality to those of the Outer Firth. Growth rates recorded for the present study varied spatially as well as temporally: growth rates were different between sites and between both years at the same site. In 2005, the highest growth rate measured by tracking the change in average length over time, was recorded at Gosford Bay (0.43 mm day^{-1}), closely followed by Blackness (0.40 mm day^{-1}) and Limekilns (0.37 mm day^{-1}); Silver Sands had the lowest growth rate measured using this method (0.29 mm day^{-1}). However, ANCOVA testing (using $\ln$ -transformed TL) indicated the estuarine sites were the same as each other, and significantly different from the Outer Firth sites, which were also the same as each other. The peak growth rates (i.e. the largest daily change recorded between two consecutive sample dates) in 2005 were slightly different, with Limekilns the highest (0.97 mm day^{-1}), Gosford Bay the next highest (0.67 mm day^{-1}) closely followed by Blackness (0.65 mm day^{-1}) and Silver Sands showed the lowest peak growth rate (0.58 mm day^{-1}). The peak and overall growth rates show the same general pattern in 2005 for each site, and suggest that Limekilns, Blackness and Gosford Bay had similar patterns of growth for this year, while Silver Sands always had the lowest growth rate.

However, the growth rates calculated by otolith increment analysis showed a slightly different pattern. The growth rates recorded using changes in length – frequency distributions were always lower than the corresponding growth rate

using otolith increments at each site, despite this, ANCOVA testing showed no significant differences between methods (Table 17). This does not disprove null hypothesis 3 and suggests that the cohort analysis method can be used to estimate growth rates. However, the otolith method gives higher growth rates than the push net method in all comparisons, thus, it may be prudent to use both methods in future work.

The growth rates measured using regressions of otolith increments on age suggest that Gosford Bay and Blackness had the highest growth rates: 0.64 and 0.61 mm day⁻¹, respectively (Figures 46 and 49). These were closely followed by Silver Sands at 0.61 mm day⁻¹ (rounding off means this is the same as Blackness, however, examination of Figures 46 and 48 shows Silver Sands was slightly lower than Blackness) and lastly Limekilns with a growth rate of 0.57 mm day⁻¹ (Figure 47). Mean and individual growth rates of at each site were slightly different: Blackness mean = 0.62, range 0.41 – 0.92 mm day⁻¹, Limekilns mean = 0.55, range 0.29 – 0.94 mm day⁻¹, Silver Sands mean = 0.58, range 0.19 – 0.90 mm day⁻¹, and Gosford Bay mean = 0.56, range 0.33 – 0.82 mm day⁻¹. However, the otolith increment growth rates were not significantly different between sites ($F = 1.178$, $df = 3, 67$, $p > 0.25$)

In 2006, measuring changes in length – frequency distributions was the only method used to estimate growth rates at the four sites. The positions of the sites in rank order were: Silver Sands and Blackness with the same growth rate (0.25 mm day⁻¹), with Limekilns and Gosford Bay lowest with the same growth rates (0.18 mm day⁻¹), (Table 12 and Figures 42 – 45). Similar to 2005, the growth rates

measured in 2006 suggest that the estuarine sites could support similar growth rates of plaice compared to the Outer Firth sites.

The only other literature that has growth rates of intertidal phase 0+ plaice in the Forth is the work of Poxton & Nasir (1985) and Nasir (1981, 1985); growth rates of plaice were measured at Broad Sands in the Outer Firth over the years 1979 – 1983. The highest growth rate recorded by Nasir (1985) was 0.54 mm day^{-1} between June and July 1983. The lowest growth rate recorded was 0.16 mm day^{-1} between September and October 1982. These growth rates were measured by tracking changes in length – frequency distributions between samples; however, Nasir (1985) used a 2 m beam trawl to collect 0+ plaice. In spite of the different gear types, the growth rates were very similar to those recorded for the present study at all sites, albeit with a higher maximum from June and July 1983. In addition, Nasir (1985) also used the same push net as used in the current study, and recorded growth rates at Broad Sands. The highest growth in length measured by Nasir (1985) using the push net was 0.37 mm day^{-1} between July and August 1982. This is lower than the growth rates recorded in 2005 at Blackness and Gosford Bay, equivalent to the overall growth rate for Limekilns and higher than Silver Sands, in the present study. Nasir (1985) growth rates are calculated using a starting date of April 1st for growth rates recorded using the change in length – frequency distribution, however, the growth rates quoted from Nasir (1985) are calculated using the same method as the peak growth rate calculations for the present study (Tables 11 and 12). Thus, they are directly comparable. As Nasir (1985) used the change in length – frequency distributions to estimate growth in length, it must be remembered that their growth rate estimates will also tend to

underestimate growth rates, due to the effects of continual settlement and settlement between sampling dates. However, the comparison demonstrates that the interannual variability recorded in the present study, as well as the actual growth rates at all four sites, were similar to those for Broad Sands. This comparison highlights again the similarity between the estuarine sites and the Outer Firth sites in the present study as well as other studies, and demonstrates the estuarine sites are potential nurseries.

To provide a larger – scale comparison of 0+ plaice growth rates measured in the current study, Table 22 provides a summary of maximum and minimum estimated growth rates (where both were noted) of 0+ plaice in other parts of the UK and the rest of Europe, where the species is found. The table also includes, where possible, gear types, method of growth rate calculation and the limits of water depths surveyed for each measurement. The growth rates shown in Table 22 are not an exhaustive review of the available literature, rather a selection of growth rates from well studied 0+ plaice habitats, and serve to indicate the range of growth rates of these animals.

Table 22: Growth rates of 0+ plaice from other areas of its range. *If* = change in length – frequency distributions over time, *oi* = otolith microincrement aging, *if* = individual tags

Area	Sub – Area	Gear, Water depth	Growth Rate (mm day ⁻¹), Method	Source
Southern North Sea	Gravelines, France	3 m Beam Trawl. 5 m	0.55 – 0.81. <i>If</i>	Amara <i>et al.</i> (2001)
	North Frisian Wadden Sea	2 m Beam Trawl. 1.4-1.8 m	0.44 – 0.96. <i>oi</i>	Berghahn <i>et al.</i> (1995)
	Western Dutch Wadden Sea	2 m Beam Trawl. Intertidal	0.34 – 0.52. <i>If</i>	Van der Veer & Witte (1993)
	"	3 m Beam Trawl. Sublittoral	0.25 – 0.36. <i>If</i>	"
English Channel	Balgzand, Dutch Wadden Sea	1.9 m Beam Trawl. Intertidal. 1-1.5 m	0.51 – 0.66. <i>If</i>	Zijlstra <i>et al.</i> (1982)
	Balgzand, Dutch Wadden Sea	2 m Beam Trawl. Intertidal. 1 – 1.5 m	0.36. <i>If</i>	Kuipers (1977)
	Dollard, Ems Estuary, Wadden Sea	2 m Beam Trawl. > 0.80 m	0.1 – 0.8. <i>If</i>	Jager <i>et al.</i> (1995)
	Bay of Canche, France	3 m Beam Trawl. 0 – 10 m	0.43. <i>If</i>	Amara (2004)
Irish Sea	La Canche estuary, France	1.5 m Beam Trawl. 0.20 – 0.60 m	0.38 – 0.61. <i>oi</i>	Amara & Paul (2003)
	Port Erin Bay, Isle of Man	2 m Beam Trawl.	0 – 0.5. <i>if</i>	Nash <i>et al.</i> (1992)

0 – 1.67 (individual), 0.1 –
0.27 (population). *if*

Nash *et al.* (1994)

“

“

“

“

“

“

“

“

“

“

“

“

“

“

Clyde Sea

Ayr Bay, Scotland

2 m Beam Trawl

0.55. *if*

Poxton *et al.* (1983)

Irvine Bay, Scotland

“

“

0.60. *if*

“

North Atlantic

Hafursfjörður, Faxaflói, Iceland

2 m Beam Trawl

0.60. *if*

Hjörleifsson & Pálsson (2001)

Central North Sea

Gullmarsfjord, Sweden

Drop Trap

0.35 – 0.80. *oi*

Modin & Pihl (1994)

Skaggerak Bays, Sweden

Drop Trap

0.39 – 0.54. *if*

Wennhage *et al.* (2007)

“

“

“

“

“

“

0.23 – 0.45. *if*

Pihl *et al.* (2000)

Firth of Forth, Outer Firth

Push Net

0.18-0.43. *if*
0.19-1.22. (individual) *oi*

Present Study

Forth Estuary

Push Net

0.18-0.40. *if*
0.29-0.94. (individual) *oi*

Present Study

Table 22 (continued).

Plaice growth rates shown in Table 22 demonstrate that the growth rates of both the Outer Firth and estuary are similar to the growth rates recorded at other, more intensively studied juvenile habitats, and actually higher than some other studies. Additionally, the large variability in individual growth rates measured in the current study (Figures 46 – 49 and Tables 18 – 21), was also found by Nash *et al.* (1994a) in Port Erin Bay in the Irish Sea.

The growth rates recorded by the two methods in the present study (otolith increment analysis and by tracking changes in length – distributions over time), suggest that Blackness and Limekilns in the estuary support growth rates of the youngest 0+ plaice, equivalent to or even greater than the growth rates measured at the two Outer Firth sites, Silver Sands and Gosford Bay. Previous work carried out on plaice in the Firth of Forth and Forth estuary has stated that plaice nurseries will only be found on the sandy habitats in the Outer Firth (Rae, 1970; Poxton & Nasir, 1985), and the very shallowest waters in the estuary have not been examined for presence of newly-settled plaice. Elliott *et al.* (1990) recorded 0+ and 1+ group plaice in the estuary, but stated that the presence of the 0+ group was caused by immigration of larger individuals from the Outer Firth into the estuary from July onwards, and that the estuary as a whole may be a nursery for 1+ group plaice. This was also claimed by Ajayi (1983). Poxton (1987) reviewed fishery studies in the Forth estuary and stated that juvenile plaice use the estuarine mudflats only during late summer and early autumn; again, plaice were found in late spring and early summer in the estuary in the two years reported for the present study (Tables 9 and 10). However, to the authors' knowledge, no attempts have previously been made to capture newly settled individuals from intertidal or

the shallowest subtidal areas in the estuary. The current work clearly demonstrates that newly – metamorphosed individuals are present in the estuary from May onwards, at the same time as similar sized individuals are present in the Outer Firth (Figures 30 – 37). Gibson (1973) demonstrated that 0+ plaice are limited by depth once they settle onto their benthic habitat, and Macer (1967) and Burrows *et al.* (2004) demonstrated that plaice have strong site fidelity in Red Wharf Bay on Anglesey, Wales, and Tralee Beach near Oban, Scotland, respectively. Thus, it is unlikely that small 0+ juveniles caught at the two estuarine sites have migrated from the Outer Firth and the 1+ group plaice in the estuary may include a large component of individuals that settled onto estuarine intertidal sites at metamorphosis.

The hypothesis stated in the introduction to the current chapter states that if the forth estuary contains nurseries for newly – metamorphosed plaice, then growth rates should be similar to growth rates in the Outer Firth. The results of the current study indicate that growth rates are similar at all four sites examined, thus disproving the proposed null hypothesis (null hypothesis 2).

Poxton & Nasir (1985) made an estimate of the size of the 0+ plaice population in the Firth of Forth based upon their density estimates, using the same data in Nasir (1985) (Poxton & Nasir (1985) is a published excerpt from Nasir's (1985) doctoral thesis), of the sandy bays around the Forth and an estimate of the available habitat by estimating the area of the sandy bays in the Firth of Forth. The similarities between the growth rates and settlement dynamics of the plaice measured in the estuary in the current study, and the growth rates measured at the

firth sites in the current study and Nasir (1985) suggest that Poxton & Nasirs' (1985) population estimate of $1 - 2 \times 10^6$ 0+ plaice, is likely to be an underestimate of the number of 0+ plaice in the whole estuarine – firth system. In addition, as the density indices (Tables 9 and 10) for the present study show, peak density could vary by as much as 3 x between years; population estimates should therefore take into account the high interannual variability in density recorded for the present study.

The difference in growth rates at all four sites between years (Table 16) is anomalous. Each site has a lower growth rate in 2006 than the growth rate measured at the same site in 2005. This is striking when the temperatures recorded by SMA SST data (Figure 29) are examined: the temperatures recorded at each site in 2005 are approximately 2 °C lower than the corresponding months in 2006. If, as suggested by other workers (Zijlstra *et al.* 1982; van der Veer *et al.*, 1990; Karakiri *et al.*, 1991), plaice growth is determined purely by prevailing water temperatures ('maximum growth/optimal food condition' theory proposed by Karakiri *et al.* (1991)), growth should have been higher in 2006 than 2005. This is reflected in the plots of hypothesised maximum growth rates based solely on temperature (Figures 38 – 45): plaice lengths of the net – caught plaice are always lower than the lengths of modelled plaice. The growth rates predicted by temperature dependent models are also higher than the growth rates estimated by otolith increment analysis in 2005, indicating growth was less than optimal. However, growth rates of some individual plaice were equal to or higher than the temperature – predicted growth rates (Tables 18 – 21). The most likely explanation for the differences in growth rates between years is that the plaice were food

limited in 2006, e.g. growth was density – dependent. A significant relationship was found between density and growth rates at all four sites (Figure 55). Much higher densities were found at Limekilns, Gosford Bay and Silver Sands in 2006 than 2005 (Tables 9 and 10). Blackness had the lowest densities of plaice in 2006, although still slightly higher than densities at Blackness in 2005. This is in agreement with the findings of Jager *et al.* (1995) for areas of the eastern Wadden Sea. Amara & Paul (2003) found density – dependent growth of newly – settled plaice on the French coast of the Eastern English Channel, however, growth of larger plaice did not appear to be density – dependent in their study. Van der Veer & Witte (1993) measured growth and feeding conditions of 0+ plaice, as well as temperature, environmental conditions and food availability in several intertidal and subtidal areas of the Western Wadden Sea. They found that growth was not density – dependent, rather plaice were limited by food availability and, if growth was indeed density – dependent, the effects were marginal compared to the effects of food quality and availability in different areas. Pihl *et al.* (2000) found density – dependent growth of plaice in bays with extremely high ($\sim 20 \text{ ind m}^{-2}$) densities, and no evidence of effects of density on growth at lower densities; similarly Modin & Pihl (1994) concluded that growth limitation due to density – dependence would be unlikely to occur unless densities were extremely high. This finding appears to be true for other species of pleuronectiformes nursery stages (Nash & Geffen, 2005). However, caution is required when interpreting the apparent limited or sub – optimal growth recorded in the present study. As van der Veer & Witte (1993) found, growth was food limited rather than negatively density – dependent, similar to the findings of Berghahn *et al.* (1995). In the current study, food availability and plaice feeding were not assessed. To determine whether growth of plaice was

actually negatively density – dependent, rather than limited by food availability, it would be necessary to estimate plaice and food productivity on each of the four sites used here, as well as the level of exploitation of the available food resources by plaice. Pihl (1985) measured consumption rates of mobile epibenthic predators, including 0+ group plaice and found that relatively little of the available food was actually consumed. This again suggests that food would only be limiting at the very highest densities of 0+ plaice. In addition, Pihl (1985) found that plaice had a similar diet to *Crangon crangon*, thus, an assessment of feeding conditions would require assessment of the level of exploitation of food resources by *C. crangon*, as this species was found in high abundance at all sites sampled for the present study. Amara (2004) found that growth of plaice during late summer in a separate study in the Bay of Canche was lower than growth predicted by Fonds *et al.* (1992) temperature dependent model; however, this could not be attributed to density – dependent effects as food conditions in this area had not been assessed and temperature measurements were of a low resolution (weekly). Bergman *et al.* (1988) and van der Veer (1986) suggest that apparent deviations from optimal growth models in the Dutch Wadden Sea were caused by differences in the timing of larval influx and settlement: years with later larval influx tended to have a lower mean length by the end of the summer. This demonstrates that settlement period must also be taken into account when assessing density – dependence of growth rates. In the case of plaice, settlement can be assessed by using otolith microstructure (e.g. Karakiri & von Westernhagen, 1989; Modin & Pihl, 1994; Amara & Paul, 2003).

The growth rates and density measurements for the current study (Figures 30 – 37, 55 and Tables 9 – 21) appear to suggest that plaice growth is density – dependent. However, the differences in growth rates between early and late settlers in 2005 (Figures 50 – 53) may contradict this. Plaice that settled later in 2005 appeared to grow faster than early settlers. Several explanations could account for the apparent differences. 1) Temperature was the sole determinant of growth: temperatures increased from April and peaked in July 2005 and remained high until the end of August 2005 (Figure 29). 2) Food availability was greater later in the season: suitable prey may have been at a lower density at the start of the season compared to late summer. 3) Newly – settled plaice densities were greater at the start of the season and, thus competition for food was reduced for late settlers. Of these 3 explanations, 1) would rule out density-dependent effects on growth, while 3) argues in favour of density–dependence of growth, 2) argues in favour of food limitation, not necessarily dependent on consumer density..

Density – dependence may appear to be operating when comparing estimated growth curves with temperature – predicted growth rates (Figures 38 – 45) however this assumption must be treated cautiously. The predicted growth curves may not take into account shorter temporal scale changes in temperature at the four sites than those actually measured *in situ* and by remote sensing. It may be the case that temperatures experienced by the plaice in the present study fluctuated around a different average than the mean temperatures estimated for the temperature – dependent model. Indeed, the effects of higher temperatures can be seen in the growth curves estimated at Limekilns in 2006 (Figure 43). The model growth curve predicted by measured water temperatures proceeds in a

similar fashion to the SMA SST predicted growth rates, but then falls steeply due to a relatively high temperature measured at that time, before proceeding almost parallel to the estimated growth rates. This could indicate that the plaice are actually growing at optimal rates for the temperatures they experienced, but the measurement protocol had insufficient temporal resolution to adequately detect them. Ideally, temperature sensors and data loggers would be attached to individual plaice to allow more accurate determinations of temperature, in order to elucidate whether plaice are growing optimally or not. However, logistically this would be difficult, if not impossible as the author is not aware of any such devices small enough for this purpose. A second, possibly more easily achieved approach to accurate growth predictions, would be to have several temperature sensors with data loggers at each site placed in several positions that plaice are known to inhabit during the 0+ phase at each site. In any case, the model-estimated growth rates at Limekilns suggest that Fonds *et al.* (1992) model may not be applicable to Firth of Forth plaice.

The densities of plaice measured in the current study were all recorded in the first hour after low tide at each site. This was a deliberate attempt to catch as many individuals as possible, as 0+ plaice are known to migrate onto the intertidal with the incoming tide (Gibson, 1973; Burrows *et al.*, 1994), maximising densities of the population susceptible to capture by the push net. Thus, the densities recorded for the four sites in the present study are indices of actual densities, rather than estimates of true density. However, as all four sites were sampled in the same way on each sampling date in both years, the densities are comparable between sites and between years. In order to separate out the effects on growth of

temperature and food conditions at each site sampled here, between – year and between – site comparisons of feeding (gut contents etc), growth rates, intraspecific and interspecific competition (*C. crangon* as well as plaice densities) and temperature would be required, e.g. van der Veer & Witte (1993).

Leaving methodological considerations aside, the water temperature (~28 °C) recorded at Limekilns in August 2006 is considered lethal for plaice (Fonds *et al.*, 1992). If this assertion is indeed true for all populations of plaice, then it must be asked why plaice would migrate into habitat that provides not only sub – optimal temperatures for growth, but also potentially lethal temperatures? One reason may be that the risk associated with low growth rates as a result of sub – optimal temperatures and the risk associated with potentially lethal temperatures, are actually lower than the risk of predation or length – dependent mortality in the necessarily deeper waters that accompany lower temperatures in estuarine habitats. If plaice are able to withstand the lethal effects of such high temperatures for a short period of time, they may reduce their risk of predation by avoiding deeper waters that possibly harbour higher densities of predators; in addition, predators of juvenile plaice may have temperature tolerances below those of the plaice themselves, forcing plaice into a temperature – driven predation refuge (Sheaves, 2001). A second reason for plaice selecting potentially sub – optimal temperatures is that food is more available in the waters with higher temperatures. Food availability may be increased on the intertidal as a result of either reduced interspecific competition, or because the higher temperatures mean that prey species are more productive on the intertidal than the same species in water with a lower mean and range of temperatures, where plaice migratory behaviour

represents a trade – off between the lower limit of growth rates imposed by the higher temperatures and the higher potential growth rates allowed for by increased food availability.

The preceding discussion allows formulation of several hypotheses of local and general interest, in terms of 0+ group plaice. The first hypothesis concerns the possible differences in settling behaviour of newly metamorphosed juveniles, between the sandy habitats (Silver Sands and Gosford Bay) and the muddy habitats (Limekilns and Blackness): plaice larvae on muddy habitats exhibit intertidal stranding at low water, while plaice larvae on sandy habitats settle in deeper water and do not exhibit intertidal stranding at low water. This hypothesis, while not showing new settlement behaviour, aims to determine the influence of sediment particle size on juvenile plaice settlement behaviour.

The second hypothesis is that growth of the forth population of 0+ plaice is food – limited and/or density – dependent, rather than determined by temperature. To test this hypothesis, several factors must be assessed. Firstly, accurate, high resolution measurements of temperature on each site are required to determine maximal growth. Secondly, feeding behaviour of 0+ plaice must be assessed using a combination of gut contents assessment, feeding rates and prey availability on each site. Thirdly, growth and settlement of plaice must be assessed using a suitable method; either otolith microstructure analysis, planktonic sampling or a combination of these approaches (e.g. Karakiri *et al.* 1991; van der Veer & Witte, 1993; Modin & Pihl, 1994). Fourth, depth stratified sampling of the range of depths occupied by all size classes of 0+ plaice, or at least sampling of the whole depth

range of 0+ plaice is required to accurately assess densities on each site at both high and low water. Lastly, several years' growth and density estimates are required to examine whether there are consistent correlations between these factors.

The final hypothesis is whether salinity in the estuary has any effect on plaice growth rates. The only study that has assessed the effects of salinity on plaice growth (Karakiri & von Westernhagen, 1989) suggested that low salinity (20 ppt in that study), had an adverse effect on somatic growth and otolith microstructure when combined with low temperatures. However, Karakiri & von Westernhagen used salinities up to only 30 ppt. The salinities at Blackness and Limekilns are slightly higher than this at between 30 and 32, while the salinities of the Outer Firth sites are fairly stable between 34 and 35 (Webb & Metcalfe, 1987). Thus, salinity may negatively affect the growth of plaice in the estuarine sites, which has implications for detecting density – dependent effects on growth in low salinity conditions. It is hypothesised that plaice are adversely affected by low salinity, causing plaice to grow more slowly at lower salinities.

3.5. Conclusion

The measurements of growth rates and densities of 0+ plaice in the forth estuary and Outer Firth show that plaice nurseries for newly – settled plaice may also be found in the estuary, forcing an upwards revision of previous population estimates for the area. Evidence for growth limitation was found on all four sites examined, however, density – dependent effects could not be unequivocally found. Settlement behaviour of plaice may differ between the estuary and the Outer Firth sites; however, much more work is required to assess these differences, if they indeed exist.

Chapter 4

Effect of Salinity on Growth Rates of Juvenile Plaice

4.1. Introduction

Much of the literature on UK plaice (*Pleuronectes platessa*) habitat records them as fully marine, rather than estuarine species (e.g. Gibson, 1973; Lockwood, 1974; Poxton & Nasir, 1985; Poxton, 1987; Nash *et al.*, 1994a; Marshall & Elliott, 1998). One reason for this proposed distribution is the sub – optimal growth rates that have been reported for plaice at low salinities (Karakiri & von Westernhagen, 1989). However, as the work in chapter 3 has shown, plaice have been found to inhabit a closely linked estuarine and marine system in the east coast of Scotland. The 0+ plaice growth rates were reported in order to demonstrate their similarity in estuarine and Outer Firth sites. However, the effects on growth rates of environmental variables other than temperature and food (Fonds *et al.*, 1992), are not as well known. It is possible that salinity differences between the estuarine sites and the firth sites also affect growth rates.

Karakiri & von Westernhagen (1989) examined the effects of salinity on somatic growth of plaice and found that low salinity (20 ppt) caused a reduction in somatic growth rates of young plaice, compared to growth rates at a higher salinity (30 ppt). However, the salinities used in that study may not be representative of the habitats in which plaice are commonly found, such as the Wadden Sea and low salinity areas and estuaries within that area (Jager *et al.*, 1993, 1995), Swedish Bays (Wennhage & Pihl, 2001) as well as higher salinity areas such as the Irish Sea nurseries (Riley & Corlett, 1965; Macer, 1967; Nash & Geffen, 2000) and the west coast of Scotland (Poxton *et al.*, 1982). Several studies have shown the influence of salinity on plaice distribution (e.g. Jager *et al.* 1993; Marshall & Elliott,

1998) and Rogers & Millner (1996) show distribution of other species is influenced by salinity. Salinity also affects behaviour, for example, Burke *et al.* (1991) found differences in settlement choices of summer and southern flounder, cued by salinity. Thus salinity is a known determinant of distribution and habitat for plaice and several other fish species.

Although food availability and temperatures have been shown to influence the growth of 0+ plaice (Zijlstra *et al.*, 1982; van der Veer *et al.*, 1993), the effects of salinity on plaice growth have been assessed only in a single study (Karakiri & von Westernhagen, 1989). Salinity has been shown to affect the growth and feeding of a range of marine species: flounder (*Platichthys flesus*) (Andersen *et al.*, 2005), gilthead sea bream (*Sparus auratus*) (Laiz – Carrion, 2005), milkfish (*Chanos chanos*) (Jana *et al.*, 2006), gray snapper (*Lutjanus griseus*) (Wuenschel *et al.*, 2004), turbot (*Psetta maxima*) (Gaumet *et al.*, 1995; Imsland *et al.*, 2001); Bouef & Payan (2001) summarised the effects of salinity on the growth of various marine, catadromous and anadromous species. In most of these studies, gross growth rates and growth efficiencies were increased at salinities lower than full strength seawater (salinity 35), only one study reviewed by Bouef & Payan (2001) showed highest growth at a salinity higher than seawater. It has been suggested that salinity acts to enhance growth rates by allowing a reduction in the energy allocated to osmoregulation (Lyndon, 1994), or by a reduction in drinking rates leading to higher absorption of food in the intestines (Jana *et al.*, 2006). Hence it can be predicted that growth of plaice in the forth estuary may be influenced by differences in salinity between the estuary and the Outer Firth, with growth possibly enhanced at low salinities.

The present study aimed to test growth performance of plaice exposed experimentally to a range of salinities more representative of the natural range experienced by 0+ plaice in the Firth of Forth and Fort Estuary than the values used by Karakiri & von Westernhagen (1989). Although distributions of plaice have been correlated with salinity (Marshall & Elliott, 1998), distribution of the 0+ plaice in the Forth estuary is unlikely to be strongly influenced by salinity, because depth imposes a limit on the habitat available for selection by such small plaice (Gibson, 1973; Gibson *et al.*, 2002), juvenile plaice do not move more than 3 km alongshore from their settlement site (Macer, 1967; Burrows *et al.*, 2004), and plaice larvae have little control over the exact area they settle in, on the scales of the salinity gradients found in the Forth (Gibson, 1973; Wennhage & Pihl, 2001).

The specific hypothesis tested here is: Plaice growth is higher at lower salinities; therefore, plaice on equal food rations will grow faster at low salinity compared to high salinity. The corresponding statistical null hypothesis is: Plaice growth is unaffected by salinity.

4.2. Methods and Materials

4.2.1. Laboratory Growth

Fish were caught at Blackness on 30th June 2006. Fish from Blackness were used in experimental treatments because the mean salinity at Blackness is the same as the middle salinity used in the experimental treatments (Webb & Metcalfe, 1987) in the present study. Thus, laboratory fish experienced higher salinity, lower salinity and the same salinity as the habitat in which they were collected. This procedure meant fish experienced a shift in salinity of the same magnitude in both directions. However, these salinities are within the range experienced by plaice in their natural habitats (salinities of the sites in the present study are: Blackness and Limekilns approximately 30, Gosford Bay and Silver Sands approximately 35). After capture, plaice were transferred to a holding tank with water at a constant temperature of 12 °C and a light regime of 12L: 12D hours; these light and temperature conditions were maintained for all fish for the duration of the experiment. After 48 hrs fish were haphazardly assigned to treatment tanks, randomly dispersed over two shelves (Figure 56), and left for a further 48 hrs to acclimatise.

Three salinity treatments were used for this experiment: a single fish each was placed in a small tank with 3.5 L water at a salinity of 25, 30 or 35. Eight replicate tanks (and fish) were used for each treatment level, giving a total of 24 tanks with 24 fish. The location of each tank in the laboratory was randomised over two shelves in case of effects of shading associated with the two different shelf

elevations, with 12 tanks per shelf (Figure 56). Salinity was maintained in each tank by a twice – weekly change of > 95 % of the water in each tank. This procedure ensured salinities were kept constant and that concentrations of nitrogenous compounds in the water were minimised.

Fish were fed on finely chopped *Nereis virens* from commercial cultures (Seabait, UK) and obtained from a local angling supplier. Fish were initially fed a minimum ration (FR1), to control for effects of salinity on feeding behaviour and allow examination of effects of salinity on metabolic and physiological performance alone. In a pilot study, minimum ration was calculated as the lowest mass of food eaten in a single day by the experimental fish: fish were fed a pre – weighed amount of food (wet weight) and the time taken to consume the food by each fish was noted. The weight of food administered was divided by the maximum number of days the fish took to consume all of this food to give a minimum daily ration.

Fish wet weights (g) and total lengths (mm) were recorded at the start of stage 1 of the experiment when fish were fed ration FR1, and these were measured approximately every fortnight from 7th July 2006 until 11th August 2006, when a different feeding regime was started.

A second feeding regime (FR2) was instigated in stage 2 of the experiment, for ethical reasons; most of the fish in the study had not increased in length and or weight during the minimum food ration regime, and hence were likely to be stressed. An *ad libitum* feeding regime was therefore initiated with the same fish in

the same treatments. Only the feeding regime was changed, the same fish, tanks and salinity treatments were used in both feeding regimes.

Fish were fed ration FR2 from 12th August 2006 until 6th September 2006. Fish were administered approximately 1.5 g wet weight of finely chopped *N. virens* each day, and any uneaten food was removed the next day, before fresh food was administered. To determine whether feeding behaviour differed between salinity treatments during FR2, consumption rates were measured over two separate periods during this feeding regime (*ad libitum*). A sub sample of fresh chopped *N. virens* was weighed wet, dried at 70 °C for 48 hours and re – weighed to provide a wet to dry weight conversion factor for administered food. This conversion was necessary because uneaten food removed from the tanks may have lost water as a result of feeding action by the fish (e.g. mastication then expulsion from the mouth was observed in a number of individuals), and as a result of being in relatively high salinity water. Uneaten food was removed from the tanks, weighed wet, dried at 70 °C for 48 hours and then re – weighed. The dry weight of food eaten by each fish was calculated as the difference between administered and uneaten dry weights, and divided by the number of days uneaten food was removed from each tank to give consumption rates in grams of dry food per day (g day⁻¹) for each fish. Time constraints meant that administered and uneaten food weights could not be measured every day during FR2. At the end of the *ad libitum* feeding regime, fish were sacrificed by overdose with MS – 222 and kept in deep freeze (-18 °C) for further analysis (not reported here).

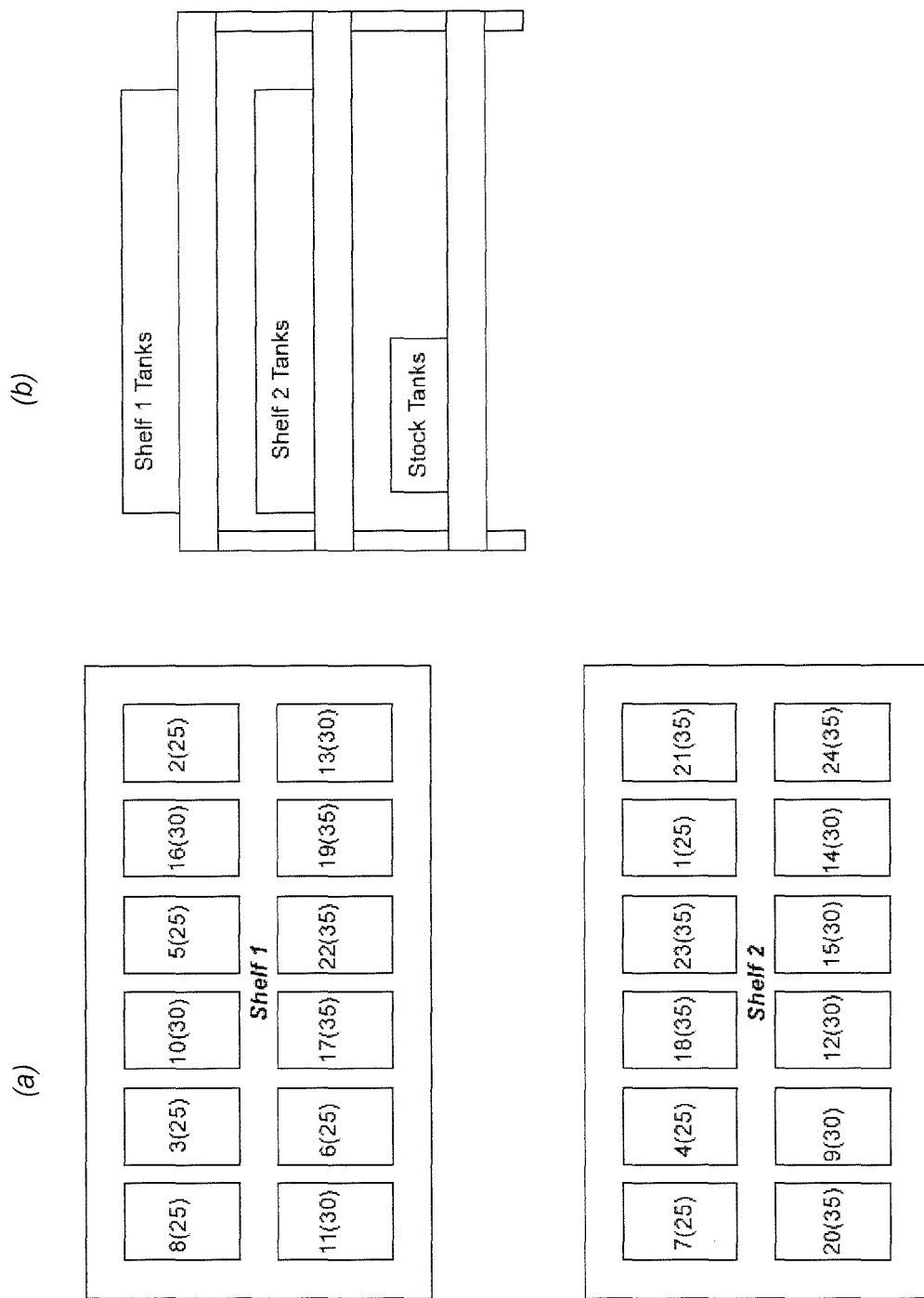


Figure 56: Layout of experimental tanks. Diagram (a) is a plan view of each shelf, diagram (b) is a side view of the apparatus, showing position of shelves in relation to each other. Numbers are unique tank identifiers, numbers in parentheses are salinities.

4.2.2. Statistical analyses

As all fish in the experimental treatments did not increase in length, only wet weights of live fish (live weights) were used in the analysis of the salinity treatments. Initial weights were tested for differences between treatments. Feeding regimes FR1 and FR2 were run for 23 days each, therefore specific growth rates (change in weight per day as % of initial body weight) were not calculated, as this would not alter the outcome of statistical testing. Weight change during FR1 was calculated as the change in live weight between 19th July 2006 and 11th August 2006, as a % of live weight measured on 19th July 2006 (the digital balance used to measure live weights on 7th July was later found to be faulty, these weights were discarded). Weight change during FR2 was calculated as the change in live weight between 11th August 2006 and the 4th September 2006, as a % of live weight measured on 11th August 2006. Percent weight changes in FR1 and FR2 regimes were tested for effects of shading as a result of different shelf elevations, with shelf as the factor.

A wet to dry weight of chopped *N. virens*. conversion ratio was calculated by regressing dry weight on wet weight. The regression co – efficient for the slope of the regression allowed a simple calculation of the dry weight of any given wet weight of chopped ragworm. Dry weights of uneaten food over two periods during FR2 were tested for differences between treatments.

For all tests performed, data were tested for normality and heteroscedasticity and were transformed where necessary before using ANOVA or the Kruskal – Wallis non-parametric equivalent.

4.3. Results

4.3.1. Experimental Growth

Initial lengths of 0+ plaice used in this experiment are shown in Table 23. The regression coefficient shown in the scatterplot in Figure 57 indicates that fresh chopped ragworm contains approximately 23 % water, and the conversion ratio to convert wet weight of ragworm to dry weight of ragworm is 0.2332.

Figure 58 shows % weight change as a percentage of the initial weight of fish during feeding regime FR1 (minimum ration), showing that at least some fish in all 3 salinity treatments lost weight. The mean % weight changes were negative in the two highest salinity treatments and positive in salinity 25. Median values of % weight change were all negative, with salinity 25 the lowest, 35 intermediate and salinity 30 the highest. Data did not meet the assumptions of ANOVA, therefore Kruskal – Wallis non – parametric test was used instead; the differences between treatments were not statistically significant ($H = 0.38$, $df = 2$, $p > 0.05$).

Table 23: Total length in mm of plaice at the start of the experiment. Rows highlighted in yellow indicate mortality by the end of the experiment.

Salinity	Tank	Total Length (mm)
25	1	40
25	2	30
25	3	36
25	4	40
25	5	33
25	6	35
25	7	42
25	8	37
30	9	33
30	10	36
30	11	38
30	12	40
30	13	38
30	14	29
30	15	43
30	16	33
35	17	37
35	18	37
35	19	43
35	20	38
35	21	32
35	22	50
35	23	38
35	24	55

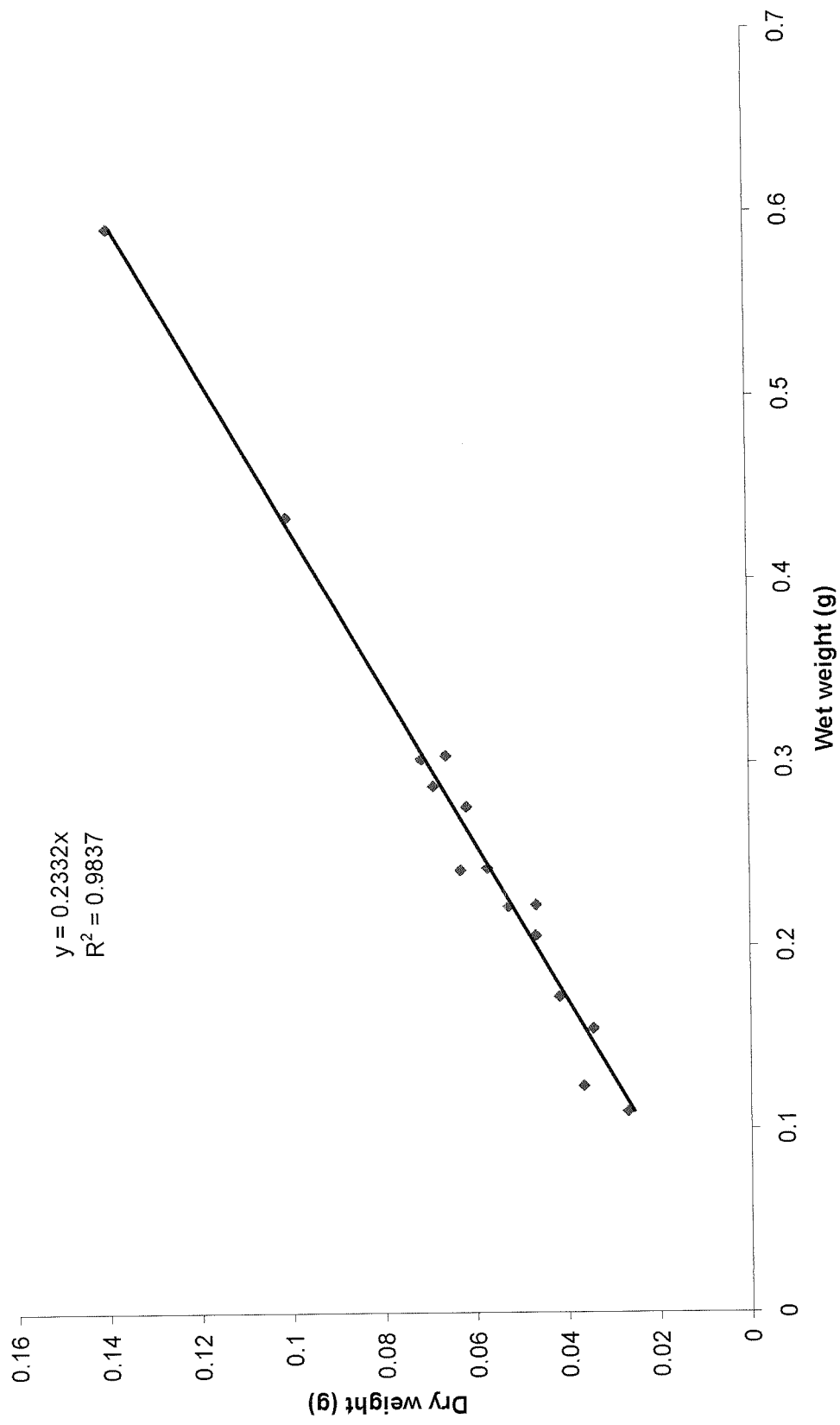


Figure 57: Scatterplot of wet weight and dry weights of chopped *Nereis virens*, the food source for plaice in the current study. Regression slope and regression equation fitted by least squares method.

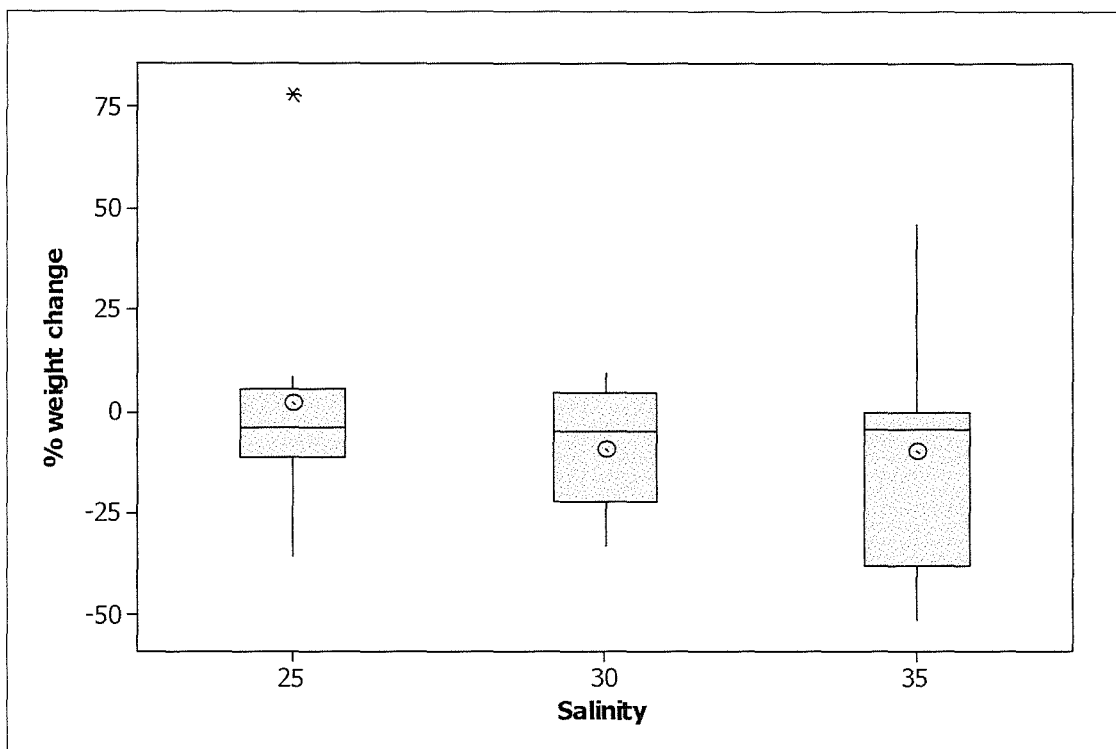


Figure 58: Boxplot of % weight changes during minimum ration feeding regime (FR1). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from $q_2 - q_3$. Asterisk above salinity 25 indicates an outlier.

Figure 59 shows % weight change during FR2 period 1, calculated as the change in weight as a percentage of weight recorded at the start of the experiment on 19th July 2006. Mean % weight change in salinity 25 was positive (fish weight increased), while the mean % weight changes in salinity treatments 30 and 35 were negative (fish weight decreased), with the greatest mean and median % weight change in salinity treatment 35. The differences between treatments were significant (ANOVA $F = 3.78$, $df = 2$, $0.05 > p < 0.01$). A Tukey post – hoc test showed that salinity treatments 25 and 35 were significantly different from each other, while 30 was not significantly different from either salinity treatment.

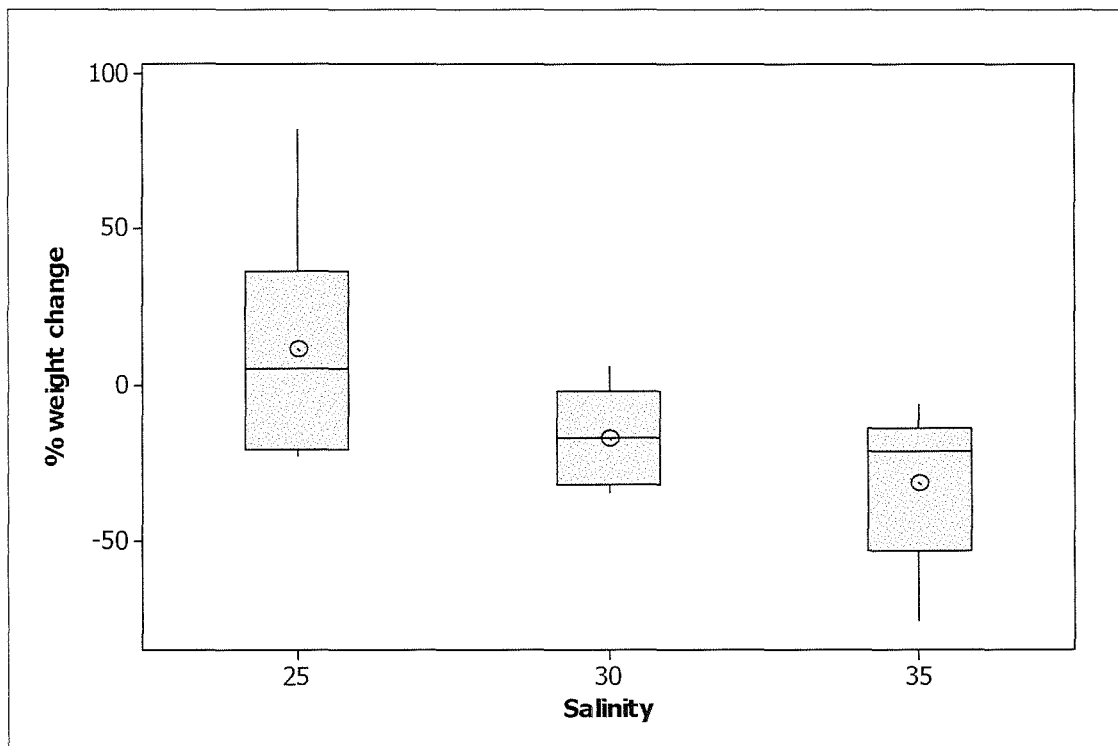


Figure 59: Boxplot of % weight changes during *ad libitum* feeding regime, using the weight measured on 19th July 2006 as the initial weight (FR2 period 1). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from q2 – q3.

Figure 60 shows % weight change during FR2 period 2, calculated as the change in weight as a percentage of weight recorded at the end of feeding regime FR1 (minimum ration). Mean % weight change in salinity 25 was positive (fish weight increased), while the mean % weight changes in salinity treatments 30 and 35 were negative (fish weight decreased), with the greatest mean and median % weight change in salinity treatment 35. The differences between treatments were highly significant (ANOVA $F = 7.69$, $df = 2$, $0.01 > p < 0.001$). A Tukey post – hoc testing indicated that salinity treatments 25 and 35 were

significantly different from each other, while salinity 30 was not significantly different from either salinity treatment.

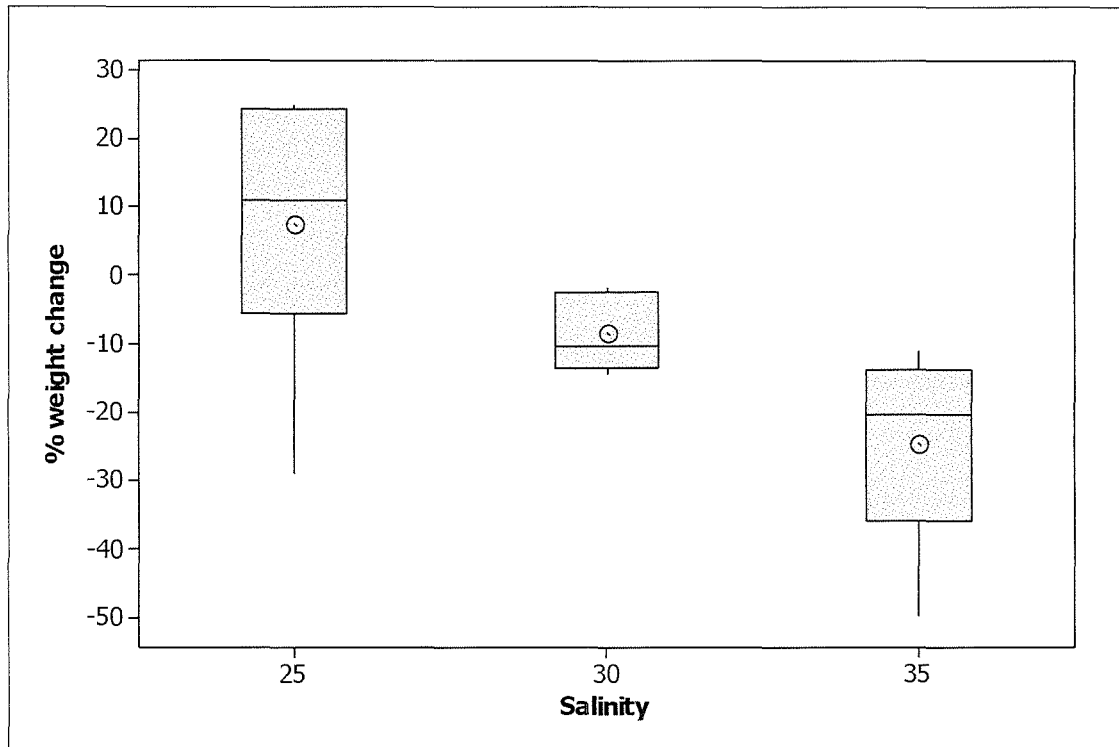


Figure 60: Boxplot of % weight changes during *ad libitum* feeding regime, using the weight measured on 11th August 2006 as the initial weight (FR2 period 2). Lines within the boxes are medians, circular symbols are means. Boxes indicate interquartile range from q2 – q3.

Consumption rates over two separate periods are shown in 61 and 62.

The first period was over 3 days, and the second period was over two days during feeding regime FR2 (*ad libitum*). During period 1 (Figure 61), salinity treatment 25 had the highest median consumption rate, salinity treatment 30 was intermediate and salinity 35 was the lowest. Data did not meet the assumptions of ANOVA, therefore Kruskal – Wallis non – parametric test was used and indicated that these differences were not statistically significant ($H = 2$, $df = 2$, $p > 0.05$). During period 2 (Figure 62), salinity treatment 25 again had the highest

median consumption rate, 35 was intermediate and salinity treatment 30 had the lowest consumption rate. However, these differences were not statistically significant (Kruskal – Wallis $H = 4.92$, $df = 2$, $p > 0.05$).

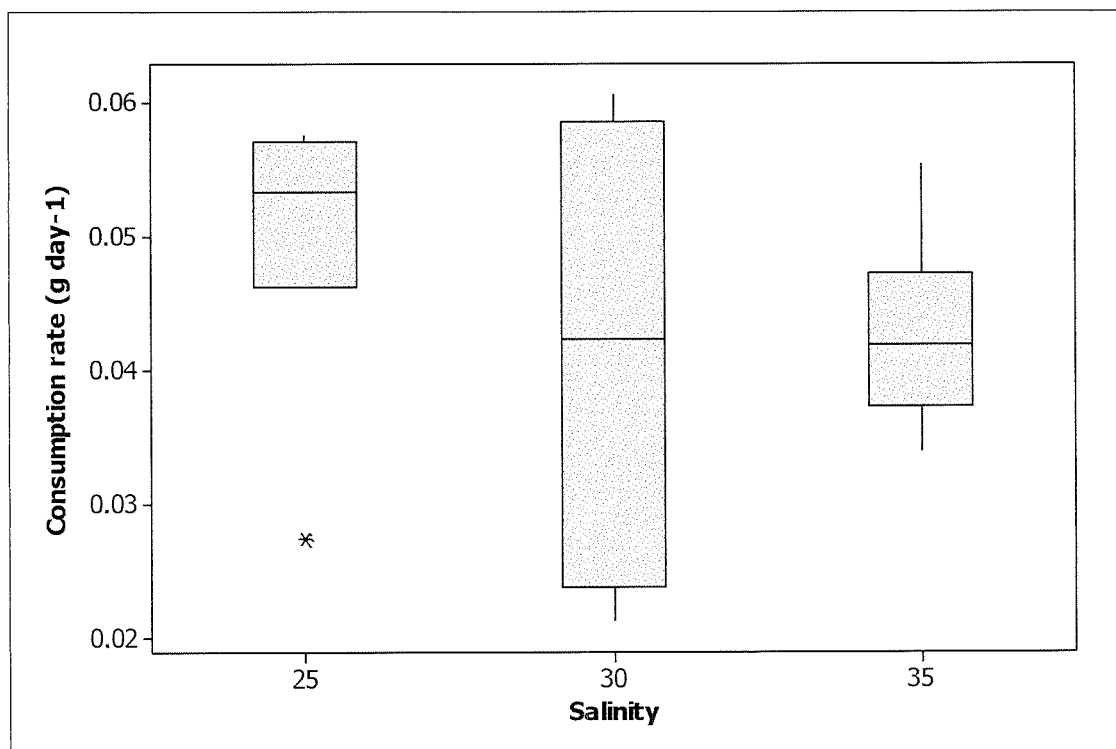


Figure 61: First period (29th-31st August 2006) consumption of chopped ragworm as dry weight (g) per day.

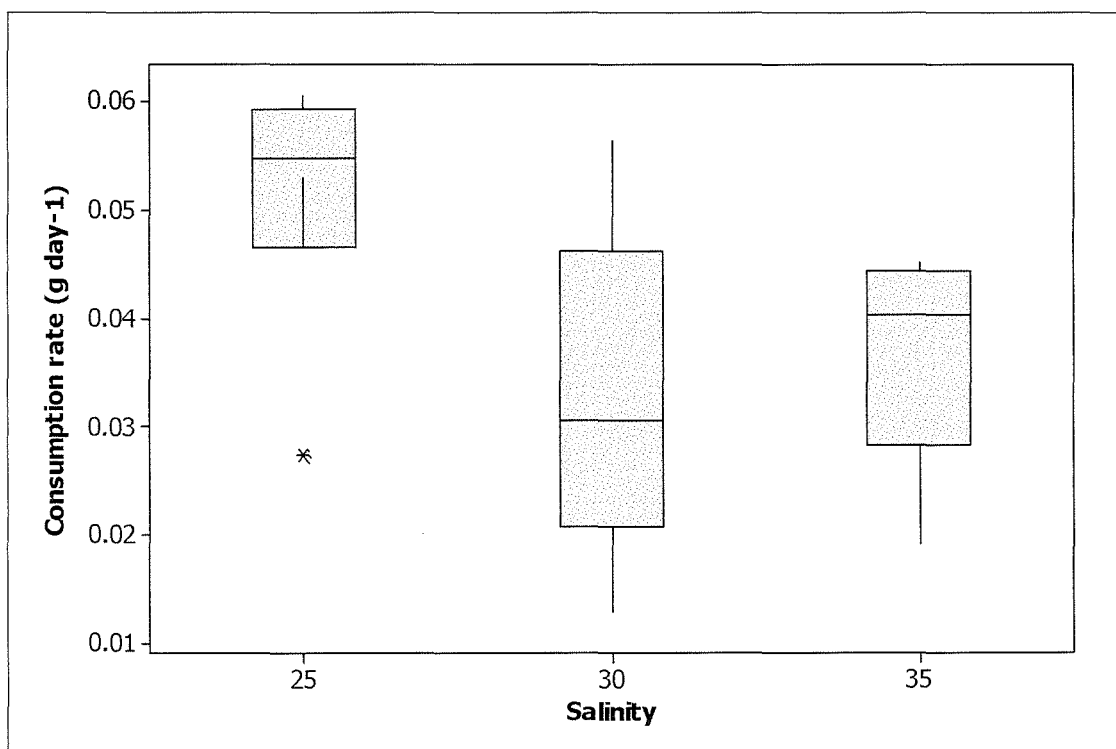


Figure 62: Second period (3rd-4th September 2006) consumption of chopped ragworm as dry weight (g) per day.

4.4. Discussion

4.4.1. Field Growth (from chapter 3)

Chapter 3 contains a full account of the growth rates of plaice on four sites in the Firth of Forth and Forth estuary. The Forth estuary has muddier sediments than the Outer Firth, with concomitant differences in faunal assemblages and 0+ plaice food composition, and has also been suggested to have much higher productivity than the Outer Firth (McLusky, 1987; Read, 1987). While food availability and composition has been shown to affect growth rates of 0+ plaice (van der Veer & Witte, 1993), the differences in salinity between the estuary and the Outer Firth may also have affected growth rates (Bouef & Payan, 2001; Andersen *et al.*, 2005)

4.4.2. Experimental Growth

The general trend in the experiment was of fish at the lowest salinity to show higher growth (or slower weight loss) than fish kept at the higher salinities. Most of the fish in the intermediate and high salinity treatments in the current study lost weight during the minimum feeding ration stage (FR1), while only some of the fish in the lowest salinity treatment lost weight. This indicates that the maintenance ration for 0+ plaice was variable but generally above the minimum food ration measured in the pilot study. The greater number of fish in lower salinity that showed an increase in weight suggests that maintenance ration was lower in salinity 25, although the differences in weight change were

only significant between salinities 25 and 35. The exact cause of differences in maintenance ration between individuals was not assessed here. Activity levels may influence the energy requirements of individuals, and some individuals were observed swimming more frequently than others, however, these behaviours were not measured and, thus effects of activity levels cannot be directly assessed. Future work could test whether different salinities affect activity levels. During the *ad libitum* feeding regime, some fish continued losing weight, although consumption rates were not significantly different, suggesting that fish could have been experiencing stress due to isolation. The measurements of consumption during *ad libitum* (FR2) feeding regime suggest that fish feeding behaviour was not affected by salinity treatment, however, fish exposed to salinity 25 did consume more than fish exposed to the other salinities. High individual variability may have obscured statistical differences in consumption rates between treatments, and only a larger sample size would determine whether the current study was of sufficient statistical power to detect differences in consumption rates between treatments. However, the differences in growth, despite the two feeding regimes, mean that the null hypothesis stated in the introduction to the current work can be rejected, supporting the hypothesis that plaice grow faster at salinity 25, than at higher salinities.

Salinity has been shown to affect growth of fish in other species and higher growth at lower salinities is almost universally accepted as applying to most marine and brackish water fish species (Bouef & Payan, 2001). The main

hypothesis relating salinity to growth suggests that fish living in hypo- and hyper-osmotic environments have additional energy costs associated with osmotic and ionic regulation, and that energy for these costs is met, at least partly, from energy used in growth that is diverted to osmoregulatory processes (e.g. Lyndon, 1994). In the current study feeding behaviour, defined as consumption rates of food, was not significantly different between salinity treatments, suggesting that the salinities used in the current study had no effect on feeding behaviour. Activity levels were not measured for the current study, thus the effect of salinity on activity cannot be assessed. Although different individual activity levels may have caused some of the variability in growth performance, there was no obvious difference in activity levels between salinity treatments.

Several physiological factors that help determine growth rates in fish could be affected by salinity. Food intake may be affected by salinity, via different drinking rates in different salinities (Tytler & Blaxter, 1988; Laiz – Carrion *et al.*, 2005) although this was not indicated for plaice in the current study. Salinity has been shown to affect hormonal control of macronutrient selection in European sea bass (Rubio *et al.*, 2005) and other species (Varsamos *et al.*, 2005).

Karakiri & von Westernhagen (1989) found an interaction between temperature and salinity on growth rates of plaice. A similar result was also found by Imsland *et al.* (2001) for juvenile turbot where optimum temperatures for growth increased at lower salinities. If the same were true for plaice, this may

also account for the differences in growth rates between plaice between Blackness and Limekilns in the present study; temperatures were similar at these two sites, yet growth was highest at Blackness, where the lowest salinity was found (Webb & Metcalfe, 1987). Similarly, plaice at the two fully marine sites may experience optimal growth conditions as a result of the interaction between temperatures and salinity, thus growth performance may be influenced only by food quality and availability. An extension of the work of Karakiri & von Westernhagen (1989) to include a greater range of salinities would answer this question

Marshall and Elliott (1998) and Thiel *et al.* (1995) showed that salinity influences both distributions and biomass of estuarine fish assemblages, including plaice. The differences in biomass they report could be accounted for by the effects of salinity on growth: younger plaice may use lower salinity areas to enhance growth, thus increasing biomass relative to areas with higher salinity. The effects of salinity on growth correlate with fish size, meaning different sizes of fish are found at different salinities. Indeed Poxton & Allouse (1982) suggest that juvenile plaice tolerate a wider range of salinities than larger plaice; the results of the current experiment indicate this could be due to higher energetic efficiency at lower salinities. However, plaice distributions are unlikely to be influenced by salinity alone: fish are influenced by a suite of environmental factors, not least of which are temperature and food availability (e.g. Fonds *et al.*, 1992; Thiel *et al.*, 1995; Attrill & Power, 2004), and plaice of this size are limited

by depth (Gibson, 1973; Riley, 1973) thus the effect of salinity on 0+ plaice distribution on the scale of an estuary may be difficult to detect.

It has been suggested that nursery quality may be influenced by the nursery's capacity for enhancing growth rates of juveniles (Gibson, 1994). Thus the effects of salinity on growth rates of 0+ plaice shown in the current study mean that salinity may be a factor in determining nursery quality. Karakiri & von Westernhagen (1989) found low growth of juvenile plaice at a salinity of 20; hence their data and the current results suggest the best plaice nurseries may be found at salinities between 20 and 30, when other determining factors (e.g. temperature, food availability) are equal. Indeed, salinity could also be considered a resource in determining ecological niches (Attrill & Power, 2004).

4.5. Conclusion

0+ plaice growth in the forth estuary is similar and possibly higher than growth of plaice in the nearby coastal firth of forth. The high growth in the estuary may partly be due to salinity. In a laboratory experiment, plaice were found to grow faster in lower salinity, but only when food was not limited. The higher growth rate at the lower salinity is likely to be due to greater gross growth efficiency, as salinity did not affect consumption rates of young plaice. Thus, salinity may be a factor in determining nursery quality.

Chapter 5

Carbon Stable Isotopes in Estuarine Sediments and their Utility as Migration Markers

5.1. Introduction

The conservation and management of aquatic organisms requires knowledge of their habitat requirements (Beck *et al.*, 2001; Gillanders *et al.*, 2003; Gillanders, 2005). Many aquatic organisms show a spatial disjunction in habitat between different life stages e.g. between adult, juvenile (nursery habitat) and pre-juvenile (egg and larval) individuals. Beck *et al.* (2001) propose a definition of nurseries based on the relative contribution to adult recruitment, which states that habitats can only be considered nurseries if they make a greater contribution per unit area to adult recruitment than other habitats occupied by conspecific juveniles. In order to determine habitat contribution, movements of individuals from each habitat must usually be traced, (however, see Mumby *et al.*, 2004). The methodologies employed to track migration directly or indirectly (and, hence, habitat contribution) vary greatly depending on factors such as species identity (varying biology), location (varying physical and chemical properties), quality of data required and resources available to investigators. The small size of juveniles of many species often means tagging or marking them is difficult (Gillanders *et al.*, 2003). One approach that has shown some success is the use of naturally-occurring stable isotopes of carbon, oxygen, nitrogen and sulphur (e.g. Gillanders *et al.*, 2003; Hobson, 1999; Peterson & Fry 1987). The value of these isotopes as tracers depends on the existence of habitat-specific concentrations of the isotope and physiological mechanisms by which the organism is 'marked' by the isotope, i.e. a tissue or structure that has an isotopic concentration that reflects the habitat the organism occupied.

Marine photosynthesis results in phytoplankton tissues with higher $\delta^{13}\text{C}$, relative to terrestrial and freshwater photosynthesisers (Peterson & Fry, 1987). In transitional and coastal waters, rivers may discharge significant masses of organic material of terrestrial and freshwater origin (terrigenous), with relatively low $\delta^{13}\text{C}$ (Riera & Richard, 1996). This allochthonous production is discharged into estuaries as dissolved (DOM) and particulate organic matter (POM) (McLusky & Elliott, 2004). Autochthonous primary production results in sediment and suspended POM and DOM with higher $\delta^{13}\text{C}$, relative to allochthonous production (Hobson, 1999; Peterson & Fry, 1987). In estuarine and coastal waters, the amount of terrigenous organic carbon (TC) in sediment and suspended material is expected to decrease with increasing distance from the source(s) of TC, with a corresponding increase in marine organic carbon (MC) (Thornton & McManus, 1994; Andrews *et al.*, 2000). The $\delta^{13}\text{C}$ value of the sediment organic carbon will reflect the proportions of TC and MC: sediments with a higher proportion of TC will have relatively low $\delta^{13}\text{C}$ and sediments with a higher proportion of MC will have relatively high $\delta^{13}\text{C}$. Graham *et al.* (2001) sampled sub-tidal sediments from the middle channel of the Forth estuary, East Central Scotland, and discovered no $\delta^{13}\text{C}$ gradient with distance from the head of the estuary near Stirling. Clarke & Elliott (1998) suggested that strong tidal scouring of the sea-bed removed all fine particulate material from the mid-channel and deposited it on the intertidal areas of the estuary. This could explain Graham *et al.* (2001) negative result and suggests that a gradient might exist in those intertidal areas used by juvenile plaice. The primary aim of the present

study was to test the hypotheses that organic material of terrestrial origin is deposited on the intertidal areas of the Forth estuary, and that a gradient in $\delta^{13}\text{C}$ caused by this TC may be used as a habitat marker, as manifested in isotopic signatures from juvenile plaice otoliths. A subsidiary null hypothesis that was investigated was that there is no difference in $\delta^{13}\text{C}$ between sandy and muddy sediments regardless of site.

A second aim was to assess the utility of otolith carbon and oxygen isotopes in identifying plaice (*Pleuronectes platessa*) nursery habitats in the estuary and shallow coastal waters of the Firth of Forth in East Central Scotland. Otoliths are calcium carbonate (aragonite polymorph) structures in the endolymphatic sacs of teleosts (Gauldie *et al.*, 1994). Carbon and oxygen isotopic composition of otoliths has been investigated for many teleost species (Kalish, 1991a, b; Gauldie, 1996; Gauldie *et al.*, 1995; Gauldie *et al.*, 1994; Edmonds & Fletcher, 1997; Weidman & Millner, 2000; Begg & Weidman, 2001; Høei *et al.*, 2004; Jamieson *et al.*, 2004), and may allow reconstruction of historic habitat use of an individual fish (which is not the case for other tissues, such as muscle, that turn-over and hence change their signature over time). Many estuarine benthic organisms are deposit and suspension feeding, and, as such, will consume some TC and some MC. Thus, it may be expected that the carbon isotope ratios of these organisms' tissues will reflect the isotopic composition of their food. Indeed, this has been shown for many different groups including fish, crustaceans, polychaetes, echinoderms, molluscs, birds, mammals and reptiles,

and has also been used to elucidate trophic interactions in coastal systems (Riera & Richard, 1996; Hobson, 1999; Waldron *et al.*, 2001; Bearhop *et al.*, 2004; Darnaude *et al.*, 2004b; Darnaude, 2005). Thus if the stable isotope signal of a juvenile habitat is taken up by the organisms inhabiting that area, it may be possible to assign adults to their juvenile habitat using stable isotope ratios. Carbon isotopic composition of otolith aragonite is influenced by both dietary sources and the dissolved inorganic carbon (DIC) of seawater. Hence otolith $\delta^{13}\text{C}$ can, in principle, be used for dietary reconstruction and habitat tracing (Kalish 1991a). In addition, otolith $\delta^{18}\text{O}$ may record differences in temperature and salinity (Kalish, 1991a; Witbaard *et al.*, 1994). Plaice (*Pleuronectes platessa*) are one of the three most commercially important demersal fish species to use intertidal areas of the Firth of Forth and estuary as juveniles (Scottish Executive, 2005; Greenwood *et al.*, 2002), and the identification of key nursery sites for this species is an important management objective. Hence they are the focal species in the current study.

5.2. Methods and Materials

5.2.1. Site description

The Forth Estuary and the Firth of Forth (Figure 63) are located on the east coast of Central Scotland, UK. The head of the estuary is at Stirling bridge (approximately 3° 52' W, 56° 07' N) and the estuary ends (and becomes the Firth of Forth) between the road and the rail bridge (approximately 3° 24' W, 56° 00' N). Seven major rivers discharge into the estuary including the Forth, Teith, Allan and Carron which, combined, constitute around 75% of the total freshwater flow to the estuary (Webb & Metcalfe, 1987). The Outer Firth is fully marine and encloses the area between the two bridges and a line drawn between Fife Ness in Fife (approximately 2° 37' W, 56° 15' N) extending south past the east of the Isle of May to Cockburnspath; East of this line is the North Sea (McLusky, 1987)

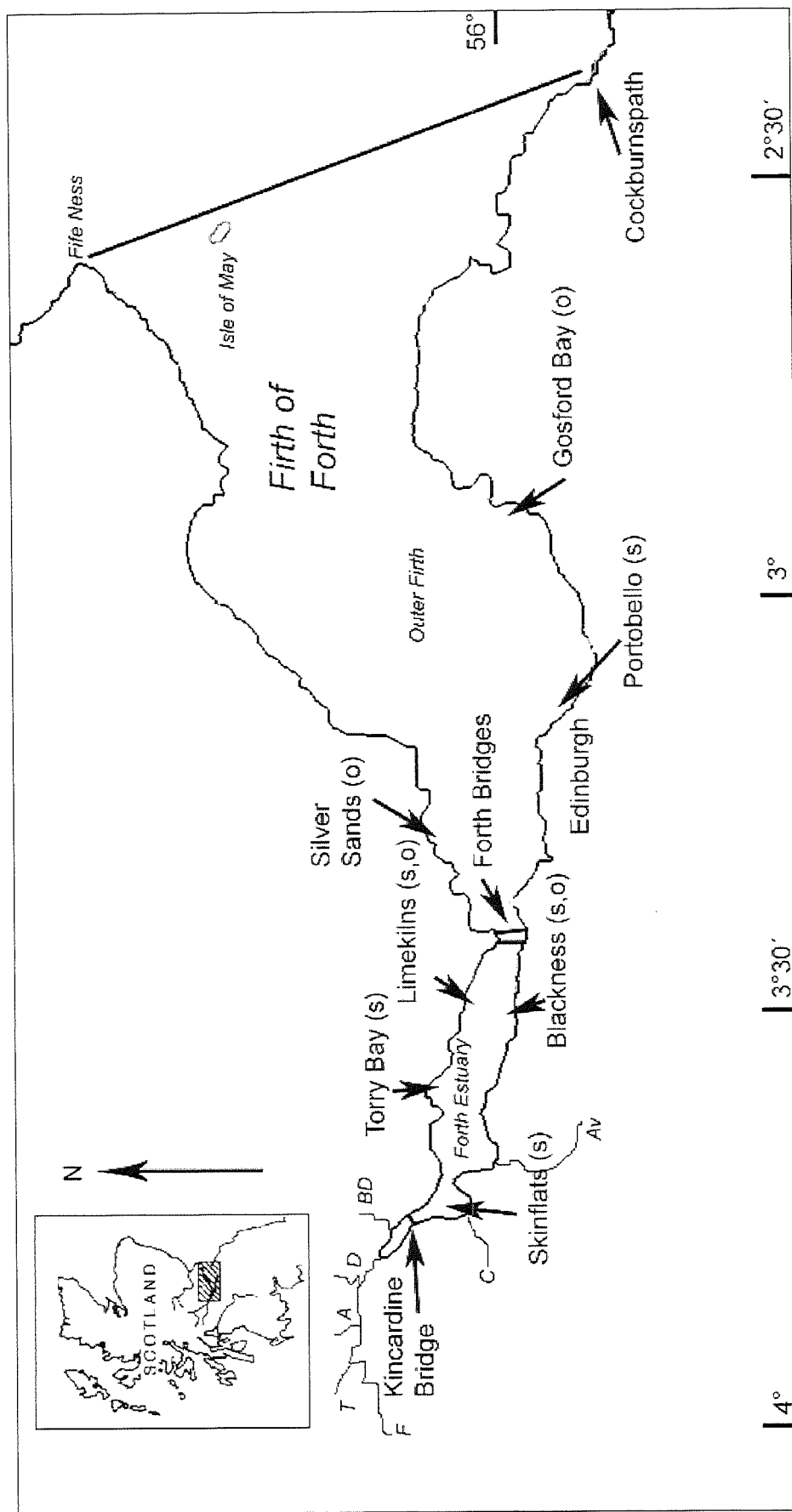


Figure 63: Map of the Forth Estuary and the Firth of Forth. Bridges are marked for delineation of physical features (see section 2.1). Arrows mark intertidal sampling sites. Letters in parentheses after the site name indicate whether otoliths (o) or sediments (s) were sampled at that site. Rivers at approximate point of discharge into the estuary are marked in italics: Forth (*F*), Teith (*T*), Allan (*A*), Devon (*D*), Black Devon (*BD*), Carron (*C*) and Avon (*Av*).

5.2.2. Sediment analysis

Samples of surface sediments were taken from four intertidal mudflats (Skinflats, Torry Bay, Blackness and Limekilns) downstream of the Kincardine Bridge in the estuary, and one site, Portobello, in the Outer Firth (Figure 63). At all four of the estuarine sites, sandy and muddy sediments were identified visually and six replicates were taken randomly from each sediment type. No muddy sediments could be observed at Portobello. Each replicate consisted of five pooled sub-samples taken with a 25 mm diameter core, to a depth of approximately 2-3 cm. At all sites, five replicates were analysed for carbon stable isotopes and one replicate was used for both granulometric and organic content analysis.

All stable isotope results are expressed as parts per thousand (‰) different from a reference material (Vienna Pee-Dee Belemnite, VPDB): $\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$ where δ is the measure of heavy to light isotope in the sample, X is the element (C or O in the current study), and R is the ratio $^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$. For isotope analysis, sediments were homogenised by mixing thoroughly before oven-drying overnight at 50 °C. After drying the sediments were again homogenised, with care taken to minimise loss of very fine particles. A sub-sample of 100 mg was taken from each replicate, placed in a 1.5ml plastic vial and acidified with 1M hydrochloric acid (HCl) to remove carbonates. After the addition of a 40 μL aliquot of HCl the sediments were dried overnight at 40 °C. Two further rounds of HCl aliquots and drying were carried out until no

effervescence was observed; this was assumed to be when all carbonates had been removed, leaving only organic carbon (Kennedy *et al.*, 2005). The vials were then sealed until ready to be analysed for carbon stable isotopes.

All sediment stable isotope analyses were carried out on a Delta^{Plus} (Thermo-Finnigan, Bremen, Germany) continuous flow, isotope ratio mass spectrometer at the National Institute of Water and Atmospheric Research (NIWA) stable isotope laboratory in Wellington. Solid samples were prepared in tin boats and combusted in a NA 1500N (Fisons Instruments, Rodano, Italy) elemental analyser combustion furnace at 1020°C in a flow of oxygen and He carrier gas. CO₂ gas was separated on a Porapak Q gas chromatograph column before being introduced to the mass spectrometer detector via an open split Conflo II interface (Thermo-Finnigan, Bremen, Germany). A CO₂ reference gas standard was introduced to the mass spectrometer with every sample analysis. ISODAT (Thermo-Finnigan) software was used to calculate $\delta^{13}\text{C}$ values against the CO₂ reference gas relative to PDB, correcting for ¹⁷O. Percent C values were calculated relative to a solid laboratory reference standard of urea (Elemental Microanalysis, U.K.) at the beginning of each run. Internal standards were routinely checked against National Institute of Standards and Technology (NIST) standards. Accuracy and precision data for NIST standard analyses are given in Table 24. The precision on repeat analyses of urea standards during batch analysis of data presented in this paper are given in Table 25. Repeat analysis of

NIST standards produces data accurate to within < 0.4 ‰ for $\delta^{13}\text{C}$ and a precision of better than 0.25 ‰.

Table 24 Comparison of %C and $\delta^{13}\text{C}$ values analysed on the NIWA Thermo-Finnigan Deltaplus mass spectrometer compared to reported NIST values. The $\pm$ values represent 1 standard deviation.

NIST standard	NIST $\delta^{13}\text{C}$ ‰ reported values	NIWA measured $\delta^{13}\text{C}$ ‰ values (n=)	NIST %C reported or calculated values	NIWA %C measured values (n=)
8541 Graphite	-15.90 $\pm$ 0.25	-15.48 $\pm$ 0.11 (10)	-	-
8542 Sucrose	-10.47 $\pm$ 0.13	-10.78 $\pm$ 0.38 (10)	42.11	43.84 $\pm$ 0.61 (9)

Table 25 Precision data for repeat analysis of urea standards during sample batch analyses. The $\pm$ values represent 1 standard deviation.

Internal Urea Standard Known value Measured value	Wt % C (n=3) 20 19.64 $\pm$ 0.14	$\delta^{13}\text{C}$ (n=5) -46.73 $\pm$ 0.14
---	--	--

Sediment granulometric and organic content analyses were conducted as per chapter 1: calculation of the percentage silt-clay by mass and calculation of organic content as percentage by mass (loss on ignition) of each sample.

5.2.3. Otolith analysis

Plaice were caught at four sites (Limekilns and Blackness in the Forth Estuary, and Silver Sands and Gosford bay in the Outer Firth; Figure 63) from May to October 2005. All fish were caught using a 1.5 m Riley push net with three tickler chains, deployed within 1 hour after low tide, in water depths ranging from 0.1 to 1.0 m. Sagittal otoliths were removed from 5 fish per site giving a total

of 20 pairs of otoliths. No fish were available from Portobello. The fish used were caught in September 2005, towards the end of the intertidal phase of 0+ group plaice; these are the offspring of adults that spawned over the preceding winter, for this particular population. Larger specimens were selected in order to allow maximum time for the habitat specific isotope signature to be picked up by the otolith aragonite. Plaice show a remarkable alongshore site fidelity (Burrows *et al.*, 2004) and clear depth selection (Gibson, 1973), thus, the isotopic composition in plaice otoliths was expected to reflect the sediment isotopic signal present at the site of capture. Juvenile plaice show a preference for fine sandy sediments in laboratory studies (Gibson & Robb, 2000); however, the plaice caught in the estuary for the present study were caught on the muddy substrates at Blackness and Limekilns. Both sagittal otoliths were removed from each fish and combined to provide sufficient material for mass spectrometry. The otoliths were cleaned in ethanol and then rinsed in distilled water. The otolith pairs were then left to air dry before being placed in plastic vials and crushed. The otolith powders were weighed into quartz buckets and plasma-ashed. Each sample in turn was reacted with a common bath of 100 % H_3PO_4 to produce CO_2 , which was cryogenically purified and analysed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ on a VG Prism II mass spectrometer. Values are reported with respect to VPDB; internal standards MAB-2B ($\delta^{13}\text{C}$ (VPDB) = + 2.48 ‰ and $\delta^{18}\text{O}$ (VPDB) = - 2.40 ‰ $\delta^{18}\text{O}$), run over the entire sample set give a s.d. of ± 0.13 and ± 0.20 for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ respectively. MAB-2B standards are periodically checked against IAEA CO-1 and IAEA CO-8 carbonates

5.2.4. Statistical analyses

The effects of distance from the upper estuary and of the sediment type on carbon isotope ratios were explored with Analysis of Covariance (ANCOVA) using site as the factor and % fines as a covariate; all data were tested for normality, equality of variances and homogeneity of slopes and transformed where necessary. To determine whether otolith isotopic ratios differed between sites, Analysis of Variance (ANOVA) was calculated once with carbon and once with oxygen isotope ratios as the response and site as the factor; all data were tested for normality and equality of variances and transformed if necessary.

5.3. Results

Cumulative frequency curves are shown in Figures 64 – 72. These curves indicate that Torry Bay sandy and silty sediments had a similar median particle size; however, the silty sediments were 24 % silt /clay, whereas the sandy sediments were approximately 5 % silt/clay. At all other silty sites, sediment median particle size was less than 62 μm , and at sandy sites, sediment median particle diameter was greater than 62 μm .

The carbon isotope ratios of the sediments varied between sites and between muddy and sandy sediments (Figure 73). The $\delta^{13}\text{C}$ values for Portobello contained an outlier with a value of -25.63 ‰. The sample that gave this value was taken from near where a small freshwater stream (Figgat Burn) discharges onto the beach. The next nearest sample to this freshwater discharge was taken approximately 20m farther away, and gave a $\delta^{13}\text{C}$ value of -21.18 ‰, demonstrating the localised influence of this stream. This outlier was the most depleted value found in the current study; it is likely therefore that this value indicated conditions representative of freshwater $\delta^{13}\text{C}$ organic carbon values. The ratios measured in sandy sediments showed a strong trend of enrichment with distance from the upper estuary. In contrast, muddy sediments showed a comparatively slight enrichment in $\delta^{13}\text{C}$ with distance down the estuary. At each site where muddy and sandy sediments were found, the muddy sediments had more depleted $\delta^{13}\text{C}$ than the corresponding sandy sediment, with the exception of Skinflats, where the sandy sediments were more depleted.

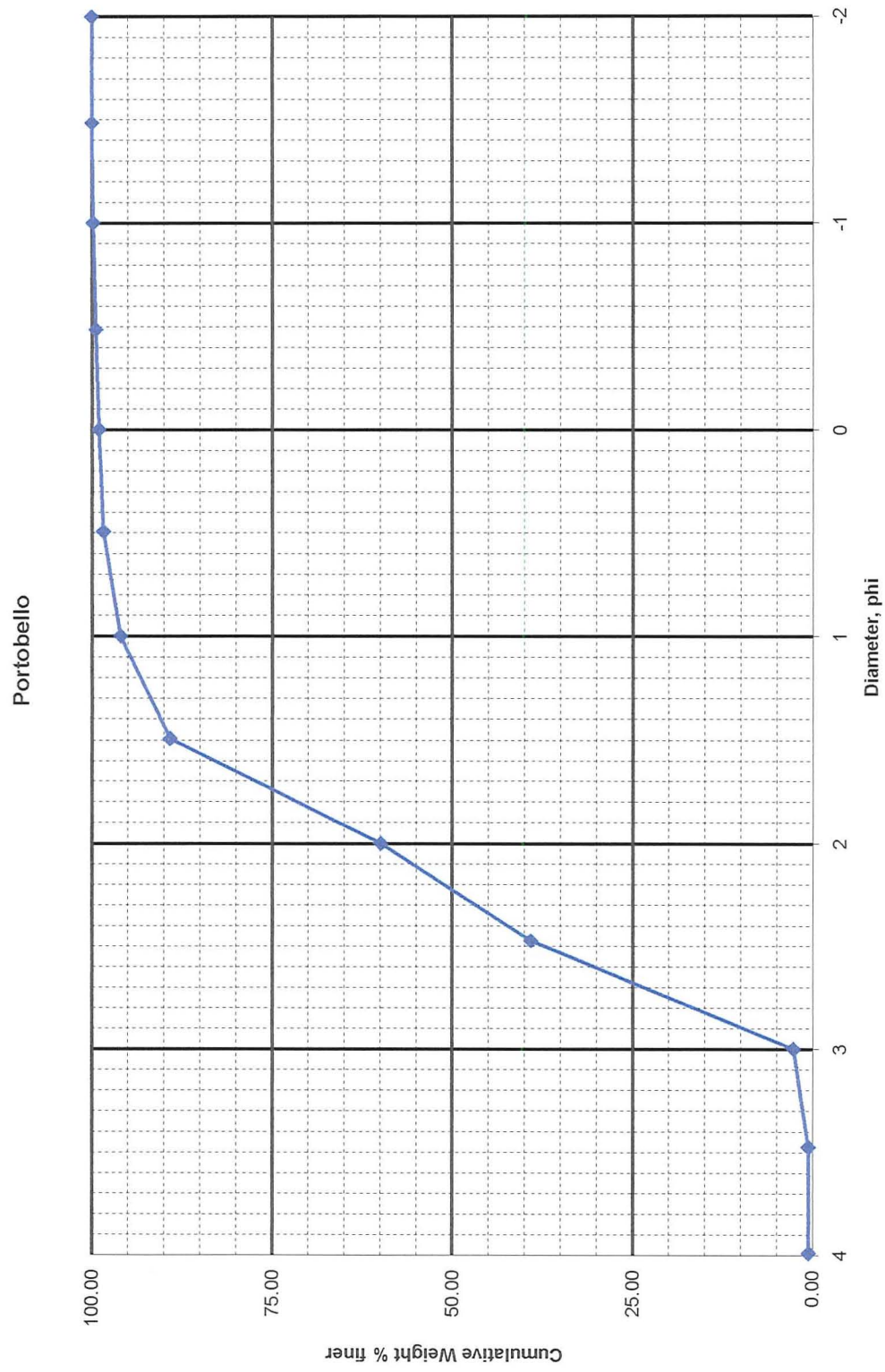


Figure 64: Cumulative frequency curve of sediments from Portobello.

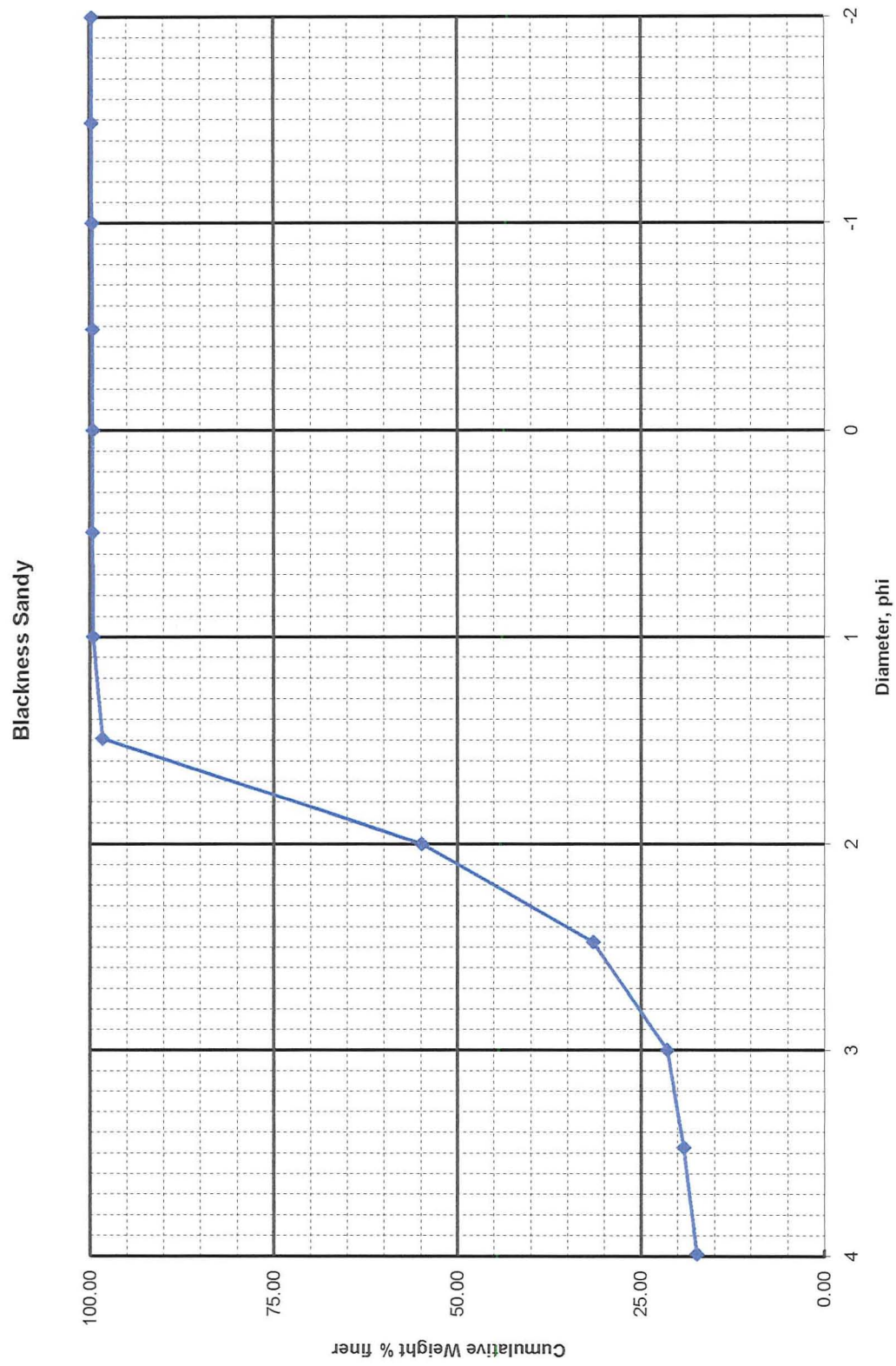


Figure 65: Cumulative frequency curve of sediments from Blackness Sandy.

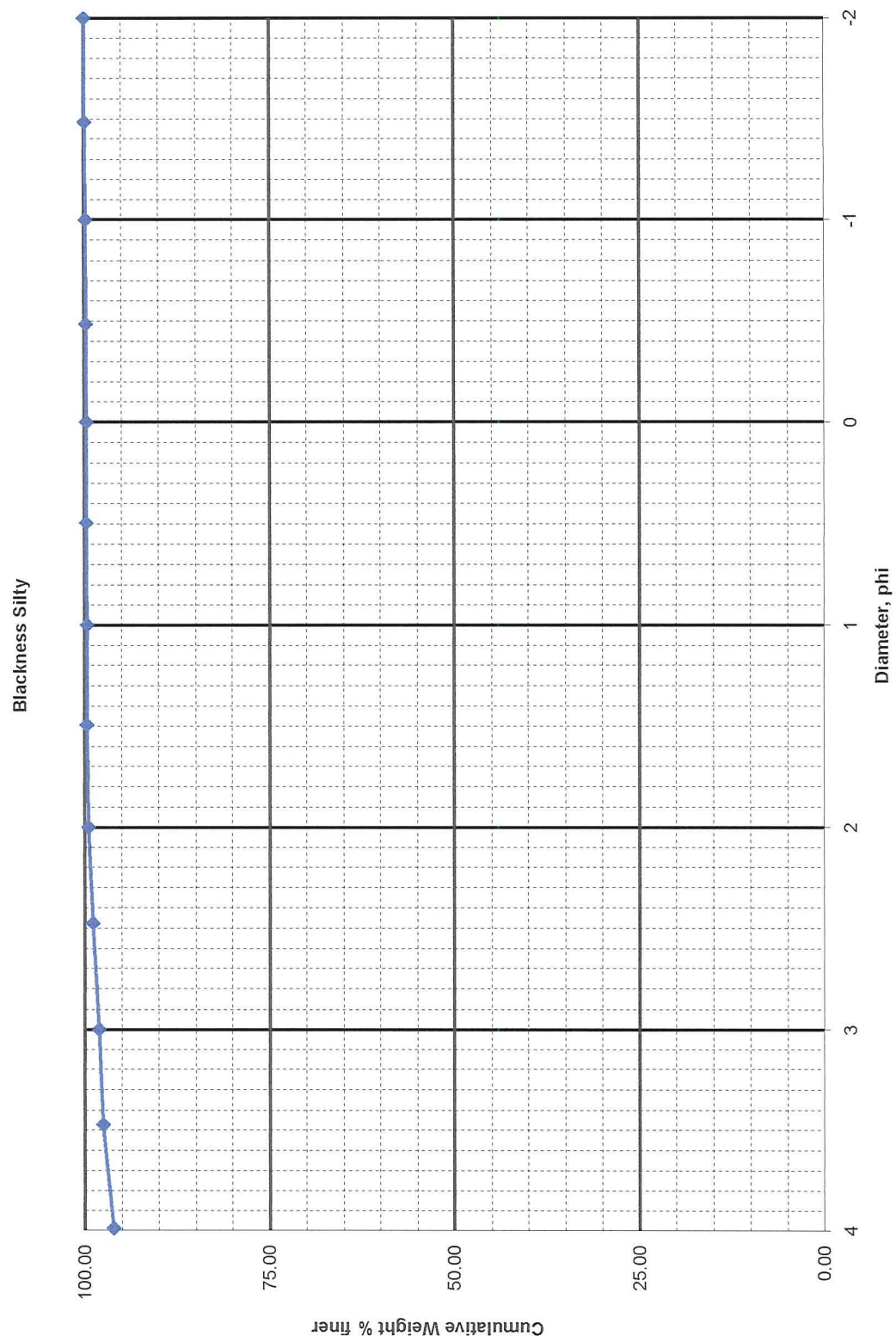


Figure 66: Cumulative frequency curve of sediments from Blackness Silty.

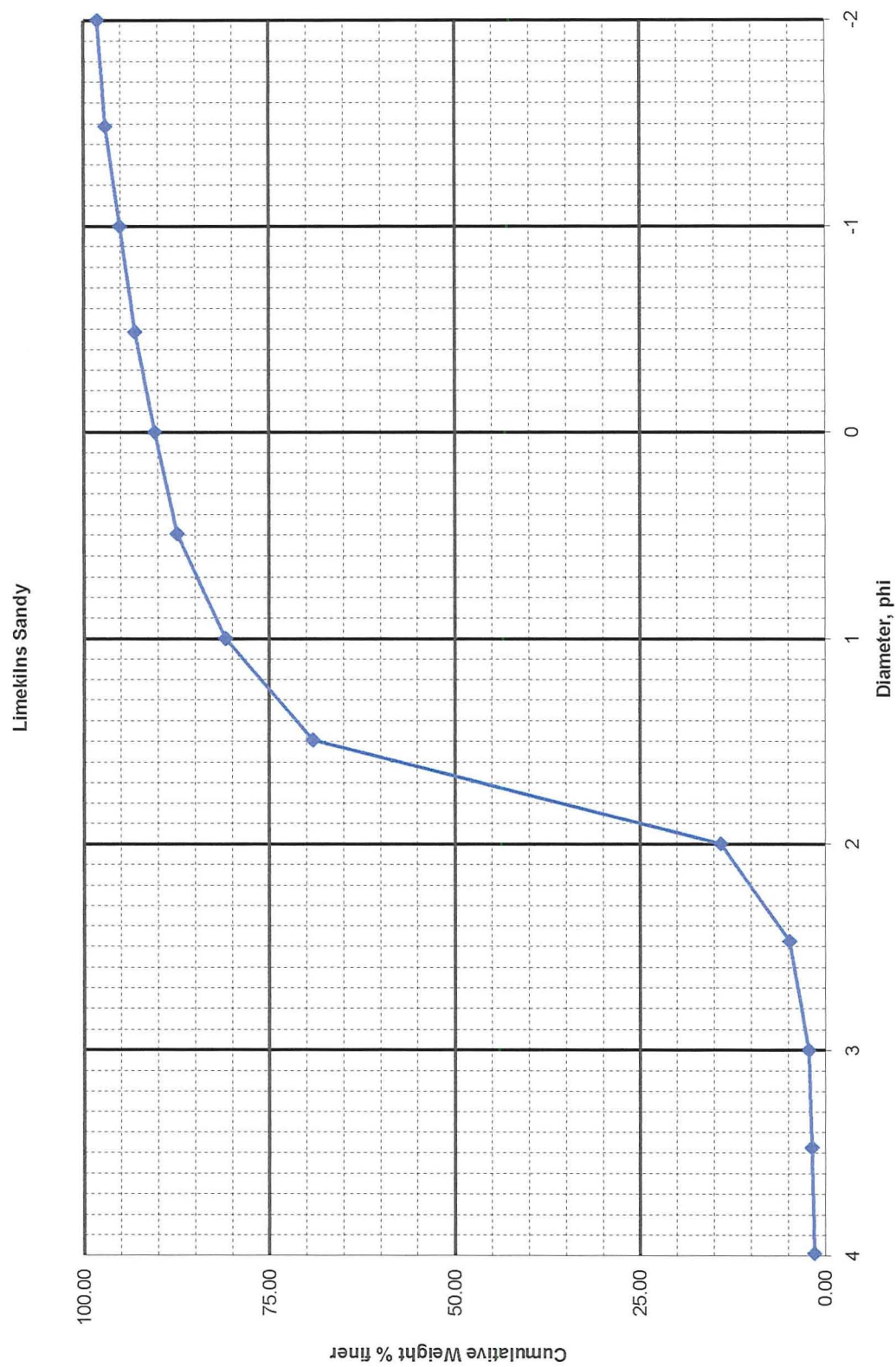


Figure 67: Cumulative frequency curve of sediments from Limekilns Sandy.

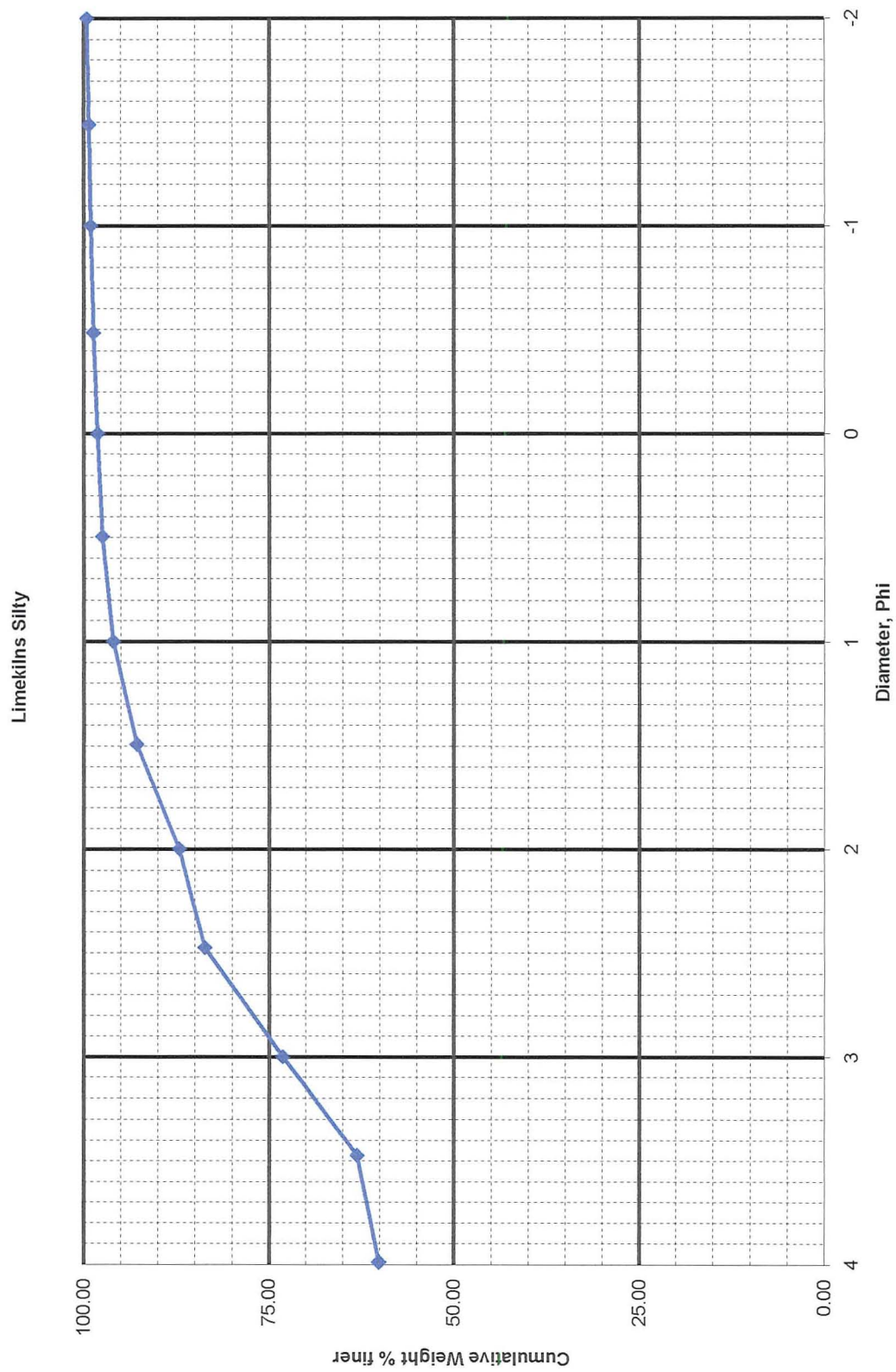


Figure 68: Cumulative frequency curve of sediments from Limekilns Silty.

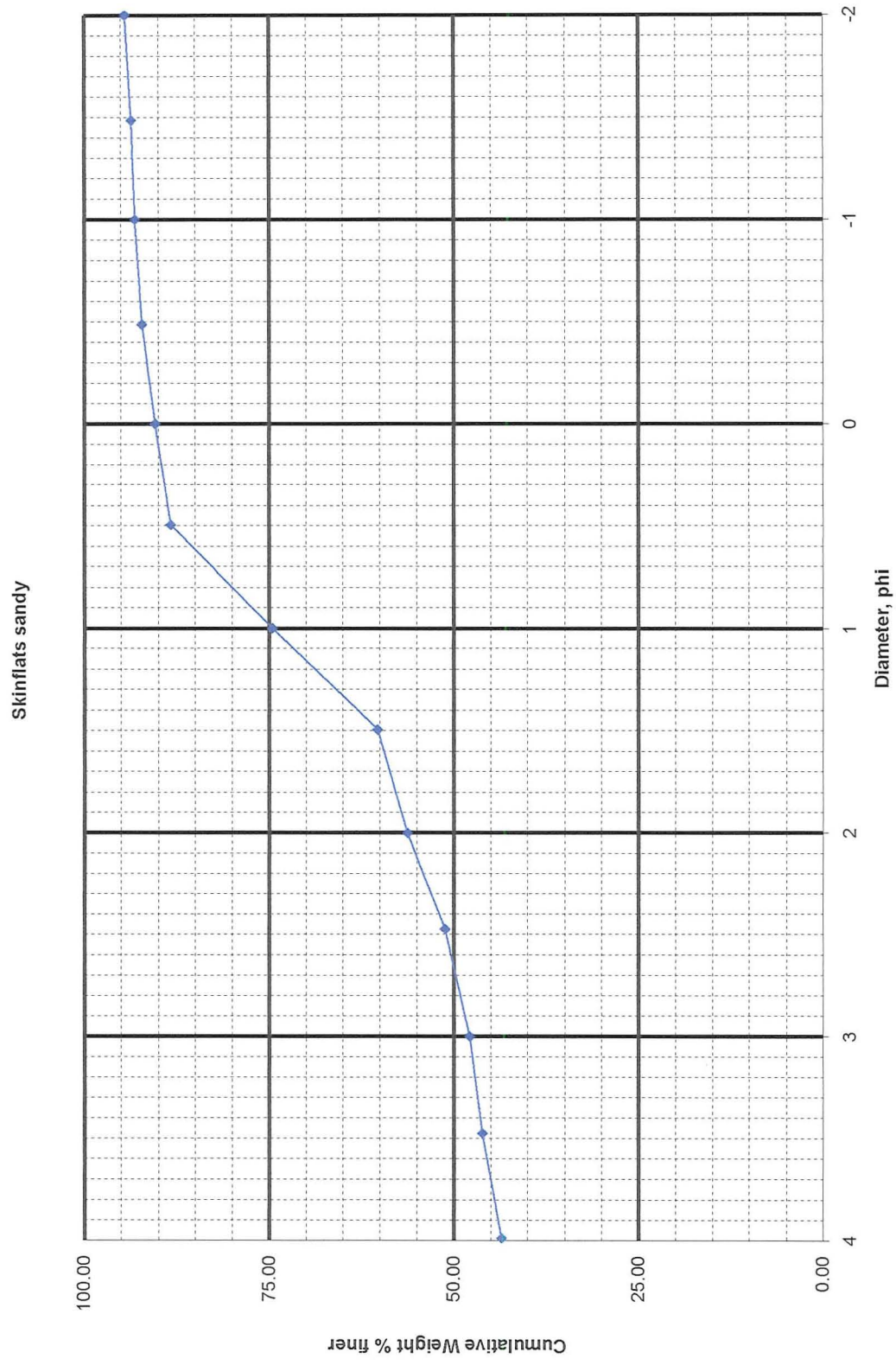


Figure 69: Cumulative frequency curve of sediments from Skinflats Sandy.

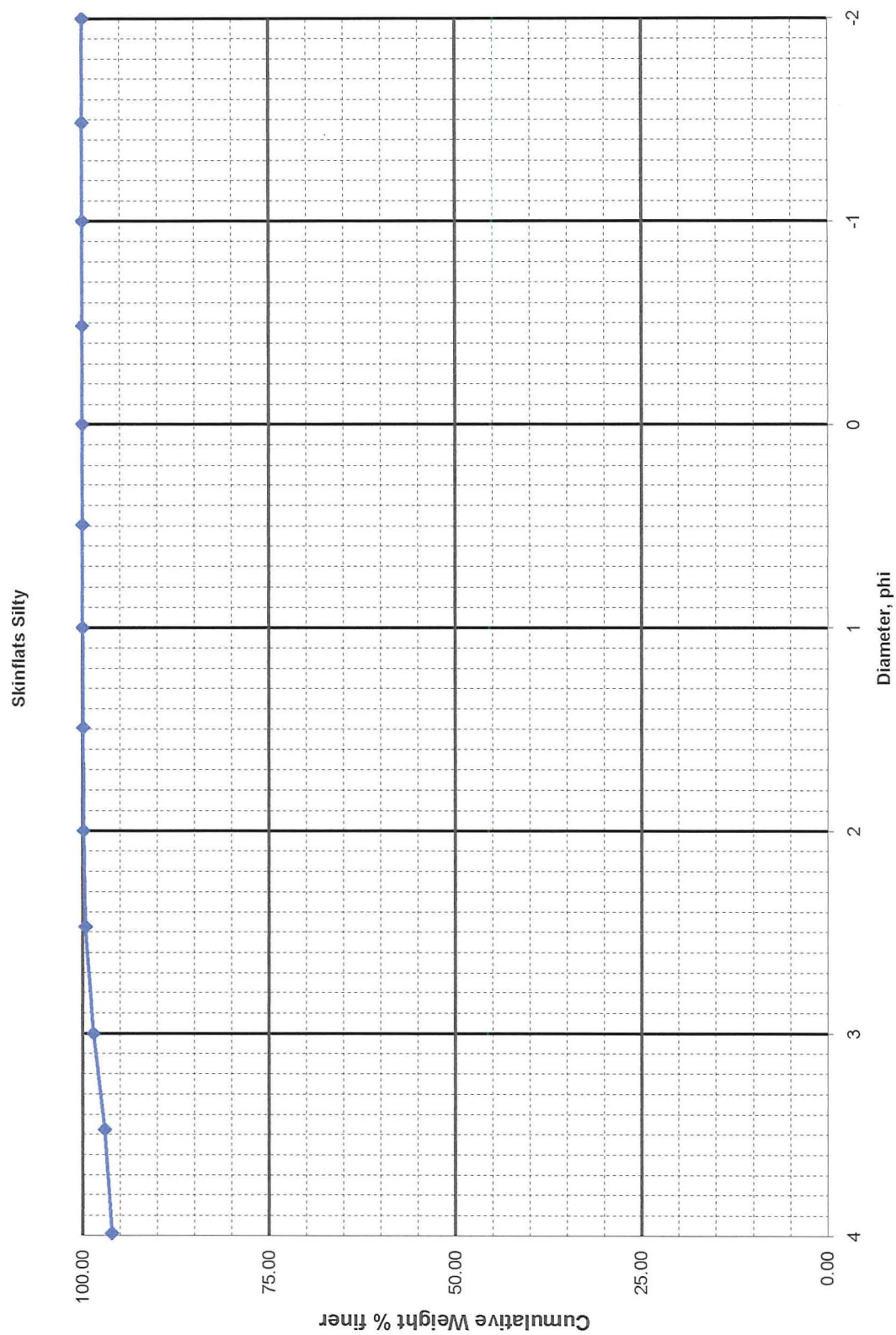


Figure 70: Cumulative frequency curve of sediments from Skinflats Silty.

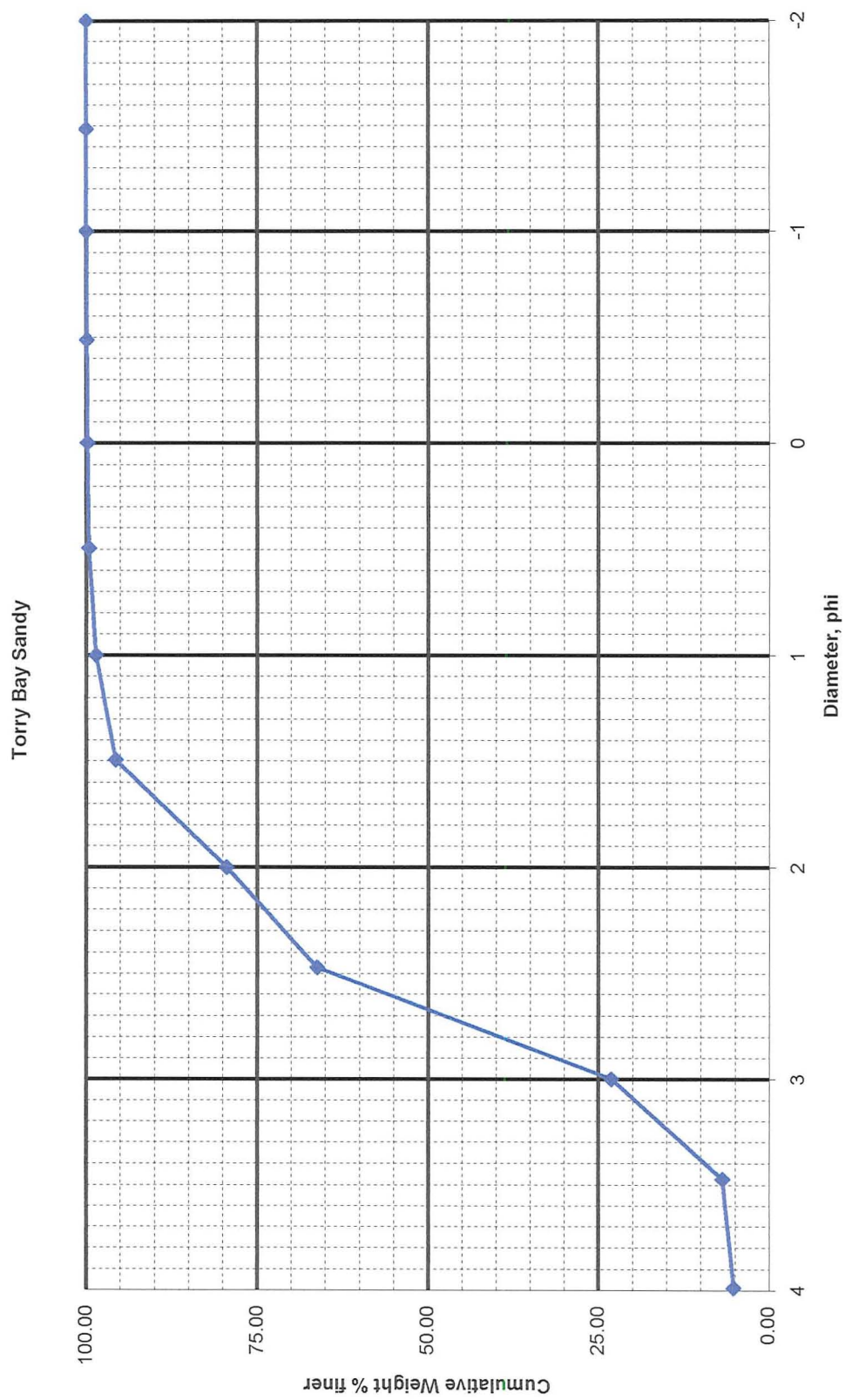


Figure 71: Cumulative frequency curve of sediments from Torry Bay Sandy.

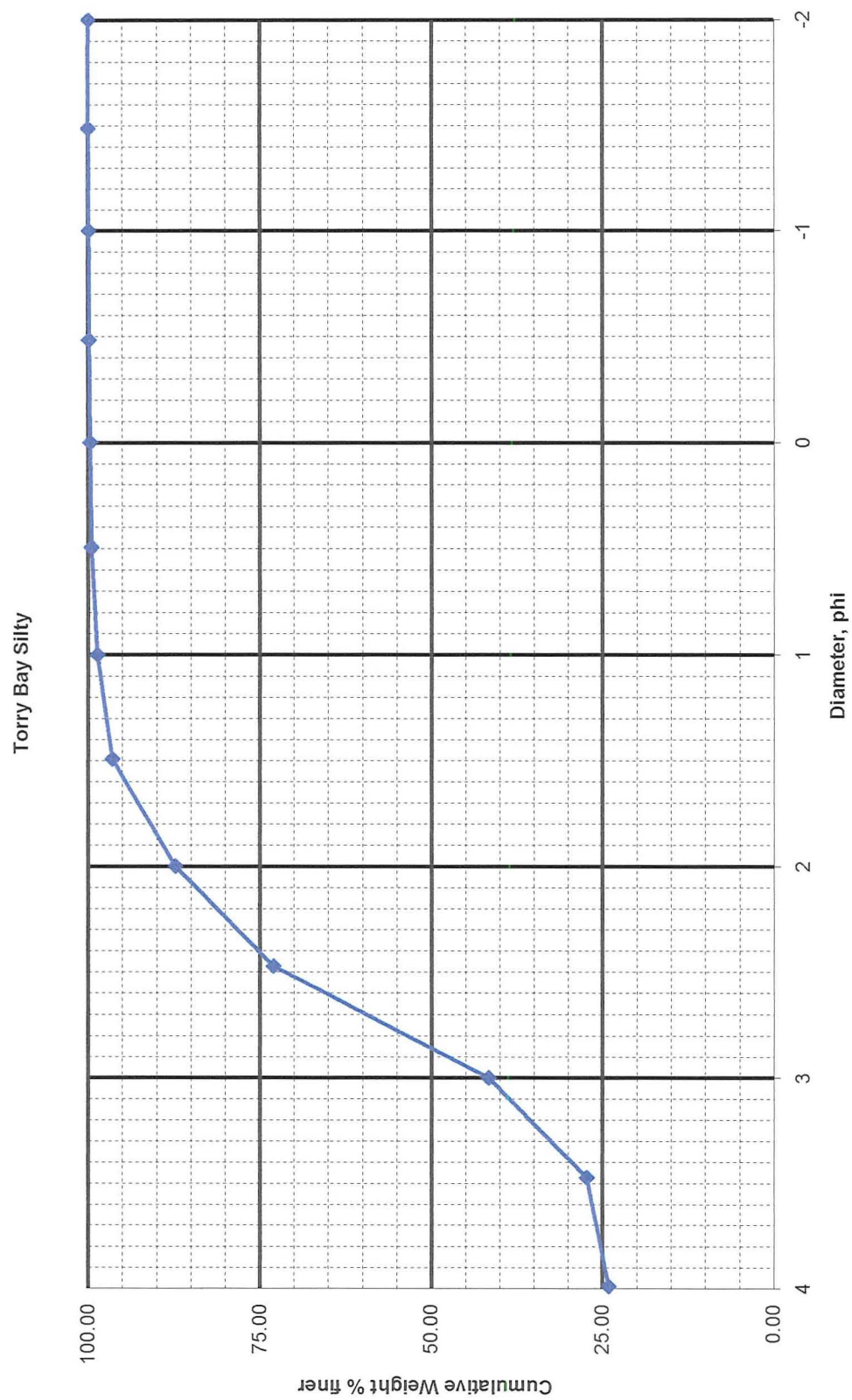


Figure 72: Cumulative frequency curve of sediments from Torry Bay Silty.

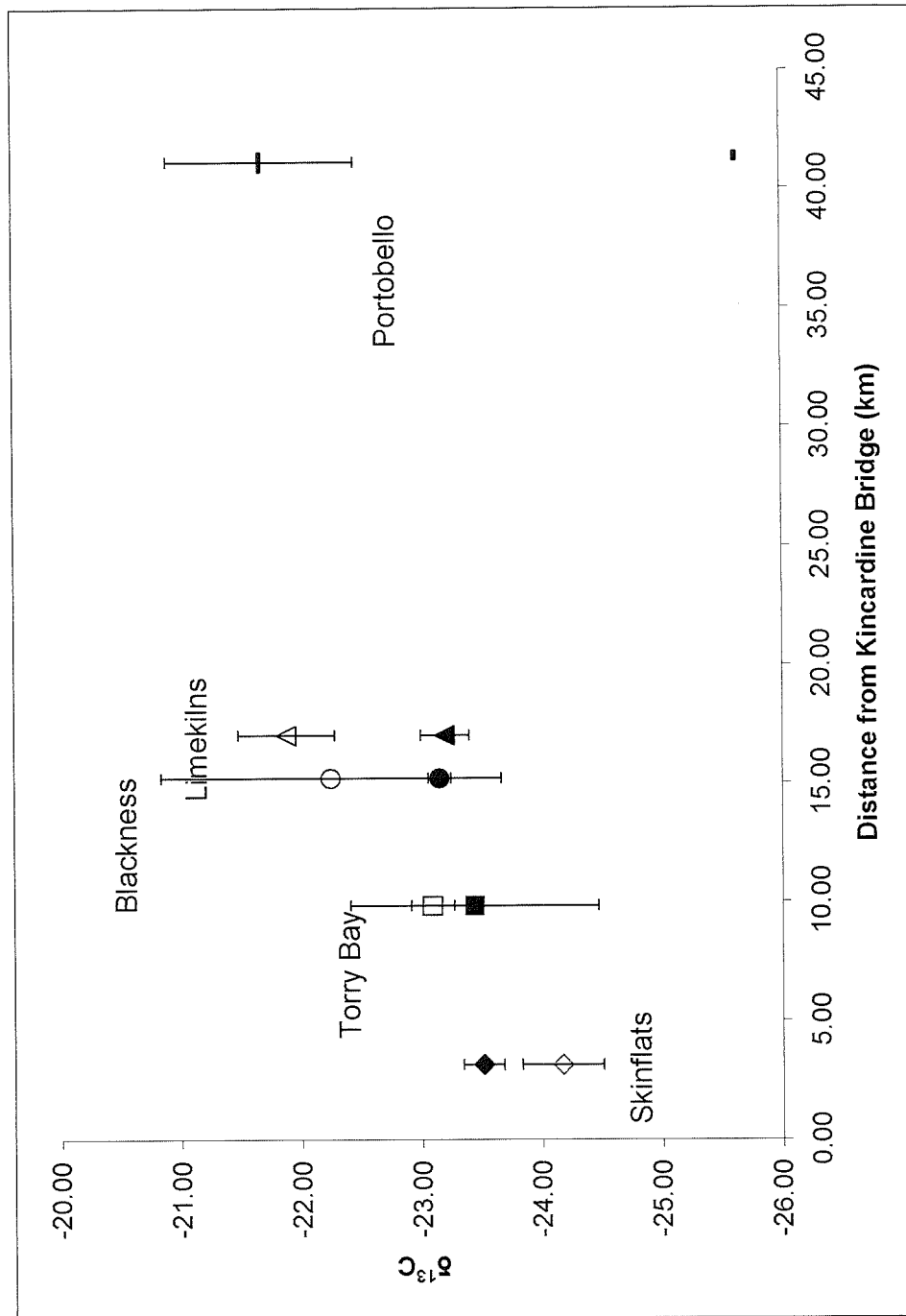


Figure 73: $\delta^{13}C$ measured at various locations in the Forth Estuary and Firth. Solid symbols indicate the sediment was predominantly muddy, outlined symbols indicate the sediment was predominantly sandy (however Torry Bay sandy sediments had intermediate silt/clay content), and Portobello sediments were sandy only. Distances from Kincardine Bridge are straight line distances (note that Blackness and Limekilns are actually the same distance from the bridge. This separation was necessary for visual clarity: Blackness is at the correct distance on this graph) Error bars are $\pm$ standard deviations. The single point at Portobello is a statistical outlier (see text).

Two separate analyses of covariance were performed to determine the influence of the outlier recorded from the Portobello samples: one with and the other without the outlier included (Table 26). $\delta^{13}\text{C}$ values were significantly different between locations with the Portobello outlier included ($p = 0.02$), however, the effect of % fines was not significant ($p = 0.06$). With the Portobello outlier removed, $\delta^{13}\text{C}$ values were highly significantly different between locations ($p < 0.0001$) and % fines had a significant effect ($p = 0.02$). Hence $\delta^{13}\text{C}$ values differed between sites, suggesting that the inputs of TC vs. MC varies with distance down the estuary; and finer sediments were more depleted in $\delta^{13}\text{C}$, indicating TC content increases with increasing silt/clay content. This is supported by the results of a correlation analysis between the percentage organic content and $\delta^{13}\text{C}$ of the sediment showing a highly significant negative relationship (Pearsons product moment correlation, $r = -0.550$, $p < 0.001$; Figure 74).

Table 26: ANCOVA was used to test for differences between locations. Percent fines (by weight) was used as a covariate. The first ANCOVA included an outlier from Portobello; the second ANCOVA excluded this outlier. The results indicate that there is a gradient in $\delta^{13}\text{C}$ with distance down the estuary, and that finer sediments are depleted in $\delta^{13}\text{C}$.

Source	<i>df</i>	MS	F	<i>p</i>
Portobello outlier included				
%fines	1	3.224	3.71	0.061
Location	4	2.9084	3.35	0.019
Error	39	0.8679		
Total	44			
Portobello outlier excluded				
%fines	1	3.224	5.73	0.022
Location	4	3.7891	6.74	0.000
Error	38	0.5623		
Total	43			

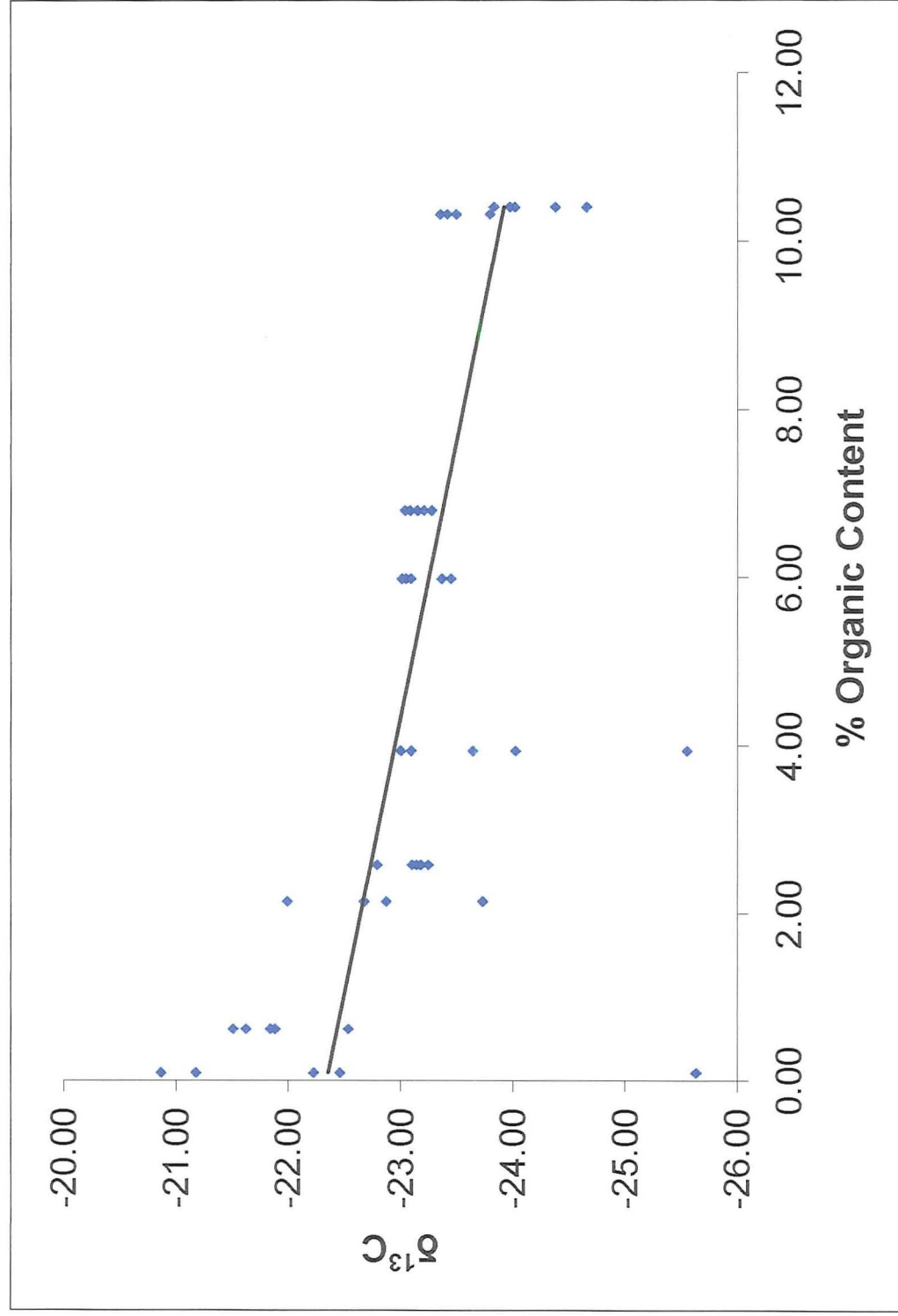


Figure 74: Correlation analysis of organic content vs. $\delta^{13}\text{C}$. Pearson's product moment correlation values of r (-0.550) and p (<0.001) suggest there is a negative linear relationship.

A simple two-source mixing model (equation 6) was used to estimate the relative inputs of TC vs. MC. This model estimates proportions of MC and assumes TC is the only other input of organic carbon.

$$x = 100 \left(\frac{y - t}{m - t} \right) \quad (6)$$

Where x is percentage of sediment that was derived from marine sources, y is measured $\delta^{13}\text{C}$ of sediment, t is $\delta^{13}\text{C}$ of terrestrial organic carbon and m is $\delta^{13}\text{C}$ of marine organic carbon. This model is similar to those used in other studies e.g. Darnaude *et al.* (2004b) In this model m and t are the only two sources contributing to sediment organic carbon, and these can be altered to reflect the $\delta^{13}\text{C}$ of each source. For the present study, values in Peterson & Fry (1987) are used to estimate the $\delta^{13}\text{C}$ of marine (-22 ‰) and terrestrial (-28 ‰) organic carbon inputs to the Forth Estuary and Firth. In addition, two further values of marine $\delta^{13}\text{C}$ recorded by Waldron *et al.* (2001) and from Limekilns in the present study were substituted for m in equation 6. These are -19 ‰ and -21.88 ‰ respectively. From this, percentages of MC and TC inputs to each locations' organic carbon were calculated and are presented in Table 27.

Table 27 % Marine vs. terrestrial organic carbon inputs, Inputs of marine organic carbon in equation 6 were assigned $\delta^{13}\text{C}$ values of -22‰ (top), -21.88‰ (middle) and -19‰ (bottom). Inputs of terrestrial carbon were assigned a value of -28‰ . Sites in italics indicate sandy sediments.

Site	mean $\delta^{13}\text{C}$ (‰)	mean % marine	mean % terrestrial
Skinflats Silty	-23.51	75	25
<i>Skinflats Sandy</i>	-24.17	64	36
Torry Bay Silty	-23.86	69	31
<i>Torry Bay Sandy</i>	-23.09	82	18
Limekilns Silty	-23.19	80	20
<i>Limekilns Sandy</i>	-21.88	102	-2
Blackness Silty	-23.15	81	19
<i>Blackness Sandy</i>	-22.25	96	4
<i>Portobello</i>	-22.47	92	8
Skinflats Silty	-23.51	73	27
<i>Skinflats Sandy</i>	-24.17	63	37
Torry Bay Silty	-23.86	68	32
<i>Torry Bay Sandy</i>	-23.09	80	20
Limekilns Silty	-23.19	79	21
<i>Limekilns Sandy</i>	-21.88	100	0
Blackness Silty	-23.15	79	21
<i>Blackness Sandy</i>	-22.25	94	6
<i>Portobello</i>	-22.47	90	10
Skinflats Silty	-23.51	50	50
<i>Skinflats Sandy</i>	-24.17	43	57
Torry Bay Silty	-23.86	46	54
<i>Torry Bay Sandy</i>	-23.09	55	45
Limekilns Silty	-23.19	53	47
<i>Limekilns Sandy</i>	-21.88	68	32
Blackness Silty	-23.15	54	46
<i>Blackness Sandy</i>	-22.25	64	36
<i>Portobello</i>	-22.47	61	39

The values for percentage contribution of marine vs. terrestrial organic carbon sources, using Peterson & Frys (1987) $\delta^{13}\text{C}$ value of -22 ‰ (Table 27), indicate that none of the sediments had greater than 36% terrestrial input. The percentage contributions of marine vs. terrestrial to estuarine sediments indicated higher percentages of MC in the lower estuary compared to the upper estuary. However, muddy sediments from Skinflats had a higher percentage of MC than the other sites at approximately the same distance from the main freshwater inputs to the estuary, Torry Bay muddy sediments (Table 27). Limekilns sandy sediments had a MC source which was in excess of 100%; this suggests actual estuarine organic carbon inputs had a more positive $\delta^{13}\text{C}$ than the -22 ‰ marine value assigned in the model.

Otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values collected at estuarine (Blackness and Limekilns) and coastal marine (Silver Sands and Gosford Bay) sites showed no clear differences between sites (Figures 75 and 76). One-way ANOVA indicated no significant differences between sites for both carbon ($F = 1.31$, $df = 3$, $p = 0.32$) and oxygen ($F = 1.05$, $df = 3$, $p = 0.41$) There was a strong correlation between the values for each isotope taken from the same fish (Pearson's product moment correlation: $r = 0.949$, $p < 0.001$; Figure 77).

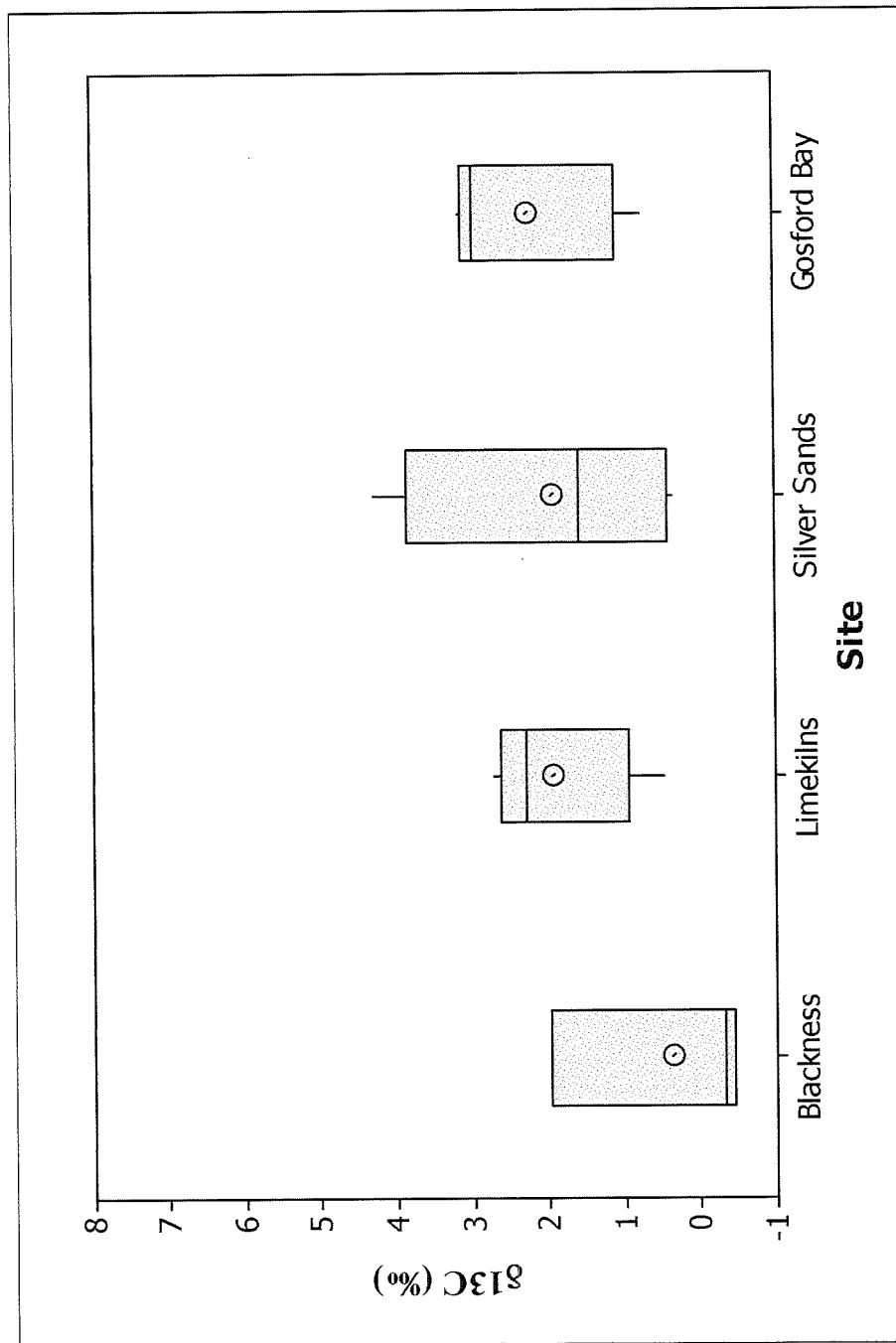


Figure 75: Boxplot of $\delta^{13}\text{C}$ of plaice otoliths caught at each site. The solid lines in each box are medians, the circles are means. Numbers of otoliths for each site are as follows: Blackness, $n = 3$, Limekilns, $n = 4$, Silver Sands, $n = 4$, Gosford Bay, $n = 5$.

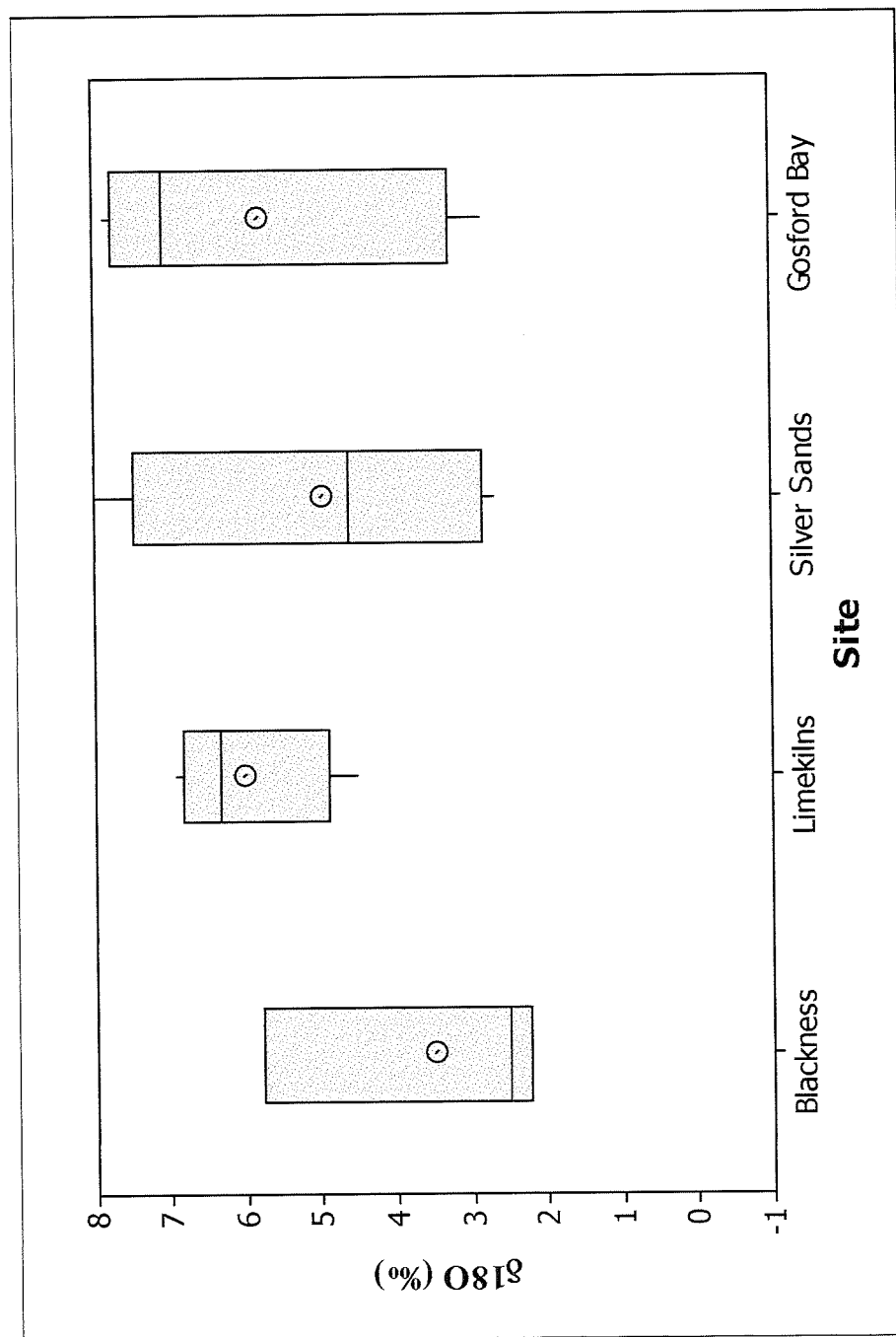


Figure 76: Boxplot of $\delta^{18}\text{O}$ of plaice otoliths. The solid lines in each box are medians, the circles are means. Numbers of otoliths for each site are as follows: Blackness, $n = 3$, Limekilns, $n = 4$, Silver Sands, $n = 4$, Gosford Bay, $n = 5$.

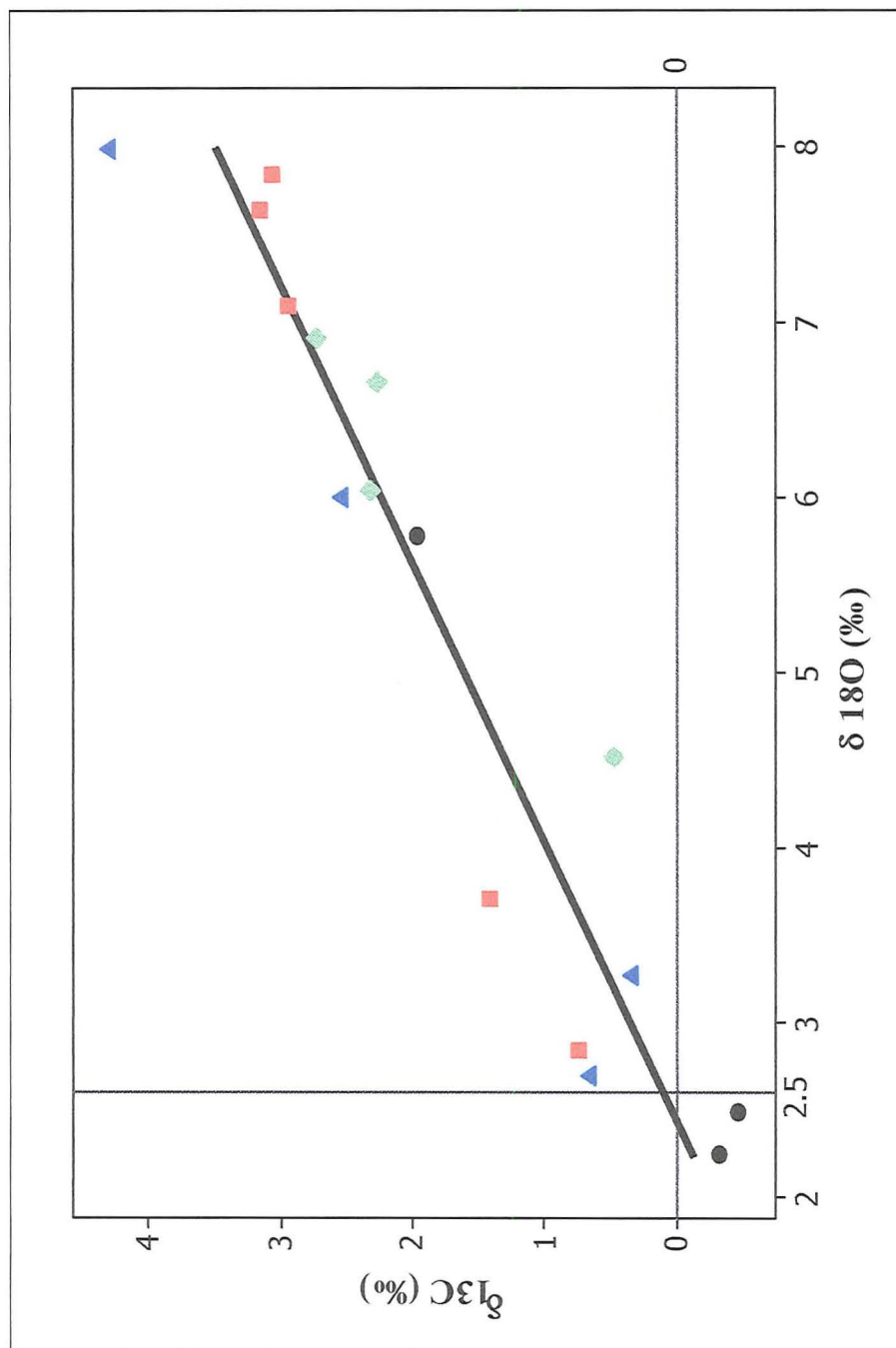


Figure 77: Scatterplot of $\delta^{13}\text{C}$ vs. $\delta^{18}\text{O}$ recorded in plaice otoliths ($n = 16$). Circles are Blackness otoliths, diamonds are Limekilns otoliths, squares are Gosford Bay otoliths and triangles are Silver Sands otoliths. Line is least squares fit. Pearson's product moment correlation values of r (0.949) and p (<0.001) indicate a very strong, positive linear relationship between the two isotopes. The two data points below the y-axis reference line and to the left of the x-axis reference line are from Blackness otoliths.

5.4. Discussion

5.4.1. Sediment isotopic composition

The present study does not show a strong gradient in terrigenous material in muddy sediments on intertidal flats from the middle to the lower Forth (Figure 73). This is in agreement with the findings of Graham *et al.* (2001). However, in contrast to their study, which concluded that there was low variability in carbon isotope content of sediments taken from the middle of the channel, the present study found higher variability in $\delta^{13}\text{C}$ of estuarine sediments, with the greatest variability observed in fully marine intertidal sediments at Portobello, and in the muddy sediments at Blackness (Figure 73). The sandy sediments in the present study did show a clear gradient in $\delta^{13}\text{C}$ with distance down the estuary (Figure 73). The present study also found slight enrichment in $\delta^{13}\text{C}$ of muddy sediments near the lower reaches of the estuary. There were significant differences between sites, and the percentage of silt/clay in the sediments was correlated with $\delta^{13}\text{C}$ (Table 26). These findings suggest that there is a greater input of terrestrial carbon in the upper estuary than the lower estuary and that terrestrial carbon is associated with finer sediments. This is consistent with terrestrial inputs to the estuary which comprise mainly dissolved organic matter that flocculates upon discharge into high salinity water (Graham *et al.*, 2001)..

The aim of the present study was to determine the utility of using $\delta^{13}\text{C}$ values of intertidal sediments to trace habitat associations, particularly in plaice. The gradient in $\delta^{13}\text{C}$ recorded here is slight (Figure 73) compared with other

systems. For example, gradients of -27 ‰ to -10 ‰ have been recorded between mangroves and adjacent seagrasses (Lepoint *et al.*, 2004; Hemminga *et al.*, 1994) and the Tay Estuary in Scotland shows a gradient of -26.2 ‰ at the head of the estuary to -23.2 ‰ at the lowest point sampled (Thornton & McManus, 1994). This suggests that isotope values of organisms' tissues may not be sufficiently different to distinguish between even the estuary and the Outer Firth. This point is exemplified by the outlier recorded from Portobello: the high variability recorded here shows that within-site variability could easily swamp between-site variability, making it unlikely that $\delta^{13}\text{C}$ would provide a useful site-specific marker in the estuary. However, as can be seen in Figure 73, the muddy sediments may show a slight enrichment with distance down the estuary, contrary to what was found in the middle channel by Graham *et al.* (2001). The muddy sediments constitute by far the largest area of intertidal habitat in the estuary (McLusky, 1987), thus, it may be expected that organisms inhabiting the estuarine intertidal, may have lower tissue $\delta^{13}\text{C}$ values than organisms inhabiting the marine sites in the Firth. Although not significant, the differences in otolith $\delta^{13}\text{C}$ recorded between fish at Gosford Bay and Blackness are consistent with this prediction.

One notable feature of the values recorded in this study is the enriched $\delta^{13}\text{C}$ of some of the sediment samples. The most enriched value measured in the current study (-19.97 ‰) came from the estuarine site at Blackness. This value is consistent with those for marine algae reported by Peterson & Fry (1987), indicating that the sources of organic carbon to the estuary are predominantly of marine origin. However, if the terrestrial sources are more enriched than assumed by Graham *et al.* (2001) and in the simple mixing model presented in Table 27, this

would mean that there is a greater input of terrestrial or other sources of organic carbon to both the intertidal and the sub-tidal sediments in the Forth Estuary. In order to fully understand this result, it would be necessary to measure $\delta^{13}\text{C}$ from all of the possible inputs of organic carbon to the Forth Estuary, including anthropogenic discharges, water column DOM and suspended POM, benthic photosynthesisers and pelagic photosynthesisers in the estuary sediments and water column. To illustrate the wide variability of possible sources of organic carbon to estuarine sediments, $\delta^{13}\text{C}$ values recorded from other temperate marine, estuarine and freshwater sources of carbon are presented in Table 28; these data demonstrate that the factors controlling $\delta^{13}\text{C}$ in different ecosystem components are complex and may depend greatly on local conditions. It should be borne in mind that the present work does not consider terrestrial inputs of inorganic nutrients (nitrogen and phosphorus salts) and the effects of these on autochthonous primary productivity.

Table 28: $\delta^{13}\text{C}$ values of various ecosystem components from published literature. Note the wide variability in values, even from locations that are relatively close.

Organic carbon source	$\delta^{13}\text{C}$ ‰	Source
Benthic microalgae	-14.9	Currin <i>et al.</i> (1995) cited in Herman <i>et al.</i> (2000)
Westerschelde estuary benthic algae	-15	Herman <i>et al.</i> (2000)
Benthic microalgae (mostly diatoms) near the mouth of the Charente River	-17.6 to -14.9	Riera and Richard (1996)
Microphytobenthos	-19.3 and -5	Herman <i>et al.</i> (2000)
Phytoplankton off Marennes-Oleron bay	-20.7 (winter) to -19.1 (spring)	Riera and Richard (1996)
Marine phytoplankton	-21.1	Currin <i>et al.</i> (1995) cited in Herman <i>et al.</i> (2000)
Oceanic phytoplankton	-21.3 (- 23.8 to -19.3)	Gearing <i>et al.</i> (1984) cited in Riera and Richard (1996)
Westerschelde estuary pelagic algae	-22 to -20	Herman <i>et al.</i> (2000)
Seawater POM	-22.36	Darnaude <i>et al.</i> (2004)
Westerschelde estuary bulk organic matter	-23.0 to -21.8	Herman <i>et al.</i> (2000)
Estuarine phytoplankton	-23.5 (-25.4 to -21.5)	Riera and Richard (1996)
Estuarine phytoplankton	-23.8 to -23.3	Fontugne and Jouanneau (1987) cited in Riera and Richard (1996)
Surface sediment	-25.06 to -24.25	Darnaude <i>et al.</i> (2004)
Rhone River plume POM	-25.37 to -23.31	Darnaude <i>et al.</i> (2004)
Terrestrial organic matter (TOM)	-26	Middelburg and Nieuwenhuize (1998)
Rhone River Particulate Organic Matter (POM)	-26.11	Darnaude <i>et al.</i> (2004)
Loch Tummel (lacustrine) sediment POM	-27.9 to -27.2	Thornton and McManus (1994)
Loch Tay (lacustrine) sediment POM	-28.0 to -25.4	Thornton and McManus (1994)
Alpine lake sediments	~ -28.0 to ~ -27.0	Cattaneo <i>et al.</i> (2004)
Riverine sediments	-31.5 to -16.3	Barth <i>et al.</i> (1998)

The two-source mixing model (equation 6) suggests that the greatest inputs of organic carbon to intertidal sediments in the estuary are of marine origin (Table 27). It is assumed that organic carbon of marine origin is from autochthonous photosynthesis. This suggests that terrestrial organic carbon accounts for no more than 36% of the intertidal sediments' total organic carbon, consistent with other measurements of marine contribution to European estuaries (McLusky & Elliott, 2004). However, the enriched values of some of the sites could mean that the value of $\delta^{13}\text{C}$ assumed for marine inputs is incorrect; some of the estuarine sites had $\delta^{13}\text{C}$ values more enriched than the - 22 ‰ assumed to be the value for MC. In fact, the mean $\delta^{13}\text{C}$ for Limekilns sandy substrate was - 21.88 ‰. The assumption that MC has a value of - 22 ‰ (m in equation 6) would then overestimate the percentage input of MC to intertidal sediment organic carbon. Waldron *et al.* (2001) measured plankton $\delta^{13}\text{C}$ in the Outer Firth near Edinburgh (see Figure 63) and recorded values of ~ - 19 ‰. This is more enriched than any of the values measured for the present study, and if the estuarine plankton have the same values for $\delta^{13}\text{C}$ would mean that TC inputs are higher than the mixing model used here suggests. Calculations using both Waldron *et al.*'s (2001) $\delta^{13}\text{C}$ value of -19 ‰ and the mean $\delta^{13}\text{C}$ value of -21.88 ‰ measured at Limekilns for the present study; show that terrestrial inputs could exceed 50% at 3 of the estuarine sites. However, Andrews *et al.* (1998) suggest that the assumption of this model, that there are only two sources of organic carbon to the intertidal sediments, can be incorrect in many cases, as demonstrated in many other studies (Table 28). Graham *et al.*'s (2001) finding of a lack of any gradient in the middle channel would mean that the intertidal $\delta^{13}\text{C}$ values measured for the present study are likely to be influenced by

autochthonous benthic primary production. Thus, the proportions of MC vs. TC calculated here should be treated as approximations only and with caution.

The relationship between organic carbon content and $\delta^{13}\text{C}$ (Figure 74) suggest that sediments with higher carbon content also have a higher proportion of TC. This is expected in an estuarine system where particulate carbon is derived largely from flocculated material, as the flocculated material is of terrestrial origin.

5.4.2. Otolith isotopic composition

Using carbon isotope ratios of plaice otoliths to determine whether an individual fish inhabited the estuary or the Outer Firth may not yield fruitful results. Analysis of variance (ANOVA) of otolith carbon isotopes showed that means of $\delta^{13}\text{C}$ were not significantly different between the four sites measured in the present study (Figure 75). However, two of the otoliths from Blackness were the most depleted of any of the otoliths used in the present study, and outside the ranges of $\delta^{13}\text{C}$ measured in otoliths at all of the other sites. The other otolith from Blackness was within the range of $\delta^{13}\text{C}$ of all of the other otoliths measured, and hence no significant differences were found. The sample sizes used in the present study were very small, due to financial constraints. If a larger and more widespread sampling of 0+ group plaice otoliths in the estuary was undertaken, a significant pattern may emerge. Hence the current negative results imply that otolith $\delta^{13}\text{C}$ is not a highly accurate marker of habitat use by any individual fish, but that it may have utility if used for population-level studies with sufficient replication.

The actual values of $\delta^{13}\text{C}$ measured in the plaice otoliths examined are over 20 ‰ higher than corresponding sediments measured in the estuary and the Outer

Firth. The higher values of otolith $\delta^{13}\text{C}$ compared to the $\delta^{13}\text{C}$ of the hypothesised metabolic source of otolith carbon (the organic fraction of sediments) is due to the majority of otolith aragonite carbon coming from DIC of seawater (Weidman and Millner, 2000; Kalish, 1991a). However, the $\delta^{13}\text{C}$ values of otolith carbon in the present study are higher than $\delta^{13}\text{C}$ measured for other species in the literature. For example, cod otolith carbon isotopic composition has been measured in several studies, yielding values for individuals of a similar age (0+ and 1+ group) of between -4.05 ‰ to 0 ‰ (Jamieson *et al.*, 2004; Weidman & Millner, 2000). The mean $\delta^{13}\text{C}$ for all plaice otoliths recorded in the present study is $1.76 \text{ ‰} \pm 1.34 \text{ s.d.}$

The more positive $\delta^{13}\text{C}$ plaice values measured here may reflect an effect of temperature on carbon isotope fractionation in plaice. This effect may be a direct (kinetic) effect at the otolith crystal surface at the time of aragonite deposition, or may be an indirect effect of temperature on metabolic rate of the individual, which affects the isotopic composition of carbon available for deposition in the individuals endolymph (Kalish, 1991a). Kalish (1991a) showed that increasing metabolic rate increased the disequilibrium between otolith $\delta^{13}\text{C}$ and DIC of seawater in several species, thus disequilibrium is minimal at lower temperatures. Without measuring $\delta^{13}\text{C}$ of seawater DIC at the sites used here, the relative contributions of DIC vs. metabolic carbon to otolith carbonate cannot be known; however, published values for seawater DIC $\delta^{13}\text{C}$ are approximately the same as those for the plaice otoliths measured here: Weidman & Millner (2000) suggest that aragonite deposited at equilibrium would have a $\delta^{13}\text{C}$ between 1.5 ‰ and 4 ‰. The plaice otoliths measured here fall within this range; hence much of the aragonite carbon may be

deposited at equilibrium with seawater DIC, suggesting a relatively small contribution from metabolic carbon in this species.

If plaice do indeed deposit most otolith carbon at isotopic equilibrium with seawater, then any differences in an individual fishes' habitat-specific carbon isotopic signature will make only a small difference in the fishes' otoliths, unless the water column isotopic composition differs between habitats. However, Blackness fish did show a non-significant depletion in otolith $\delta^{13}\text{C}$ (Figures 75 and 77), which could be caused by differences in DIC between the two areas. Again this may be caused by temperature differences, or possibly differences in the functioning of phytoplankton between the two areas (Weidmann & Millner, 2000). However, as $\delta^{13}\text{C}$ of estuarine and firth seawater DIC has not been measured, temperature and plankton effects on DIC cannot be assessed.

It was expected that oxygen would be deposited in otoliths at isotopic equilibrium with seawater, and that the estuarine sites may be more depleted than the marine sites due to differences in salinity (Witbaard *et al.*, 1994). The mean salinity of Blackness and Limekilns is 30. Thus, the expected depletion of oxygen in otoliths from the estuarine sites, using the values of Witbaard *et al.* (1994), was $\sim -1.2\%$. However, only Blackness was depleted by approximately the expected amount ([mean $\pm$ s.d.] Blackness mean $\delta^{18}\text{O} = 3.50 \pm 1.98$, Silver Sands mean $\delta^{18}\text{O} = 5.00 \pm 2.47$, Gosford Bay mean $\delta^{18}\text{O} = 5.83 \pm 2.36$ and Limekilns mean $\delta^{18}\text{O} = 6.04 \pm 1.07$; Figure 76). This relative depletion may mean that it is possible to distinguish groups of fish on the basis of the $\delta^{18}\text{O}$ of their otoliths. However, the differences in otolith $\delta^{18}\text{O}$ between the 4 sites were not significant (ANOVA, $F =$

1.05, $df = 3$, $p > 0.05$), and the overlapping variability of all the sites means that all of the fish (with the exception of two from Blackness, with otolith $\delta^{18}\text{O}$ values of 2.24 and 2.49 ‰) in the present study could have come from any one of the sites. Hence oxygen, like carbon, may allow a statistical identification of nursery sites given large sample sizes but will not provide an accurate marker for individual fish. At present, the extent of juvenile plaice penetration up the estuary is not fully known. To determine whether the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signals present at Blackness hold true for other possible plaice juvenile habitat in the estuary, a more widespread and intensive sampling of otoliths and 0+ plaice distribution in the estuary is required.

Carbon and oxygen isotopic composition of juvenile plaice otoliths reported in the current study are higher than other published accounts of fish otolith isotopes. There are several possibilities that, while not assessed in the current study, are suggested for future work on plaice otolith isotopes. The first possibility is related to the method of otolith preparation used in the current study: whole otoliths were analysed for carbonate isotopic composition. This method measures the isotopic composition of both juvenile and larval stages of otoliths. Plaice otoliths form during the late egg stages, shortly before hatching (Karakiri & von Westernhagen, 1989), and hence may include carbon that is derived from egg material. The isotopic composition of plaice embryonic and larval otoliths has not been published; if these stages of plaice have relatively high $\delta^{13}\text{C}$, this may affect the $\delta^{13}\text{C}$ measured using the methods in the current study. Newer methods of isotopic ratio analysis (e.g. laser ablation inductively coupled plasma mass spectrometry or LA-ICPMS) allow isotopic analysis of much smaller amounts of material than possible in the current study, thus permitting the measurement of the

isotopic composition of juvenile stage otoliths alone. Measurement of isotopic composition of plaice pre-juvenile stages would further elucidate the influence of larval sections of otoliths on whole otolith isotopic composition measurements.

Plaice at the age used in the present study are caught in very shallow water (<1m), and thus are exposed to relatively high temperatures for periods of the day during their nursery ground phase. As water temperature data of sufficient temporal resolution are not available for the present study, this possibility cannot be fully assessed. However, temperatures recorded during the present work peaked at over 28°C at Limekilns (pers. obs.). Despite the differences in absolute values of both isotopes between plaice and cod otoliths, Figure 77 shows a strong linear relationship between both isotopes in plaice otoliths. This is consistent with otolith isotopes in other species, and it has been suggested that the same mechanism responsible for isotopic fractionation of oxygen in otoliths also affects the isotopic fractionation of carbon in otoliths, e.g. temperature (Kalish, 1991a).

The present study has not conclusively ruled out the use of otolith isotopic composition as a habitat tracer on the relatively small scale of the estuary and adjacent marine system of the Firth of Forth and Forth estuary. On larger scales it has been shown that cod otoliths show latitudinal differences in isotopic composition (e.g. Schwarcz *et al.*, 1998; Weidman & Millner, 2000; Jamieson *et al.*, 2004). These differences are attributed to latitudinal gradients in temperature. As plaice inhabit the North Sea, the Baltic Sea, the Irish Sea and the Atlantic coast of Ireland, it may be possible to determine which of the putative nursery habitats for each of these areas contribute the most to adult populations. This would require

unique isotope signals associated with each geographic area. For example, if there are differences in isotopic composition between otoliths from Wadden Sea, Eastern English Channel and Swedish west coast 0+ plaice, then the contribution from each of these important habitats can be calculated and, hence their nursery value can be determined. The present study provides a baseline value for isotopic composition of 0+ group plaice from the Firth of Forth and Forth estuary.

5.5. Conclusion

The aim of the present chapter was to determine whether stable isotopes of carbon in the organic component of estuarine and coastal sediments could be used to reconstruct organisms' historical habitat associations. The presence of large variability in sediment $\delta^{13}\text{C}$ values in the Forth Estuary and only a small gradient between the middle and lower reaches, suggests that their usefulness as habitat markers on their own may be limited. This is supported by the isotopic composition of plaice otoliths, which showed no significant differences in carbon or oxygen isotopes between the Forth Estuary and the Outer Firth. However, because values from otoliths did show the expected trend, the use of these isotopes in larger studies with more statistical power, and in studies on a larger (latitudinal) scale, may prove effective.

Chapter 6

Summary And Conclusions

6.1. Summary and Conclusions

The preceding work was conducted in an attempt to elucidate some of the dynamics and functioning of the Forth estuary and Firth of Forth as a nursery habitat for newly – settled plaice. The initial prompt for the current work was the nursery definition of Beck *et al.*, (2001) and the possibility of assessing the use of carbon stable isotopes in fish otoliths as a tool to assess habitat contribution by inferring migrations from habitats spaced along an estuarine – marine gradient in carbon isotopic composition. The research questions for the current study are restated here, in order to place the conclusion in the relevant context

The definition of a nursery as stated by Beck *et al.*, (2001) is:

“A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur.”

Thus, nurseries can only truly be identified after the juveniles in question have recruited to the adult population, i.e. migrations from each of the putative nurseries must be somehow traced or inferred. The first research question was: What percentage of the adult plaice population that spawns at Fife Ness comes from each of the bays in the Firth of Forth and estuary? To answer this question, a method of tracing migrations of juvenile plaice was required, and a review of the literature highlighted stable isotopic composition of fish otoliths as a possible candidate. This method relies on two conditions in order to work. First juvenile habitats must demonstrate unique isotopic signatures, in the current work it was proposed that these could arise as a result of a carbon isotope gradient from the terrestrial to the marine ends of the Forth. Second, this signal must be taken up by juvenile fish, and be preserved in the juvenile portions of adult otoliths.

In the process of reviewing the literature on juvenile plaice, especially from the area studied here, it was apparent that the role of the estuaries as nursery grounds for newly settled plaice had not been fully assessed, because the intertidal areas had largely been ignored; it was suggested that they were not utilised by very young plaice, based upon meagre evidence (Poxton & Nasir, 1985); and in spite of the published accounts of the enormously important juvenile plaice habitats of the Southern North Sea (e.g. Kuipers, 1977; Zijlstra *et al.*, 1982). It was decided that the best way, in terms of probability of success as well as relevance to the research questions, to determine the role of the estuary was to begin to collect data on growth and density of plaice as an indicator of the possible importance of the estuarine habitats as plaice nurseries. Many studies of nurseries, regardless of the nursery definition used, have cited growth as a major factor in determining the quality of a particular habitat as a nursery; additionally, much of the substantial literature on plaice population dynamics, has also stated the importance of early growth in determining the functioning of the adult populations. This is why there have been many published accounts of growth rates of juvenile plaice. This prompted the next research question: Do the juvenile plaice in the estuary grow as fast as the juvenile plaice in the Outer Firth, and do plaice in the Forth in general grow optimally? Thus this research question, although requiring a significant effort to answer, was more localised in scope than the use of isotope 'tags' in fish otoliths. In order to answer this question, a method of estimating growth rates of juveniles was also required. Traditionally, beam trawls have been used to sample fish and the change in length – frequency distributions over time has been assessed. However, the shallow water depths at the current field sites precluded the use of boats (necessary for beam trawls), and it was decided that a push net,

similar to a beam trawl in most respects, would suffice. This then posed more questions: how efficient is the push net and is it size selective for the range of fish lengths required, and does the habitat sampled affect these two factors? A second method of growth rate estimation was used, otolith increment counts, in order to determine the accuracy of the net – estimated growth rates. Finally, when the first years' estimates of growth had been completed, it was clear that the estuarine fish grew as fast, and possibly faster, than the fish on Outer Firth habitats. From previous literature reviews, it was known that the effects of food and temperature on plaice growth were very well described, however, the effects of salinity, which has an important effect on growth of other species, had not been fully assessed. As the plaice in the Forth estuary experience lower salinities, the next research question was: does salinity affect growth of 0+ group plaice? This was best answered in a controlled experiment, and the experiment reflected the typical salinities plaice may be expected to experience naturally.

The chapters presented thus contain hypotheses (and corresponding statistical null hypotheses) designed to answer the questions posed above. The findings of hypothesis testing and the wider relevance of these are summarised in order to highlight the research contribution that this thesis represents.

The results of gear efficiency trials demonstrate that the coarse efficiency of the net is determined largely by the habitat it is deployed in; where the greatest difference between habitat types is the structure of the sediments. The net appears less efficient in sediments with greater silt/clay content and finer median particle size than on habitats with coarser sediments and lower silt/clay content. The

differences in efficiency are likely to be due to either the functioning of the net as it is swept across the area sampled or due to the greater effort required to recover animals from the net once they have been caught. Thus, the difference in gear efficiency between habitat types has been shown for the first time, with a suggestion for further work: why is the net less efficient on muddier habitats and is the net an appropriate tool in these habitats?

The estimates of growth rate of juvenile plaice have shown that the intertidal areas of the Forth estuary support similar growth rates of plaice as the Outer Firth intertidal areas, and therefore may also contain putative nurseries for plaice, which contradicts some of the assertions of previous work (Poxton & Nasir, 1985), and highlights the need for revision of population estimates for the area. The comparison of growth rates with predicted growth from temperature – dependent models showed sub – optimal growth rates at all sites, with some evidence that these reductions in growth are density – dependent, which has rarely been shown for plaice nurseries. Future work to assess if growth of these plaice is density – dependent, is therefore suggested: the assessment of plaice growth, feeding rates and food conditions on these habitats. The growth rates estimated by otolith increment counts also highlight the problem of continual settlement of juveniles onto the studied habitats when using changes in length – frequencies to estimate growth rates and the need to assess this settlement when conducting similar growth studies.

The experimental assessment of the effects of salinity on growth rates of plaice suggests that plaice are similar to other marine fish, in that lowered salinity

can enhance growth rates. This result has implications for plaice nurseries: if growth is indeed important for recruitment to adult populations then, all other factors being equal, habitats with less than full strength marine salinity may be expected to support higher growth rates and, hence, contribute more individuals to recruitment than fully marine habitats, further highlighting the importance of European estuaries. In addition, the results of this experiment suggest that plaice may have an 'optimal' salinity for growth somewhere between 20 and 30.

Finally, the measurement of sediment isotopic composition of estuarine and Outer Firth sediments suggests that different sediment types have different sources of organic carbon: coarser sediments may have a smaller component of organic carbon derived from terrestrial sources than muddier sediments. However, a determination of this would require measurement of isotopic composition of all possible sources of organic carbon to these intertidal sediments. The sediment isotopic composition measured in the estuarine intertidal may also suggest that inputs of riverine organic carbon (including terrigenous carbon) are deposited on the intertidal areas only, when the results of the current study are compared with previous work in the estuary. The isotopic composition of plaice otoliths does not allow determination of juvenile habitat on the relatively small scale of the bays surveyed in the current study. However, the isotopic composition of plaice otoliths provides a baseline for the current area for larger, latitudinal – scale studies.

7. References

Ajayi, O. D., (1983). Some aspects of the ecology of flatfish in the Firth of Forth.
PhD thesis, Heriot –watt university, Edinburgh

Alhossaini, M., Liu, Q., & Pitcher, T. J., (1989). Otolith microstructure indicating growth and mortality among plaice, *Pleuronectes platessa* L, Post-Larval Sub-Cohorts. Journal of Fish Biology, 35:81-90

Alhossaini, M., & Pitcher, T. J., (1988). The relation between daily rings, body growth and environmental factors in plaice, *Pleuronectes platessa* L, juvenile otoliths. Journal of Fish Biology, 33:409-418

Amara, R., (2004). 0-group flatfish growth conditions on a nursery ground (Bay of Canche, Eastern English Channel). Hydrobiologia 518:23-32

Amara, R., Mahe, K., LePape, O., & Desroy, N., (2004). Growth, feeding and distribution of the solenette *Buglossidium luteum* with particular reference to its habitat preference. Journal of Sea Research 51:211-217

Amara, R., & Paul, C., (2003). Seasonal patterns in the fish and epibenthic crustaceans community of an intertidal zone with particular reference to the population dynamics of plaice and brown shrimp. Estuarine, Coastal and Shelf Science 56:807-818

- Amara, R., Laffargue, P., Dewarumez, J. M., Maryniak, C., Lagardere, F., & Luczac, C., (2001). Feeding ecology and growth of O-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). *Journal of Fish Biology* 58:788-803
- Andersen, A. K., Schou, J., Sparrebohn, C.R., Nicolajsen, H., & Stottrup, J.G., (2005). The quality of release habitat for reared juvenile flounder, *Platichthys flesus*, with respect to salinity and depth. *Fisheries Management and Ecology* 12:211-219
- Andrews, J.E., Greenaway, A.M., & Dennis, P.F., (1998). Combined carbon isotope and C/N ratios as indicators of source and fate of organic matter in a poorly flushed, tropical estuary: Hunts Bay, Kingston Harbour, Jamaica. *Estuarine, Coastal and Shelf Science* 46:743-756
- Andrews, J.E., Samways, G., Dennis, P.F., & Maher, B.A., (2000). Origin, abundance and storage of organic carbon and sulphur in the Holocene Humber Estuary: emphasising human impact on storage changes. In: I. Shennan and J.E. Andrews, (Eds.), *Holocene land–ocean interaction and environmental change around the North Sea* (2000). The Geological Society of London, London, Special Publications, 166, 145–170.
- Attrill, M.J., & Power, M., (2002). Climatic influence on a marine fish assemblage. *Nature* 417:275-278

- Attrill, M.J., & Power, M., (2004). Partitioning of temperature resources amongst an estuarine fish assemblage. *Estuarine, Coastal and Shelf Science* 61:725-738
- Augley, J., Huxham, M., Fernandes, T.F., Lyndon, A.R., & Bury, S., (2007). Carbon stable isotopes in estuarine sediments and their utility as migration markers for nursery studies in the Firth of Forth and Forth Estuary, Scotland. *Estuarine, Coastal and Shelf Science* 72:648-656
- Bailey, K.M., Brown, E.S., & Duffy-Anderson, J.T., (2003). Aspects of distribution, transport and recruitment of Alaska plaice (*Pleuronectes quadrituberculatus*) in the Gulf of Alaska and eastern Bering Sea: comparison of marginal and central populations. *Journal of Sea Research* 50:87-95
- Bale, A.J., & Kenny, A.J. (2005). Sediment analysis and seabed characterisation, In: *Methods for the study of marine benthos*. A. Eleftheriou and A.D.Mcintyre (editors), third edition., 43-86
- Barth, J. A. C., Veizer, J., & Mayer, B., (1998). Origin of particulate organic carbon in the upper St. Lawrence: isotopic constraints. *Earth and Planetary Science Letters* 162:111-121.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., & Macleod, H., (2004).

Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73:1007-1012

Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., & Weinstein, M. R., (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51:633-641.

Begg, G. A., & Weidman, C. R., (2001). Stable $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopes in otoliths of haddock *Melanogrammus aeglefinus* from the northwest Atlantic Ocean. *Marine Ecology-Progress Series* 216:223-233.

Berghahn, R., Ludemann, K., & Ruth, M., (1995). Differences in individual growth of newly settled O-group plaice (*Pleuronectes platessa* L) in the intertidal of neighbouring Wadden Sea areas. *Netherlands Journal of Sea Research* 34:131-138

Bergman, M.J.N., van der Veer, H.W., & Zijlstra, J.J., (1988). Plaice nurseries - Effects on recruitment. *Journal of Fish Biology* 33:201-218

Beverton, R.J.H., (1995) Spatial limitation of population size; The concentration hypothesis. *Netherlands Journal of Sea Research* 34:1-6

- Beverton, R.J.H., & Iles, T.C., (1992). Mortality rates of 0-group plaice (*Pleuronectes platessa* L), dab (*Limanda limanda* L) and turbot (*Scophthalmus maximus* L) in European waters .2. Comparison of mortality rates and construction of life Table for 0-group plaice. Netherlands Journal of Sea Research 29:49-59
- Beyst, B., Hostens, K., & Mees, J., (2001). Factors influencing fish and macrocrustacean communities in the surf zone of sandy beaches in Belgium: temporal variation. Journal of Sea Research 46:281-294
- Blaber, S.J.M., & Blaber, T.G., (1980). Factors affecting the distribution of juvenile estuarine and inshore fish. Journal of Fish Biology 17:143-162
- Boeuf, G., & Payan, P., (2001). How should salinity influence fish growth? Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 130:411-423
- Bolam, S. G. & Whomersley, P., (2005). Development of macrofaunal communities on dredged material used for mudflat enhancement: a comparison of three beneficial use schemes after one year. Marine Pollution Bulletin 50: 40-47
- Borg, J. A., Attrill, M. J., Rowden, A. A., Schembri, P. J., & Jones, M. B. (2002) A quantitative technique for sampling motile macroinvertebrates in beds of the seagrass *Posidonia oceanica* (L.) Delile. Scientia Marina 66:53-58

- Bowman, A., (1914). The distribution of plaice eggs in the northern North Sea. I.
Scientific Investigations of the Fishery Board for Scotland 2:1-67
- Bowman, A., (1921). The distribution of plaice eggs in the northern North Sea. II.
Scientific Investigations of the Fishery Board for Scotland 1:1-33
- Buchanan, J. B., 1984. Sediment Analysis. In. A. Holme., A. D. McIntyre, (Eds.).
Methods for the study of the marine benthos. Blackwell Scientific Publishing,
Oxford. pp 41-65
- Bunn, N.A., Fox, C.J., & Webb, T., (2000). A literature review of studies on fish egg
mortality: implications for the estimation of spawning stock biomass by the
annual egg production method. Sci. Ser. Tech. Rep. CEFAS, Lowestoft,
vol. 111. 37 pp.
- Burke, J.S., (1995). Role of feeding and prey distribution of summer and southern
flounder in selection of estuarine nursery habitats. Journal of Fish Biology
47:355-366
- Burke, J.S., Miller, J.M., & Hoss, D.E., (1991). Immigration and settlement pattern
of *Paralichthys dentatus* And *Paralichthys lethostigma* in an estuarine
nursery ground, North-Carolina, USA. Netherlands Journal of Sea Research
27:393-405

Burrows, M.T., (2001). Depth selection behaviour during activity cycles of juvenile plaice on a simulated beach slope. *Journal of Fish Biology* 59:116-125

Burrows, M.T., Gibson, R.N., Robb, L., & MacLean, A., (2004). Alongshore dispersal and site fidelity of juvenile plaice from tagging and transplants. *Journal of Fish Biology* 65:620-634

Burrows, M.T., Gibson, R.N., Robb, L. & Comely, C.A., (1994). Temporal patterns of movement in juvenile flatfishes and their predators - underwater television observations. *Journal of Experimental Marine Biology and Ecology* 177:251-268

Burrows, M. T. & Gibson, R. N., (1995) The effects of food, predation risk and endogenous rhythmicity on the behavior of juvenile plaice, *Pleuronectes platessa* L. *Animal Behaviour* 50:41-52

Butcher, A., Mayer, D., Smallwood, D., & Johnston, M., (2005). A comparison of the relative efficiency of ring, fyke, fence nets and beam trawling for estimating key estuarine fishery populations. *Fisheries Research* 73:311-321

Campana, S.E., (1992). Measurement and interpretation of the microstructure of fish otoliths. P. 59-71. *In*: D.K. Stevenson and S.E. Campana [eds]. *Otolith microstructure examination and analysis*. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Campana, S.E., & Neilson, J.D., (1985). Microstructure of Fish Otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032

Cattaneo, A., Manca, M., & Rasmussen, J.B., (2004). Peculiarities in the stable isotope composition of organisms from an alpine lake. Aquatic Sciences 66:440-445.

Chapman, M.R., & Kramer, D.L., (2000). Movements of fishes within and among fringing coral reefs in Barbados. Environmental Biology of Fishes 57:11-24

Clarke, S., & Elliott, A.J.. (1998) Modelling suspended sediment concentrations in the firth of forth. Estuarine, Coastal and Shelf Science 47:235-250 Rapports et Procès-verbaux des Réunions du Conseil International pour l'Exploration de la Mer 30: 117-195

Coull, K.A., Johnstone, R., & Rogers, S.I., (1998). Fisheries sensitivity maps in British waters. In: United Kingdom offshore Operator's Association Ltd. (UKOOA)

Cushing, D.H., (1969). The regularity of the spawning season in some fishes. Journal du Conseil - Conseil International pour l'Exploration de la Mer 33:81-92

Cushing, D.H., (1982). Fisheries Biology: A Study in Population Dynamics, 2nd edn. University of Wisconsin Press, Madison, Wisconsin, USA

Cushing, D.H., (1990). Plankton production and year-class strength in fish populations - an update of the match mismatch hypothesis. *Advances in Marine Biology* 26:249-293

Dahlgren, C.P., Kellison, G. T. Adams, A. J. Gillanders, B. M. Kendall, M. S. Layman, C. A. Ley, J. A. Nagelkerken, I. & Serafy, J. E. (2006). Marine nurseries and effective juvenile habitats: concepts and applications. *Marine Ecology-Progress Series* 312:291-295

Darnaude, A.M., (2005). Fish ecology and terrestrial carbon use in coastal areas: implications for marine fish production. *Journal of Animal Ecology* 74:864-876

Darnaude, A.M., Salen-Picard, C., & Harmelin-Vivien, M.L., (2004a). Depth variation in terrestrial particulate organic matter exploitation by marine coastal benthic communities off the Rhone River delta (NW Mediterranean). *Marine Ecology-Progress Series* 275:47-57

Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., & Harmelin-Vivien, M.L., (2004b). Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138:325-332

DEFRA (2003). United Kingdom Sea Fisheries Statistics 2003. Department for Environment Food and Rural Affairs. Accessed from
<<http://www.defra.gov.uk/corporate/publications/pubcat/ag.htm#fishstat>>

de la Moriniere, E.C., Pollux, B.J.A., Nagelkerken, I., & van der Velde, G., (2002). Post-settlement life cycle migration patterns and habitat preference of coral reef fish that use seagrass and mangrove habitats as nurseries. *Estuarine, Coastal and Shelf Science* 55:309-321

De veen, J.F., (1978). On selective tidal transport in the migration of north sea plaice (*Pleuronectes platessa*) and other flatfish species. *Netherlands Journal of Sea Research* 12: 115- 147.

Dickey-Collas, M., Fox, C.J., Nash, R.D.M., & O'Brien, C.M., (2003). Plaice egg mortality: can we determine survivorship? *Journal of Sea Research* 50:211-225

Dorenbosch, M., van Riel, M.C., Nagelkerken, I., & van der Velde, G., (2004). The relationship of reef fish densities to the proximity of mangrove and seagrass nurseries. *Estuarine, Coastal and Shelf Science* 60:37-48

Edgar, G.J., Barrett, N.S., Graddon, D.J., & Last, P.R., (2000). The conservation significance of estuaries: a classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. *Biological Conservation* 92:383-397

- Edmonds, J.S., & Fletcher, W.J., (1997). Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. Marine Ecology-Progress Series 152:241-247
- Elliott, M., & Cutts, N.D., (2004). Marine habitats: loss and gain, mitigation and compensation. Marine Pollution Bulletin 49:671-674
- Elliott, M., O'Reilly, M.G., & Taylor, C.J.L., (1990). The Forth Estuary - a Nursery and Overwintering Area for North-Sea Fishes. Hydrobiologia 195:89-103
- Ellis, T., & Gibson, R.N., (1995). Size-Selective Predation of 0-Group Flatfishes On A Scottish Coastal Nursery Ground. Marine Ecology-Progress Series 127:27-37
- Environment Agency (2002). The Water Framework Directive – Guiding principles on the technical requirements. Environment Agency, Bristol, UK. 81 pages.
- Fonds, M., Cronie, R., Vethaak, A.D., & Vanderpuy, P., (1992). Metabolism, food-consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. Netherlands Journal of Sea Research 29:127-143
- Fowler, J., Cohen, L. & Jarvis, P. (2004) Practical Statistics for Field Biology. John Wiley & Sons, Chichester, UK. p61-68.

- Fox, C.J., McCloghrie, P., Young, E.F., & Nash, R.D.M., (2006). The importance of individual behaviour for successful settlement of juvenile plaice (*Pleuronectes platessa* L.): a modelling and field study in the eastern Irish Sea. *Fisheries Oceanography* 15:301-313
- Gauldie, R.W., West, I.F., Coote, G., & Merrett, N., (1995). Strontium variation and the oxygen-isotope water temperature record in the otoliths of the deep-water fishes *Trachyrincus murrayi* and *Coryphaenoides mediterraneus*. *Comparative Biochemistry And Physiology A-Physiology* 110:271-280
- Gauldie, R. W., (1996). Biological factors controlling the carbon isotope record in fish otoliths: Principles and evidence. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 115:201-208.
- Gauldie, R. W., Thacker, C. E., & Merrett, N. R., (1994). Oxygen and carbon isotope variation in the otoliths of *Beryx splendens* and *Coryphaenoides profundicolus*. *Comparative Biochemistry and Physiology A-Physiology* 108:153-159.
- Gaumet, F., Boeuf, G., Severe, A., Leroux, A., & Mayergonstan N (1995). Effects of salinity on the ionic balance and growth of juvenile turbot. *Journal of Fish Biology* 47:865-876

- Geffen, A.J., (1992). Validation of otolith increment deposition rate. In: Stevenson, D.K., Campana, S.E. (Eds.), Otolith Microstructure Examination and Analysis, pp. 101–113.
- Gibson, R.N., (1994). Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32:191-206
- Gibson, R.N., (1997). Behaviour and the distribution of flatfishes. Journal of Sea Research 37:241-256
- Gibson, R.N., Pihl, L., Burrows, M.T., Modin, J., Wennhage, H., & Nickell, L.A., (1998). Diel movements of juvenile plaice *Pleuronectes platessa* in relation to predators, competitors, food availability and abiotic factors on a microtidal nursery ground. Marine Ecology-Progress Series 165:145-159
- Gibson, R.N., & Robb, L., (1992). The relationship between body size, sediment grain-size and the burying ability of juvenile plaice, *Pleuronectes platessa* L. Journal of Fish Biology 40:771-778
- Gibson, R.N., & Robb, L., (1996). Piscine predation on juvenile fishes on a Scottish sandy beach. Journal of Fish Biology 49:120-138
- Gibson, R.N., Robb, L., Wennhage, H., & Burrows, M.T., (2002). Ontogenetic changes in depth distribution of juvenile flatfishes in relation to predation risk

and temperature on a shallow-water nursery ground. Marine Ecology-
Progress Series 229:233-244

Gibson, R. N., & Robb, L., (2000). Sediment selection in juvenile plaice and its
behavioural basis. Journal of Fish Biology 56:1258-1275.

Gibson, R. N., (1973). The intertidal movements and distribution of young fish on a
sandy beach with special reference to the plaice (*Pleuronectes platessa* L.).
Journal of Experimental Marine Biology and Ecology 12:79.

Gillanders, B.M., & Kingsford, M.J., (2000). Elemental fingerprints of otoliths of fish
may distinguish estuarine 'nursery' habitats. Marine Ecology-Progress
Series 201:273-286

Gillanders, B. M., (2005). Using elemental chemistry of fish otoliths to determine
connectivity between estuarine and coastal habitats. Estuarine Coastal and
Shelf Science 64:47-57.

Gillanders, B. M., Able, K. W., Brown, J. A., Eggleston, D. B., & Sheridan, P. F.,
(2003). Evidence of connectivity between juvenile and adult habitats for
mobile marine fauna: an important component of nurseries. Marine Ecology-
Progress Series 247:281-295.

Gilliers, C., Le Pape, O., Desaunay, Y., Morin, J., Guerault, D., & Amara, R.,
(2006). Are growth and density quantitative indicators of essential fish

habitat quality? An application to the common sole *Solea solea* nursery grounds. Estuarine, Coastal and Shelf Science 69:96-106

Graham, M. C., Eaves, M. A., Farmer, J. G., Dobson, J., & Fallick, A. E., (2001). A study of carbon and nitrogen stable isotope and elemental ratios as potential indicators of source and fate of organic matter in sediments of the Forth Estuary, Scotland. Estuarine Coastal and Shelf Science 52:375-380.

Greenwood, M. F. D., Hill, A. S., & McLusky, D. S., (2002). Trends in abundance of benthic and demersal fish populations of the lower Forth Estuary, East Scotland, from 1982-2001. Journal of Fish Biology 61:90-104.

Harden-Jones, F. R., (1968). Fish migration. Edward Arnold, London, uk. 325 pp.

Healey, E.G., (1999). The skin pattern of young plaice and its rapid modification in response to graded changes in background tint and pattern. Journal of Fish Biology 55:937-971

Heck KL, Hays G, & Orth RJ (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. 253:123-136

Hemminga, M. A., Slim, F. J., Kazungu, J., Ganssen, G. M., Nieuwenhuize, J., & Kruijt, N. M., (1994). Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral-reefs (Gazi Bay, Kenya). Marine Ecology-Progress Series 106:291-301.

- Herman, P. M. J., Middelburg, J. J., Widdows, J., Lucas, C. H., & Heip, C. H. R., (2000). Stable isotopes' as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Marine Ecology-Progress Series* 204:79-92.
- Hjorleifsson, E., & Palsson, J., (2001). Settlement, growth and mortality of 0-group plaice (*Pleuronectes platessa*) in Icelandic waters. *Journal of Sea Research* 45:321-324
- Hjort, J., (1914). Fluctuations in the great fisheries of Northern Europe. *Rapports et Procès-verbaux des Réunions du Conseil International pour l'Exploration de la Mer* 20:1-228
- Hobson, K. A., (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314-326.
- Høie, H., Otterlei, E., & Folkvord, A., (2004). Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). *ICES Journal of Marine Science* 61:243-251.
- Hunter, E., Metcalfe, J.D., Arnold, G.P., & Reynolds, J.D., (2004). Impacts of migratory behaviour on population structure in North Sea plaice. *Journal of Animal Ecology* 73:377-385

- Huxham, M., Kimani, E., Newton, J. & Augley, J., (2007). Stable isotope records from otoliths as tracers of fish migration in a mangrove system. *Journal of Fish Biology* 70: 1554-1567
- Huxham, M., Kimani, E., Augley, J., (2004). Mangrove fish: a comparison of community structure between forested and cleared habitats. *Estuarine, Coastal and Shelf Science* 60:637-647
- Imslund, A.K., Foss, A., Gunnarsson, S., Berntssen, M. H. G., FitzGerald, R., Bonga, S. W., Von Ham, E., Naevdal, C., & Stefansson, S. O. (2001). The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 198:353-367
- Jager, Z., Kleef, H.L., & Tydeman, P., (1993). The distribution of O-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dollard (Ems Estuary, Wadden Sea). *Journal of Fish Biology* 43:31-43
- Jager, Z., Kleef, H.L., & Tydeman, P., (1995). Mortality and growth of O-group flatfish in the brackish Dollard (Ems Estuary, Wadden Sea). *Netherlands Journal of Sea Research* 34:119-129
- Jamieson, R. E., Schwarcz, H. P., & Bratney, J., (2004). Carbon isotopic records from the otoliths of Atlantic cod (*Gadus morhua*) from eastern Newfoundland, Canada. *Fisheries Research* 68:83-97.

- Jana, S.N., Garg, S.K., & Patra, B.C., (2006). Effect of inland water salinity on growth performance and nutritional physiology in growing milkfish, *Chanos chanos* (Forsskal): field and laboratory studies. *Journal of Applied Ichthyology* 22:25-34
- Jones, G.P., Milicich, M.J., Emslie, M.J., & Lunow, C., (1999). Self-recruitment in a coral reef fish population. *Nature* 402:802-804
- Kalish, J. M., (1991a). ^{13}C and ^{18}O Isotopic disequilibria in fish otoliths - metabolic and kinetic effects. *Marine Ecology-Progress Series* 75:191-203.
- Kalish, J. M., (1991b). Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Marine Biology* 110:37-47.
- Kamenos, N.A., Moore, P.G., & Hall-Spencer, J.M., (2004a). Attachment of the juvenile queen scallop (*Aequipecten opercularis* L.) to Maerl in mesocosm conditions; juvenile habitat selection. *Journal of Experimental Marine Biology And Ecology* 306:139-155
- Kamenos, N.A., Moore, P.G., & Hall-Spencer, J.M., (2004b). Maerl grounds provide both refuge and high growth potential for juvenile queen scallops (*Aequipecten opercularis* L.). *Journal of Experimental Marine Biology And Ecology* 313:241-254

- Kamenos, N.A., Moore, P.G., & Hall-Spencer JM (2004c). Nursery-area function of maerl grounds for juvenile queen scallops *Aequipecten opercularis* and other invertebrates. *Marine Ecology-Progress Series* 274:183-189
- Karakiri, M., Berghahn, R., & van der Veer, H.W., (1991). Variations in settlement and growth of 0-group plaice (*Pleuronectes platessa* L.) in the Dutch Wadden Sea as determined by otolith microstructure analysis. *Netherlands Journal of Sea Research* 27:345-351
- Karakiri, M., Berghahn, R., & von Westernhagen, H., (1989). Growth differences in O-group plaice *Pleuronectes platessa* as revealed by otolith microstructure analysis. *Marine Ecology-Progress Series* 55:15-22
- Karakiri, M., & von Westernhagen, H., (1988). Apparatus for grinding otoliths of larval and juvenile fish for microstructure analysis. *Marine Ecology-Progress Series* 49:195-198
- Karakiri, M., & von Westernhagen, H., (1989). Daily growth patterns in otoliths of larval and juvenile plaice (*Pleuronectes platessa* L.): influence of temperature, salinity, and light conditions. *Rapports et Procès-verbaux des Réunions du Conseil International pour l'Exploration de la Mer* 191:390-399.
- Kell, L.T., O'Brien, C.M., Smith, M.T., Stokes, T.K., & Rackham, B.D., (1999). An evaluation of management procedures for implementing a precautionary

approach in the ICES context for North Sea plaice (*Pleuronectes platessa* L.). ICES Journal of Marine Science 56:834-845

Kennedy, P., Kennedy, H., & Papadimitriou, S., (2005). The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. Rapid Communications in Mass Spectrometry 19:1063-1068.

Kraus, R.T., & Secor, D.H., (2005). Application of the nursery-role hypothesis to an estuarine fish. Marine Ecology-Progress Series 291:301-305

Kuipers, B. R., (1975). On the efficiency of a two metre beamtrawl in juvenile plaice (*Pleuronectes platessa*). Netherlands Journal of Sea Research 9: 69-85

Kuipers, B.R., (1977). On the ecology of juvenile plaice on a tidal flat in the Wadden Sea. Netherlands Journal of Sea Research 11:56-91

Kuipers, B.R., Maccurrin, B., Miller, J.M., van der Veer, H.W., & Witte, J.I.J., (1992). Small trawls in juvenile flatfish research - their development and efficiency. Netherlands Journal of Sea Research 29:109-117

Laiz-Carrión, R., Sangiao-Alvarellos, S., Guzmán, J.M., del Río, M.P.M., Soengas, J.L., & Mancera, J.M., (2005). Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: Implications for osmoregulation and energy metabolism. Aquaculture 250:849-861

Lamont, J.M., (1964). Plaice investigations in Scottish waters. I. Size composition of the stocks. *Marine Research* 1:1-29

Lazzari, M.A., Sherman, S., & Kanwit, J.K., (2003). Nursery use of shallow habitats by epibenthic fishes in Maine nearshore waters. *Estuarine, Coastal and Shelf Science* 56:73-84

Le Pape, O., Chauvet, F., Mahevas, S., Lazure, P., Guerault, D., & Desaunay, Y. (2003). Quantitative description of habitat suitability for the juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. *Journal of Sea Research* 50:139-149

Leggett, W.C., & Deblois, E., (1994). Recruitment in marine fishes - is it regulated by starvation and predation in the egg and larval stages. *Netherlands Journal of Sea Research* 32:119-134

Lepoint, G., Dauby, P., & Gobert, S., (2004). Applications of C and N stable isotopes to ecological and environmental studies in seagrass ecosystems. *Marine Pollution Bulletin* 49:887-891.

Litvak, M.K., & Leggett, W.C., (1992). Age and size-selective predation on larval fishes - the bigger- is-better hypothesis revisited. *Marine Ecology-Progress Series* 81:13-24

- Lockwood, S.J., (1974). The settlement, distribution and movements of O-group plaice *Pleuronectes platessa* (L.) in Filey Bay, Yorkshire. *Journal of Fish Biology* 6:465-477
- Lyndon, A.R., (1994). A method for measuring oxygen-consumption in isolated-perfused gills. *Journal of Fish Biology* 44:707-715
- MacCall, A.D., (1990). *Dynamic geography of marine fish populations*. University of Washington Press, Seattle
- Macer, C.T., (1967). The food web in Red Wharf Bay (North Wales) with particular reference to young plaice (*Pleuronectes platessa*) Helgoland Marine Research 15:560-573
- Marshall, S., & Elliott, M., (1998). Environmental influences on the fish assemblage of the Humber estuary, UK. *Estuarine, Coastal and Shelf Science* 46:175-184
- McLusky, D. S., & Elliott, M., (1987). *The Estuarine Ecosystem ecology, threats and management*. Oxford University Press, Oxford. 222 pages.
- McLusky, D. S., & Elliott, M., (2004). *The Estuarine Ecosystem: Ecology, threats and management, Third Edition*. Oxford University Press, Oxford. 222 pages.

- McLusky, D. S., (1987). Intertidal habitats and benthic macrofauna of the Forth Estuary, Scotland. Proceedings of The Royal Society of Edinburgh Section B-Biological Sciences 93:389-399.
- Meng, L., Powell, J.C., & Taplin, B., (2001). Using winter flounder growth rates to assess habitat quality across an anthropogenic gradient in Narragansett Bay, Rhode Island. Estuaries 24:576-584
- Middelburg, J. J., & Nieuwenhuize, J., (1998). Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. Marine Chemistry 60:217-225.
- Miller, J.M., Maccurrin, B., & van der Veer, H.W., (1992). Growth and mortality in juvenile flatfish - the design of optimal sampling programs. Netherlands Journal of Sea Research 29:93-96
- Minello, T.J., Able, K.W., Weinstein, M.P., & Hays, C.G., (2003). Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. 246:39-59
- Modin, J., Fagerholm, B., Gunnarsson, B., & Pihl, L., (1996). Changes in otolith microstructure at metamorphosis of plaice, *Pleuronectes platessa* L. ICES Journal of Marine Science 53:745-748

Modin, J., & Pihl, L., (1994). Differences in growth and mortality of juvenile plaice, *Pleuronectes platessa* L, following normal and extremely high settlement.

Netherlands Journal of Sea Research 32:331-341

Mumby, P. J., Edwards, A. J., Arias-Gonzalez, J. E., Lindeman, K. C., Blackwell, P. G., Gall, A., Gorczynska, M. I., Harborne, A. R., Pescod, C. L., Renken, H., Wabnitz C. C. C., & Llewellyn, G., (2004). Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature* 427:533-536.

Nagelkerken, I., Dorenbosch, M., Verberk, W., de la Moriniere, E.C., van der Velde, G., (2000a). Importance of shallow-water biotopes of a Caribbean bay for juvenile coral reef fishes: patterns in biotope association, community structure and spatial distribution. *Marine Ecology-Progress Series* 202:175-192

Nagelkerken, I., van der Velde, G., Gorissen, M.W., Meijer, G.J., van't Hof, T., & den Hartog, C.,(2000b). Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. *Estuarine, Coastal and Shelf Science* 51:31-44

Nagelkerken, I., Kleijnen, S., Klop, T., van den Brand, R., de la Moriniere, E.C., van der Velde, G., (2001). Dependence of Caribbean reef fishes on mangroves and seagrass beds as nursery habitats: a comparison of fish faunas between bays with and without mangroves/seagrass beds. *Marine Ecology-Progress Series* 214:225-235

NASA (2007). OBPG SeaWiFS/Modis_Aqua Monthly Global 9-km Products. In,
vol. 2007. NASA

Nash, R.D.M., (1998). Exploring the population dynamics of Irish Sea plaice,
Pleuronectes platessa L., through the use of Paulik diagrams. Journal of
Sea Research 40:1-18

Nash, R.D.M., & Geffen, A. J., (2005). Age and Growth. In: Gibson RN (ed)
Flatfishes Biology and Exploitation, 1st edn. Blackwell Publishing, Oxford,
UK; Ames, Iowa, pp 138-153

Nash, R.D.M., & Geffen, A.J., (2000). The influence of nursery ground processes
in the determination of year-class strength in juvenile plaice *Pleuronectes*
platessa L. in Port Erin Bay, Irish Sea. Journal of Sea Research 44:101-110

Nash, R.D.M., Geffen, A.J., & Hughes, G., (1992). Winter growth of juvenile plaice
on the port-erin bay (Isle-of- Man) nursery ground. Journal of Fish Biology
41:209-215

Nash, R.D.M., Geffen, A.J., & Hughes, G., (1994a). Individual growth of juvenile
plaice (*Pleuronectes platessa* L) on a small Irish Sea nursery ground (Port-
Erin Bay, Isle of Man, UK). Netherlands Journal of Sea Research 32:369-
378

- Nash, R.D.M., Santos, R.S., Geffen, A.J., Hughes, G., & Ellis, T.R., (1994b). Diel variability in catch rate of juvenile flatfish on 2 small nursery grounds (Port- Erin Bay, Isle of Man and Porto Pim Bay, Faial, Azores). *Journal of Fish Biology* 44:35-45
- Nasir, N.A., (1981). Ecology of flatfish on the nursery areas in the Firth of Forth. In: Department of Brewing & Biological Sciences (Marine Biology), vol. MSc Thesis. Heriot-Watt University, Edinburgh, p 192
- Nasir, N.A., (1985). Ecology of the Plaice (*Pleuronectes platessa* L.) in Broad Sands Bay, Firth of Forth. In: Department of Brewing and Biological Sciences (Marine Biology), vol. Ph. D Thesis. Heriot-Watt, Edinburgh, p 324
- Norcross, B.L., Holladay, B.A., & Muter, F.J., (1995). Nursery area characteristics of Pleuronectids in coastal Alaska, USA. *Netherlands Journal of Sea Research* 34:161-175
- Nybakken, J.W., (2001). *Marine Biology: An Ecological Approach*, 5th Edition. Benjamin Cummings, San Francisco
- Orth, R. J., & J. van Montfrans., (1987). Utilization of a seagrass meadow and tidal marsh creek by blue crabs *Callinectes sapidus*. I. Seasonal and annual variations in abundance with emphasis on post-settlement juveniles. *Marine Ecology Progress Series* 41:283-294.

Peterson, B. J., & Fry, B., (1987). Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320.

Phelan, B.A., Goldberg, R., Bejda, A. J. Pereira, J. Hagan, S. Clark, P. Studholme, A. L. Calabrese, & A. Able, K. W. (2000). Estuarine and habitat-related differences in growth rates of young-of-the-year winter flounder (*Pseudopleuronectes americanus*) and tautog (*Tautoga onitis*) in three northeastern US estuaries. Journal of Experimental Marine Biology And Ecology 247:1-28

Piet, G.J., & Rijnsdorp, A.D., (1998). Changes in the demersal fish assemblage in the south-eastern North Sea following the establishment of a protected area ("plaice box"). ICES Journal of Marine Science 55:420-429

Pihl, L., (1985). Food selection and consumption of mobile epibenthic fauna in shallow marine areas. Marine Ecology-Progress Series 22:169-179

Pihl, L., Modin, J., & Wennhage, H., (2000). Spatial distribution patterns of newly settled plaice (*Pleuronectes platessa* L.) along the Swedish Skagerrak archipelago. Journal of Sea Research 44:65-80

Pihl, L., & Rosenberg, R., (1982). Production, abundance, and biomass of mobile epibenthic marine fauna in shallow waters, Western Sweden. Journal of Experimental Marine Biology and Ecology 57:273-301

- Pihl, L., & van der Veer, H.W., (1992). Importance of exposure and habitat structure for the population-density of 0-group plaice, *Pleuronectes platessa* L, in coastal nursery areas. Netherlands Journal of Sea Research 29:145-152
- Poxton, M.G., (1987). Fishery studies in the estuary and Firth of Forth, Scotland. Proceedings of The Royal Society of Edinburgh Section B-Biological Sciences 93: 495-508
- Poxton, M.G., & Allouse, S.B., (1982). Water quality criteria for marine fisheries. Aquaculture Engineering 1:223–228
- Poxton, M.G., Eleftheriou, A., & McIntyre, A.D., (1982). The population-dynamics of 0-group flatfish on nursery grounds in the clyde sea area. Estuarine, Coastal and Shelf Science 14:265-282
- Poxton, M.G., Eleftheriou, A., & McIntyre, A.D., (1983). The food and growth of 0-group flatfish on nursery grounds in the Clyde Sea area. Estuarine, Coastal and Shelf Science 17:319-337
- Poxton, M.G., & Nasir, N.A., (1985). The distribution and population-dynamics of 0-group plaice (*Pleuronectes platessa* L) on nursery grounds in the Firth of Forth. Estuarine, Coastal and Shelf Science 21:845-857

Rae, B.B., (1970). The distribution of flatfishes in Scottish and adjacent waters.

Department of Agriculture and Fisheries for Scotland: Marine Research 2:1-

38

Read, P., (1987). The intertidal benthos and sediments of particulate shores in the

Firth of Forth, Scotland, with particular reference to waste-water discharges.

Proceedings of The Royal Society of Edinburgh Section B-Biological

Sciences 93: 401-413

Riera, P., & Richard, P., (1996). Isotopic determination of food sources of

Crassostrea gigas along a trophic gradient in the estuarine bay of

Marennes-Oleron. Estuarine Coastal and Shelf Science 42:347-360.

Rijnsdorp, A.D., Van Beek, F. A. Flatman, S. Millner, R. M. Riley, J. D. Giret, M. &

De Clerck, R., (1992). Recruitment of sole stocks, *Solea solea* (L), in the

Northeast Atlantic. Netherlands Journal of Sea Research 29:173-192

Rijnsdorp, A.D., Grift, R.E., & Kraak, S.B.M., (2005). Fisheries-induced adaptive

change in reproductive investment in North Sea plaice (*Pleuronectes*

platessa)? Canadian Journal of Fisheries and Aquatic Sciences 62:833-843

Rijnsdorp, A.D., Vanstralen, M., & van der Veer, H.W., (1985). Selective tidal

transport of north-sea plaice larvae *Pleuronectes platessa* in coastal nursery

areas. Transactions of The American Fisheries Society 114:461-470

- Rijnsdorp, A.D., & Vingerhoed, B., (2001). Feeding of plaice *Pleuronectes platessa* L. and sole *Solea solea* (L.) in relation to the effects of bottom trawling. *Journal of Sea Research* 45:219-229
- Riley, J.D., (1973). Movements of O-group plaice *Pleuronectes platessa* L as shown by latex tagging. *Journal of Fish Biology* 5:323-343
- Riley, J. D. & Corlett, J. (1965). The numbers of O-group plaice in Port Erin Bay, 1964–66. *Report of the Marine Biological Station at Port Erin* 78, 51–56.
- Riley, J.D., (1971). The Riley push net. In: A. Holme., A. D. McIntyre, (Eds) *Methods for the study of marine benthos*. Blackwell Scientific Publications, Oxford, pp. 286-29
- Rogers, S.I., & Millner, R.S., (1996). Factors affecting the annual abundance and regional distribution of English inshore demersal fish populations: 1973 to 1995. *Ices Journal of Marine Science* 53:1094-1112
- Rooker, J.R., Secor, D.H., Zdanowicz, V.S., De Metrio, G., & Relini, L.O., (2003). Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. *Fisheries Oceanography* 12:75-84
- Ross, S.W., (2003). The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. *Fishery Bulletin* 101:384-404

- Rozas, L.P., & Minello, T.J., (1997). Estimating densities of small fishes and decapod crustaceans in shallow estuarine habitats: A review of sampling design with focus on gear selection. *Estuaries* 20:199-213
- Rozas, L.P., & Odum, W.E., (1987). Fish and macrocrustacean use of submerged plant beds in tidal fresh-water marsh creeks. *Marine Ecology-Progress Series* 38:101-108
- Rubio, V.C., Sanchez-Vazquez, F.J., & Madrid, J.A., (2005). Effects of salinity on food intake and macronutrient selection in European sea bass. *Physiology & Behavior* 85:333-339
- Ryland, J. S., (1963). The swimming speeds of plaice larvae. *Journal of Experimental Biology* 40, 285—99.
- Ryland, J.S., (1966). Observations on the development of Larvae of the plaice, *Pleuronectes platessa* L. in aquaria. *Journal of Experimental Biology*
- Schwarcz, H. P., Gao, Y., Campana, S., Browne, D., Knyf, M., & Brand, U., (1998). Stable 15carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 55:1798-1806.
- Scottish Executive., 2005. Scottish Fisheries Statistics 2004. Scottish Executive, Edinburgh. 34 pages.

Secor, D.H., (2002). Historical roots of the migration triangle. ICES Marine Science Symposia 215:329-335

Serchuk, F.M., Kirkegaard, E., & Daan, N., (1996). Status and trends of the major roundfish, flatfish, and pelagic fish stocks in the North Sea: Thirty-year overview. ICES Journal of Marine Science 53:1130-1145

Sette, O. E., (1943). Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 1: early life history, including the growth, drift, and mortality of the egg and larval populations. Fishery bulletin 38, 91pp

Sheaves, M. (2001) Are there really few piscivorous fishes in shallow estuarine habitats? Marine Ecology Progress Series 222:279-290

Sogard, S.M., (1992). Variability in growth-rates of juvenile fishes in different estuarine habitats. Marine Ecology-Progress Series 85:35-53

Spencer, K., Shafer, D.J., Gauldie, R.W., & DeCarlo, E.H., (2000). Stable lead isotope ratios from distinct anthropogenic sources in fish otoliths: a potential nursery ground stock marker. Comparative Biochemistry And Physiology A-Molecular And Integrative Physiology 127:273-284

Symes, D., (1998). The integration of fisheries management and marine wildlife conservation. In: JNCC Report, vol. No. 287. JNCC

- Thiel, R., Sepulveda, A., Kafemann, R., & Nellen, W., (1995). Environmental-factors as forces structuring the fish community of the Elbe Estuary. *Journal of Fish Biology* 46:47-69
- Thornton, S. F., and McManus, J., (1994). Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic-matter provenance in estuarine systems - evidence from the Tay Estuary, Scotland. *Estuarine Coastal and Shelf Science* 38:219-233.
- Tytler, P., & Blaxter, J.H.S., (1988). The effects of external salinity on the drinking rates of the larvae of herring, plaice and cod. *Journal of Experimental Biology* 138:1-15
- van der Veer, H.W., (1986). Immigration, settlement, and density-dependent mortality of a larval and early postlarval 0-Group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Marine Ecology-Progress Series* 29:223-236
- van der Veer, H. W., Witte, J. I., Beumkes, H. A., Dapper, R., Jongejan, W. P., & van der Meer, J. (1992) Intertidal fish traps as a tool to study long-term trends in juvenile flatfish populations. *Netherlands Journal of Sea Research* 29:119-126

van der Veer, H.W., Berghahn, R., Miller, J.M., & Rijnsdorp AD (2000a).

Recruitment in flatfish, with special emphasis on North Atlantic species:

Progress made by the Flatfish Symposia. ICES Journal of Marine Science

57:202-215

van der Veer, H.W., Geffen, A.J., & Witte, J.I.J., (2000b). Exceptionally strong year

classes in plaice *Pleuronectes platessa*: are they generated during the

pelagic stage only, or also in the juvenile stage? Marine Ecology-Progress

Series 199:255-262

van der Veer, H.W., Berghahn, R., & Rijnsdorp, A.D., (1994). Impact of juvenile

growth on recruitment in flatfish. Netherlands Journal of Sea Research

32:153-173

van der Veer, H.W., & Bergman, M.J.N., (1986). Development of tidally related

behavior of a newly settled O-group plaice (*Pleuronectes platessa*)

population in the Western Wadden Sea. Marine Ecology-Progress Series

31:121-129

van der Veer, H.W., & Bergman, M.J.N., (1987). Predation by crustaceans on a

newly settled O-group plaice *Pleuronectes platessa* population in the

Western Wadden Sea. Marine Ecology-Progress Series 35:203-215

van der Veer, H.W., Pihl, L., & Bergman, M.J.N., (1990). Recruitment mechanisms in North Sea plaice *Pleuronectes platessa*. Marine Ecology-Progress Series 64:1-12

van der Veer, H.W., & Witte, J.I.J., (1993). The maximum growth optimal food condition hypothesis - a test for 0-group plaice *Pleuronectes platessa* in the Dutch Wadden Sea. Marine Ecology-Progress Series 101:81-90

Vance, D.J., Haywood, M.D.E., Heales, D.S., Kenyon, R.A., Loneragan, N.R., & Pendrey, R.C., (1996). How far do prawns and fish move into mangroves? Distribution of juvenile banana prawns *Penaeus merguensis* and fish in a tropical mangrove forest in northern Australia. Marine Ecology-Progress Series 131:115-124

Varsamos, S., Nebel, C., & Charmantier, G., (2005). Ontogeny of osmoregulation in postembryonic fish: A review. Comparative Biochemistry And Physiology A-Molecular & Integrative Physiology 141:401-429

Waldron, S., Tatner, P., Jack, I., & Arnott, C., (2001). The impact of sewage discharge in a marine embayment: A stable isotope reconnaissance. Estuarine Coastal and Shelf Science 52:111-115.

Webb, A. J., & Metcalfe, A. P., (1987). Physical aspects, water movements and modeling studies of the Forth Estuary, Scotland. Proceedings of The Royal Society of Edinburgh Section B-Biological Sciences 93:259-272.

- Wegner, G., Damm, U., Purps, M., (2003). Physical influences on the stock dynamics of plaice and sole in the North Sea. *Scientia Marina* 67:219-234
- Weidman, C. R., and Millner, R., (2000). High-resolution stable isotope records from North Atlantic cod. *Fisheries Research* 46:327-342.
- Wennhage, H., (2002). Vulnerability of newly settled plaice (*Pleuronectes platessa* L.) to predation: effects of habitat structure and predator functional response. *Journal of Experimental Marine Biology and Ecology* 269:129-145
- Wennhage, H., & Gibson, R.N., (1998). Influence of food supply and a potential predator (*Crangon crangon*) on settling behaviour of plaice (*Pleuronectes platessa*). *Journal of Sea Research* 39:103-112
- Wennhage, H., Gibson, R.N., & Robb, L., (1997). The use of drop traps to estimate the efficiency of two beam trawls commonly used for sampling juvenile flatfishes. *Journal of Fish Biology* 51:441-445
- Wennhage, H., Pihl, L., (2001). Settlement patterns of newly settled plaice (*Pleuronectes platessa*) in a non-tidal Swedish fjord in relation to larval supply and benthic predators. *Marine Biology* 139:877-889

- Wennhage, H., Pihl, L., & Stal, J., (2007). Distribution and quality of plaice (*Pleuronectes platessa*) nursery grounds on the Swedish west coast. *Journal of Sea Research* 57:218-229
- Wimpenny, R.S., (1953). The Plaice. Edward Arnold & Co., London
- Witbaard, R., Jenness, M. I., Vanderborg, K., and Ganssen, G., (1994). Verification of annual growth increments in *Arctica islandica* L from the North-Sea by means of oxygen and carbon isotopes. *Netherlands Journal of Sea Research* 33:91-101.
- Wuenschel, M.J., Jugovich, A.R., & Hare, J.A., (2005). Metabolic response of juvenile gray snapper (*Lutjanus griseus*) to temperature and salinity: Physiological cost of different environments. *Journal of Experimental Marine Biology And Ecology* 321:145-154
- Zar, J.H., (1999). Biostatistical Analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ.
- Zijlstra, J.J., Dapper, R., & Witte, J.I., (1982). Settlement, growth and mortality of post-larval plaice (*Pleuronectes platessa*) In The Western Wadden Sea. *Netherlands Journal of Sea Research* 15:250-272
- Zijlstra, J.J., & Witte, J.I., (1985). On the recruitment of O-group plaice in the North-Sea. *Netherlands Journal of Zoology* 35:360-376