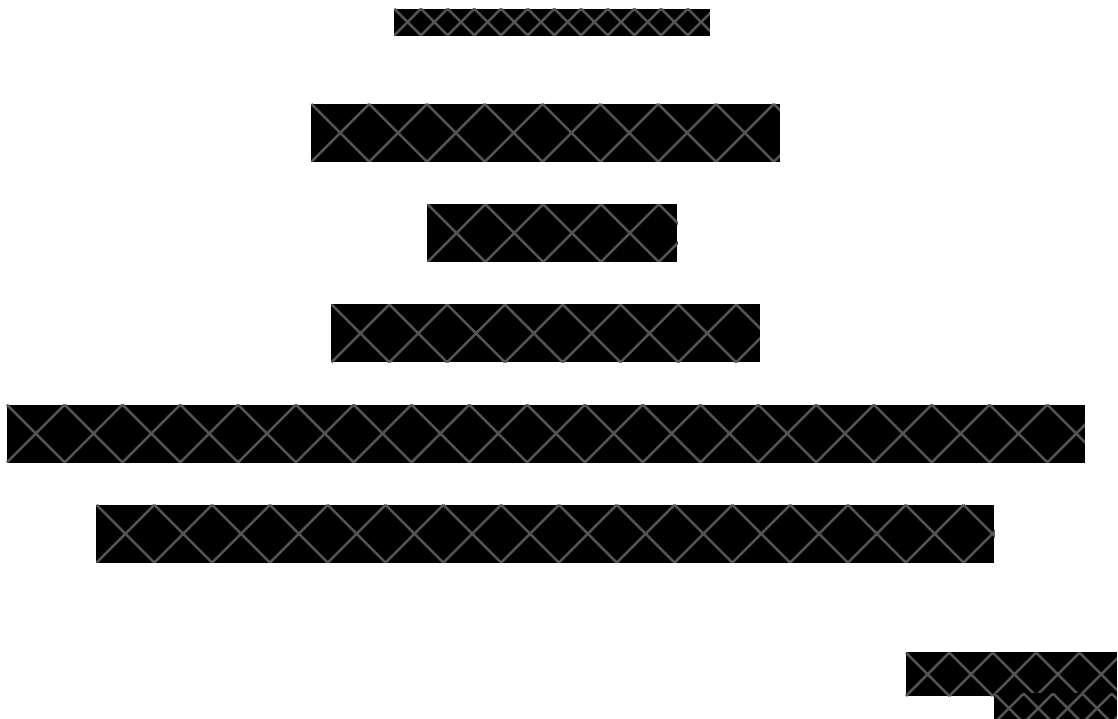


Investigating the effects of abiotic factors on the spawning cycle of the Native Oyster, *Ostrea Edulis* within Lough Foyle.



Contents

ABSTRACT	2
INTRODUCTION	2
AIMS AND OBJECTIVES	4
NULL HYPOTHESES	4
MATERIAL AND METHODS	5
STUDY AREA	5
SPAWNING STAGES	6
WATER TEMPERATURE AND SALINITY	6
LARVAL COUNTS	7
STATISTICAL ANALYSIS	7
RESULTS	8
LENGTH AND WEIGHT TRENDS	8
GONAD STAGES AND WEEKLY INTERVALS	10
GONAD STAGES AND BED VARIANCE	11
LARVAL COUNTS AND TEMPERATURE VARIATIONS	13
LARVAL COUNTS AND SALINITY	14
GONAD STAGE WITH TIME AND LOCATION VARIANCE	15
DISCUSSION	15
LIMITATIONS	16
CONCLUSION	17
ACKNOWLEDGMENTS	17
REFERENCES	18

Abstract

The effects of abiotic factors such as temperature and salinity on the spawning cycle of *Ostrea Edulis* has been widely researched in the past 30 years. (McKelvey, 1996; McGonigle et al, 2016; Brander, 2016; Harvell et al, 2002 and O'Connor et al, 2006, 2007). The basis of all the previous studies have concluded that temperature and salinity are the main influencers of spawning activity of the *Ostrea Edulis*. The data for this study was collected by the Aquaculture department of the Lough's Agency, Derry~Londonderry for the 2016 Spawning Survey of Lough Foyle which began in June 2016 and finished in September 2016. 30 oyster samples were taken from five density popular oyster beds within Lough Foyle at weekly intervals. Lengths, Weights and the gonad stage of each sample was then recorded. Water temperature and salinity were carried out using the Seabird 19+ CTD. Statistical analysis was carried out on the various factors through tests such as the Friedman Repeated Measures Non-Parametric test, Chi-Square Test of Association and One-Way ANOVA using IBM SPSS Statistics 24 and Microsoft Excel 2016 Software. The results and discussion have aided in the statement that when temperature has increased across the weeks the spawning stages have also increased into the more mature stages of gonad level found in late summer. The results have also shown that there is little variation between each of the bed locations and temperature, salinity and each of the sampled gonad stages. In conclusion, there are significant relationships between all factors which have an impact on the spawning behaviour of the *Ostrea edulis* species. It has also been suggested further research is needed from the basis of this study which implies the correlating relationship between abiotic factors such as temperature and salinity with the gonad stages of species *O. edulis* within Lough Foyle.

Introduction

Climate change is an enormous impact that is mentioned in countless articles and the impacts of this process affect various environments. Rijnsdorp et al, (2009), suggest that due to the changes in climate change there will be some effects on species distribution. However, even seasonal changes impact water temperatures. Seasonal changes are a key component in the regulation of temperatures on a global scale. In relation to seasonal changes and water temperatures, changes regulate the thermocline which has various impacts across the seafloor. Kaiser et al (2011), composed that whilst air temperatures dramatically fluctuate, sea surface temperatures create a buffering feature therefore giving an insight on the temperature changes within the water column. The processes and impacts change from season to season. For example, in winter with the low levels of light it is inevitable that there are low levels of growth of species during this season and with the absence of a thermocline it is also known that there will be no sediment transport therefore no allowance of dispersal of larvae if *O. edulis* was to spawn during this season. In contrast within the summer months, there is an abundance of light allowing photosynthesis and the growth of species and an increase of nutrients in the water column therefore creating good feeding conditions.

Native Oysters (*Ostrea edulis*) can be found in habitats of highly productive estuarine areas or shallow coastal areas. The species prefers areas of very weak to weak tidal strengths within depths of 0-80m. *O. edulis* have a size range of 0.2cm to 11cm whilst reaching sexual maturity around 5cm. the larval stage of this species lasts around 11-30 days with dispersal ranges of up to 5km depending on temperature and feeding conditions. The larval stage also represents the only mobile period stage in the life cycle of the oyster in which they can spend around 7 to 17 days in the dispersal route. (McKelvey 1996; MarLIN, 2017).

Prior to the dispersal the oysters must go through the spawning cycle process in which needs a series of good temperatures and valuable food supply around early spring to ensure the oysters which are sexual mature are ready to spawn, this is known as conditioning. The greater the length of time of conditioning the more likely it is that wide spawning results will occur. This species is larviparous which means that within the mantle cavity fertilisation occurs and the larvae brood in this cavity until ready to enter the dispersal stage before settling upon suitable substrate. (McGonigle et al, 2016).

Some studies have focussed on the collective effects of several abiotic environmental elements on bivalves. (Lemos et al, 1994; Hutchinson and Hawkins. 1992). However, McKelvey (1996), states that due to the changing environmental conditions within estuarine habitats it is essential that oysters have a susceptible adaptation to changing environmental conditions. Many of the studies have mainly focussed on the effects of changing salinity and temperature on *Ostrea edulis*. Allan et al (2007), suggest that temperature can even determine the metabolic rate of organisms and Brander (2007) adds that increasing water temperatures has certain benefits such as range expansion in terms of dispersal, longer growing seasons, increased growth rates as well as increased food conversion efficiencies. However, Brander also mentions the disadvantages of the increased water temperatures in which the species may undergo stress due to the increased oxygen demand, decline in pH and the uncertain supply of nutrient filled water that also occur with increased water temperatures. This point of stress that Brander (2007) discusses could also cause a failure in immune response to disease which Harvell et al, (2002) confers that the growth in temperature is associated to the heightened spreading of disease.

Numerous studies on the reactions of the native oyster to variance in water temperatures have been carried out in various geographical settings across the globe it is widely accepted that water temperatures are the most substantial influence affecting the reproduction of oysters. O'Connor et al (1992), suggests that spawning usually occurs around 16°C whilst McKelvey (1996), furthers this by stating that larval development is again dependent on water temperature with the inclusion of feeding conditions. McKelvey (1996), also states that the development rate can be influenced by water temperature with reference to temperatures of more than 20°C having a high development rate whilst temperatures of 18°C having a slow rate. Wilson and Simons (1985) have also stated that temperature is a normal impulse of inducing sexual activity in molluscs.

Houde (1987), carried out a study investigating the effects of climate change on fishery production which also focused on the impacts of life stage variation. In this study it was found that larval survival is quite important in recruitment dynamics, however, McKelvey (1993), stated that successful oyster recruitment is a function massively influenced by water temperature and in relation to Lough Foyle successful oyster recruitment only occurs every three to four years. In further study McKelvey (1996), then stated that settlement stages of the *O. edulis* life cycle are also dependent on water temperature. However, factors such as food availability, invasive species, and changes in the hydrodynamic regime, habitat availability and exposure to diseases are influential on the survival of these populations. (McGonigle et al, 2016).

Aims and Objectives

The aim of this dissertation is to thoroughly investigate the effects of abiotic factors such as water temperatures and salinity on the spawning activity of the native oyster, *Ostrea edulis*, within Lough Foyle, Co. Londonderry.

The objectives of this study will involve

- Deriving the water temperature and salinity data from various locations within Lough Foyle and investigate trends at weekly, monthly, seasonal or annual stages.
- Carry out a full investigation of the gonad stages of oysters from Lough Foyle. Sampled on a weekly basis and find trends within the data.
- Investigate the relationships between the brooded larval counts with temperature and salinity.
- Find a substantial corresponding relationship between changing water temperatures and the spawning activity of *Ostrea edulis* using SPSS software.
- Statistically analyse the variations over bed and time between gonad stages, larval counts, temperature and salinity using IBM SPSS Statistics 24 and Microsoft Excel 2016.

Null Hypotheses.

For this investigation the following null hypotheses had been created to focus this study.

H₀¹: The abiotic factors, temperature and salinity have no influence on the biological, chemical or physiological characteristics of *Ostrea edulis*.

H₀²: Temperature variation is the sole influence in the spawning activity of *Ostrea edulis*.

H₀³: There is no inter bed variability within Lough Foyle impacting the spawning activity of *Ostrea Edulis*.

(2010), who used a minimum of 30 oysters and a maximum of 48 oyster from 12 global locations from several types of settlements including wild populations, hatchery's and ponds. These samples were then returned to the laboratory where measurements were recorded on the following characteristics Length (mm) and Weight (g). The lengths were recorded using digital callipers. Whilst the weights were recorded using a scale balance.

Spawning Stages

The spawning stages of each sample were also recorded using the following classification created by Helm et al. (2004).

- Mature/Developed: The gonad is filled or filling.
- White Sick: Gonad Empty and white Substance covering gills
- Grey Sick: Larvae is present in shell and there is a grey substance covering the gills.
- Black Sick: Larvae has grown and is ready to spawn.
- Spent: No gonad material remains, and the oyster has spawned.



FIGURE 3, VISUAL GUIDE OF THE GONAD STAGES RANGING FROM WHITE SICK TO BLACK SICK. ([HTTP://WWW.FAO.ORG/DOCREP/007/Y5720E/Y5720E09.HTM](http://www.fao.org/docrep/007/y5720e/y5720e09.htm))

These guidelines aided in the investigation of identifying samples which have reached sexual maturity within the sites.

Water Temperature and Salinity

Water temperature data was collected through Seabird 19+ CTD equipment which had been deployed in the same five locations within Lough Foyle. Engelhard et al, (2014), used the annual means of sea surface temperatures interpolated to 1° latitude by 1° longitude whilst also focussing on the wind speed and direction of westerly winds across the North Atlantic to gain an insight perspective on water temperatures whilst Dunn et al, (2016), used standardised bottom temperatures from autumn and spring surveys for each day of the year for each survey and depth using linear regression. In this study standard deviation of the median temperatures across of years of each season were studied. However, Monari et al (2007), conducted a controlled investigation on clams, with controlling the temperatures at 20°, 25° and 30° intervals for 7 days. However, for this investigation water temperatures and salinity data was collected through the deployment of the Seabird 19+ CTD at each sampled location. The CTD was deployed at depths up to 1m off the seafloor to avoid disturbance. The CTD recorded data at 10 second intervals to create an insight into the different salinity levels and water temperatures at each depth.

Larval Counts

The investigation of larvae in the water column was also taken from the Spawning Survey 2016 study. In the field the samples were collected using a 500mm diameter plankton net with a 60micron mesh size which was deployed vertically to depths above the seabed to avoid disturbance. A manual flow meter was attached to the mouth of the net to calculate the deployment depth. On retrieval the exterior of the net and bucket was washed using a seawater deck hose. The sample was then stored in a 250ml plastic bottle with the date, time and location labelled.

In the laboratory three 1ml samples were taken from the hand mixed 250ml sample using 1ml sampling pipettes which had been changed between each sub-sample. The 1ml sample was then put onto a glass Sedgewick rafter counting cell and all bivalve larvae was counted. The results of each sub-sample were then averaged and converted into density per m³ using the following calculation.

Bivalve Larvae in Sample

$$\text{Bivalve Larvae in Sample} = \frac{\text{Mean Number per 1ml} \times \text{Sample Volume 1ml}}{\text{Volume of Water Sampled}}$$

Bivalve Larvae per m³

$$\text{Bivalve Larvae per m}^3 = \frac{\text{Bivalve Larvae in sample}}{\text{Volume of Water sampled m}^3}$$

(McGonigle et al, 2016)

Statistical Analysis

Using IBM SPSS Statistics 24 software and Microsoft Excel 2016 software the statistical analysis for this study was carried out. The Friedman Non-parametric repeated measure test explores the level of differentiation between two variables. For this study the Friedman test was used to explore the differences between gonad stages and bed, gonad stages at weekly intervals, brooded larval counts and temperature as well as investigating the differences between brooded larval counts and salinity. This was carried out using the IBM SPSS Statistics software. (Dytham. C, 2003)

A one-way Analysis of Variance (ANOVA) (Dytham. C, 2003) test was used to investigate the level of variance between Gonad stages at weekly intervals and again used to explore the variance between gonad stages and Bed Locations. This was carried out using the IBM SPSS Statistics software.

A Chi-square test explores the level of association between two variables. (Dytham. C, 2003). In the present study, the first Chi-Square test explored association between gonad stages over weekly intervals for each oyster bed. The Chi-square test was carried out using excel by first calculating the expected values and then using the formula function CHISQ.TEST which calculated the association between the actual observations and the expected observations.

$$\text{Expected} = (\text{Sum of each Gonad Stage over all weeks}) \times \frac{\text{Sum of Oysters Sampled in one week}}{\text{Number of total Oysters Samples}}$$

Results

Length and Weight Trends

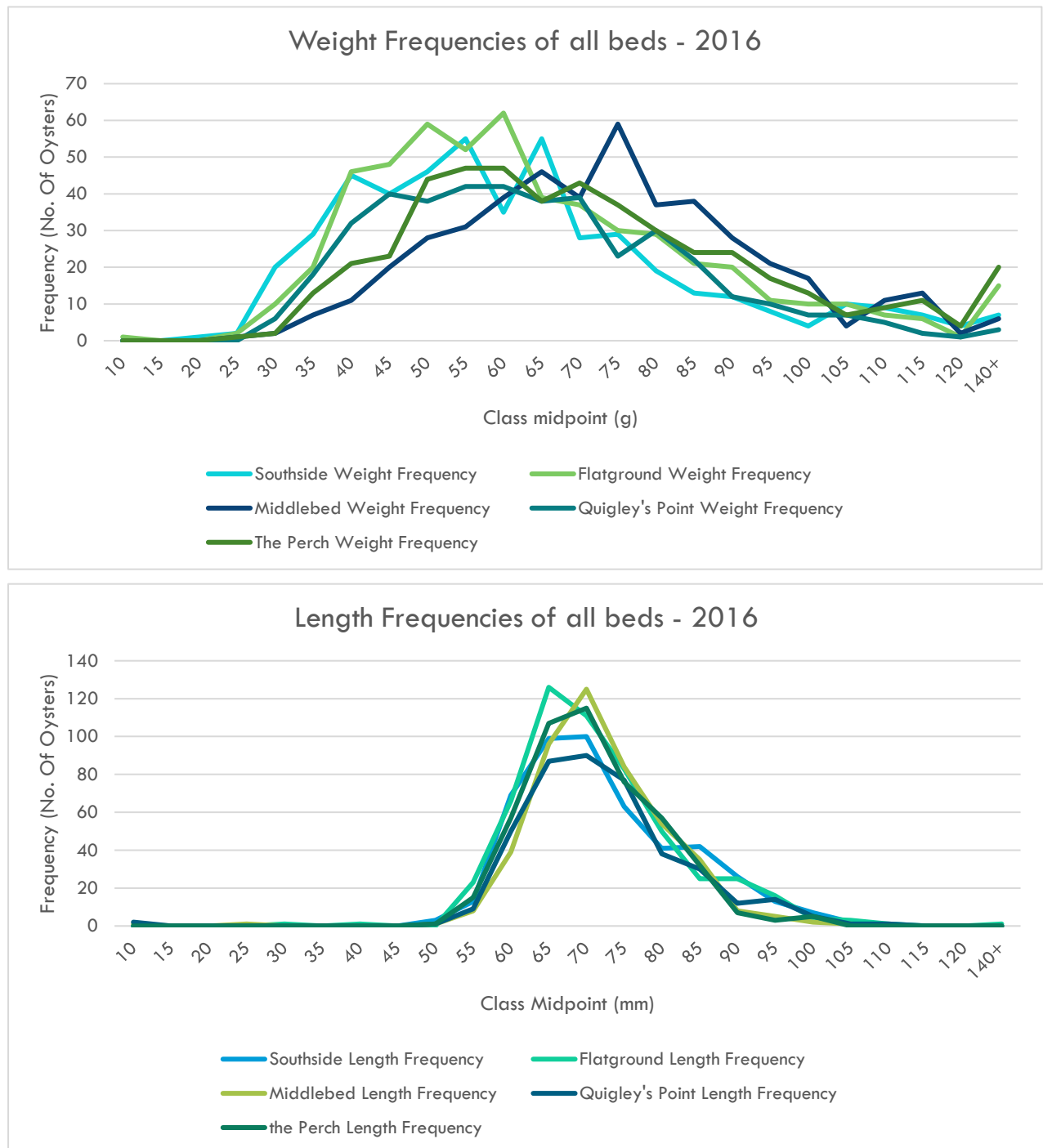


FIGURE 4, THE LENGTH AND WEIGHT FREQUENCY OF ALL BEDS, SPAWNING SEASON 2016

Figure 4, highlights trends between length and weight frequency across all beds. From this figure, the weight frequency increases the length frequency also increases. However, the length frequency varies from class midpoint 10 to 140+ whilst weight frequency has the same variation. The mode of length frequency was 126 oysters at class midpoint 65mm and the mode weight frequency was 62 oysters at class midpoint 60g.

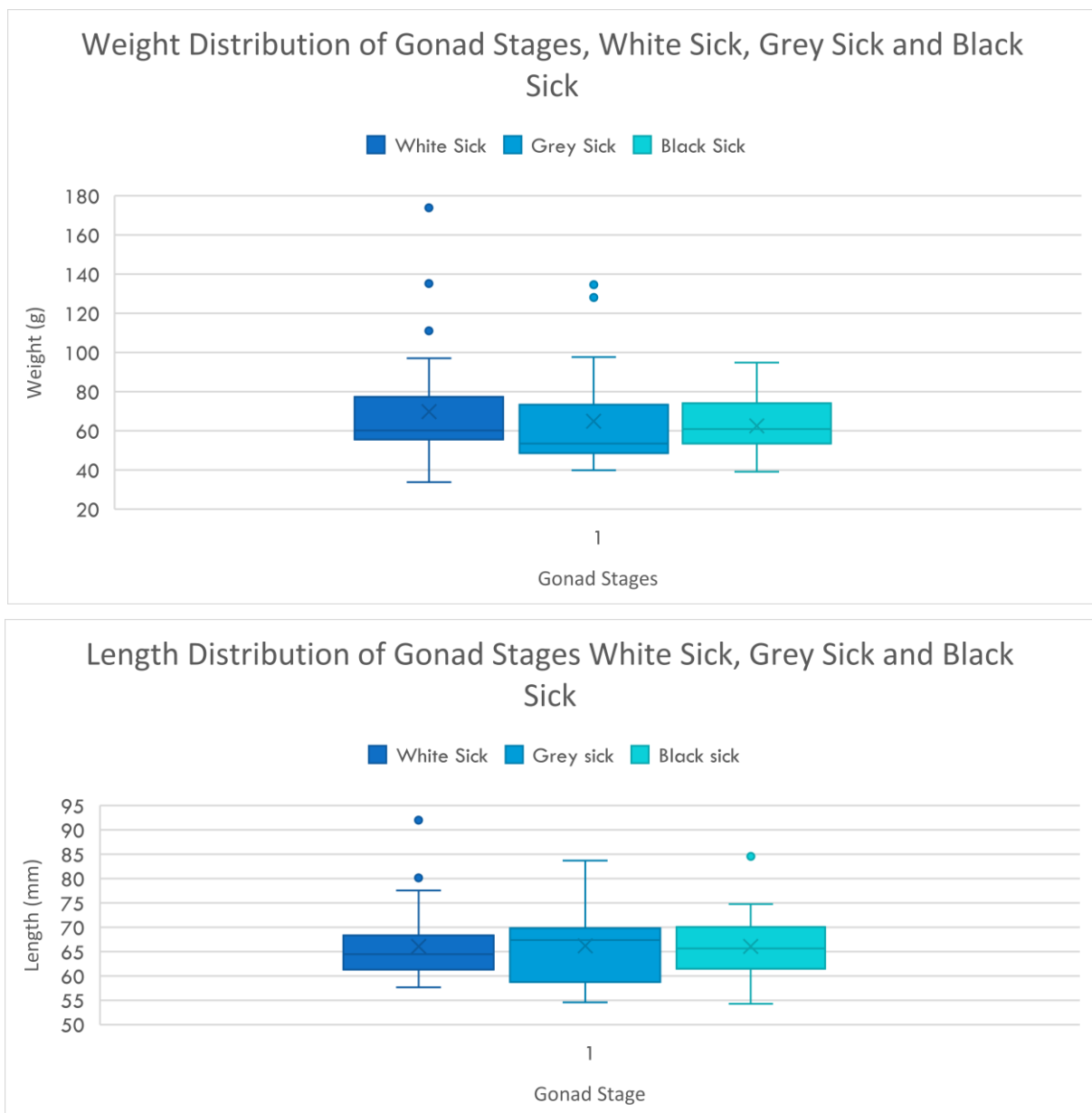


Figure 5, Length and Weight Distribution across gonad stages

Figure 5, illustrates the distribution of length and weight values across gonad stages White Sick, Grey Sick and Black Sick. The largest distribution is from the Grey Sick with a low length value of 54.61mm and a high length value of 83.71mm, the median value was 67.26mm. The distribution of White Sick varies from 57.71mm to 77.59mm whilst Black Sick varies from 54.3mm to 74.78mm. It can be observed that the largest weight is from the white sick stage with the lowest weight value of 33.8g and the heaviest weight value of 97.1g however there are values of 111.1g, 114g, 135.2g and 173.g outside of the quartile. Grey sick varies from 39.9g to 97.7g whilst Black sick varies from 39.2g to 94.9g.

Gonad Stages and Weekly Intervals

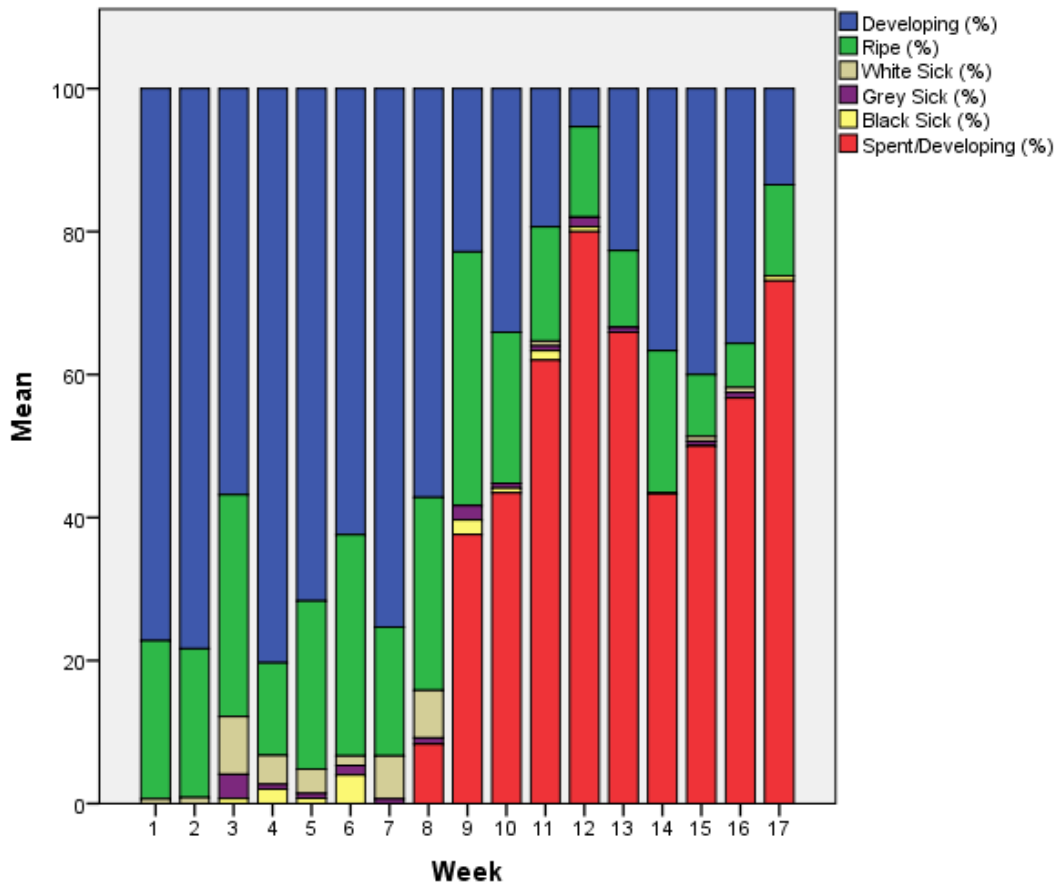


FIGURE 6, DISTRIBUTION OF GONAD STAGES PER WEEK

Figure 6 illustrates the changes in distribution in the percentage of oysters transitioning from the developing stage right through to the Spent stage. This is an observation that highlights that change in time coincides with a change in Gonad stages. The Friedman non-parametric test results found that all gonad stages changes are significant to the weekly variations with values at a high of 0.03 and therefore less than 0.05 directing to a highly significant relationship of both variables.

Gonad Stages and Bed Variance

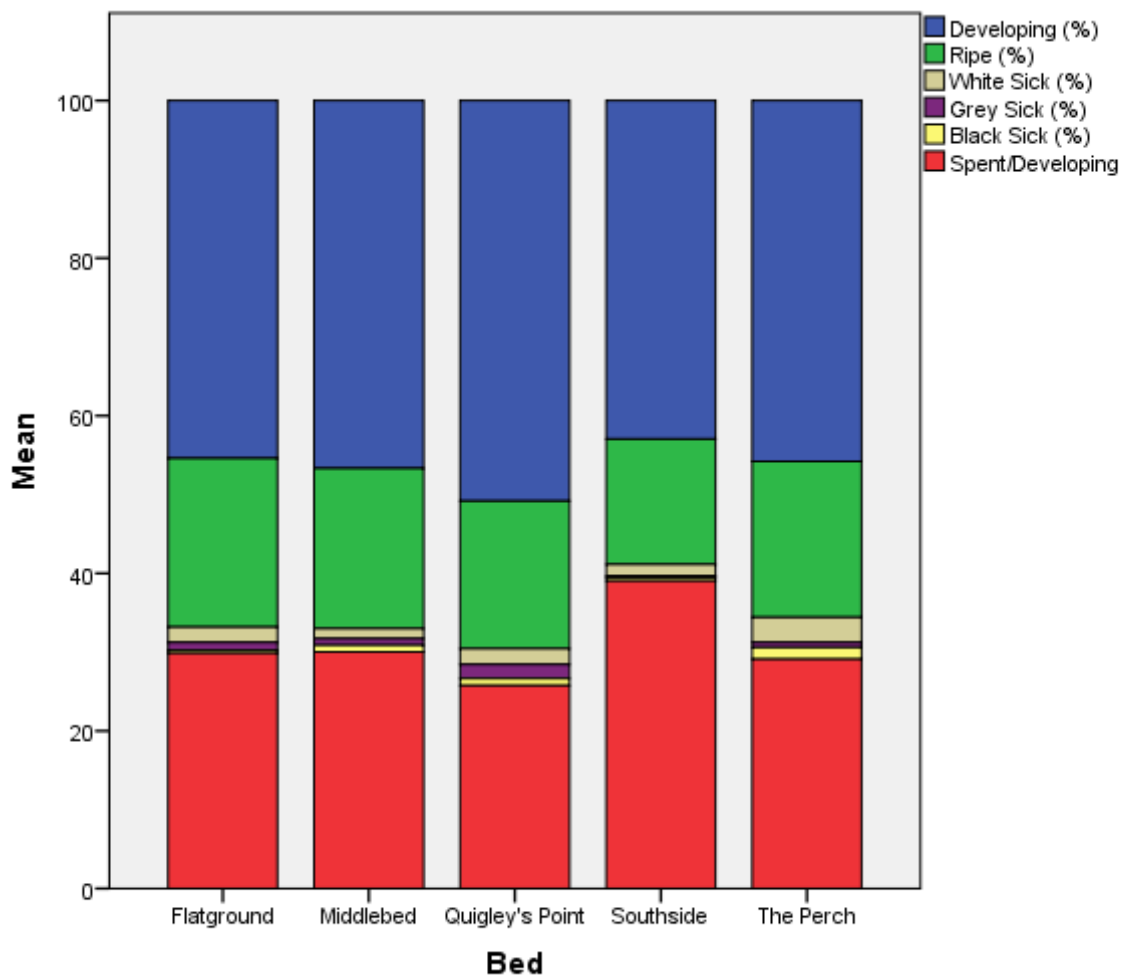


FIGURE 7, DISTRIBUTION OF GONAD STAGES PER BED

Figure 7, displays that although there are variances between each of these beds they are relatively small when assessed collectively over time. The results from the ANOVA test (Table 1) further analysed these variances and found significance values of .960 for the developing stage meanwhile Grey Sick was .251. However, none of these values are less than 0.05 and therefore had no significant relationship in terms of gonad stage and bed variations. The Friedman test found that again there are no significance between these two variables with values such as 1.00 between the gonad stages and beds.

TABLE 1, ANOVA RESULTS BETWEEN GONAD STAGES AND BED

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Developing (%)	Between Groups	507.367	4	126.842	.156	.960
	Within Groups	61117.758	75	814.903		
	Total	61625.124	79			
Ripe (%)	Between Groups	283.988	4	70.997	.421	.793
	Within Groups	12657.779	75	168.770		
	Total	12941.767	79			
White Sick (%)	Between Groups	36.669	4	9.167	.649	.629
	Within Groups	1058.590	75	14.115		
	Total	1095.259	79			
Grey Sick (%)	Between Groups	21.144	4	5.286	1.374	.251

	Within Groups	288.606	75	3.848		
	Total	309.750	79			
Black Sick (%)	Between Groups	13.081	4	3.270	.931	.451
	Within Groups	263.475	75	3.513		
	Total	276.556	79			
Spent/Developing	Between Groups	1541.063	4	385.266	.337	.852
	Within Groups	85825.895	75	1144.345		
	Total	87366.958	79			

Larval Counts and Temperature Variations

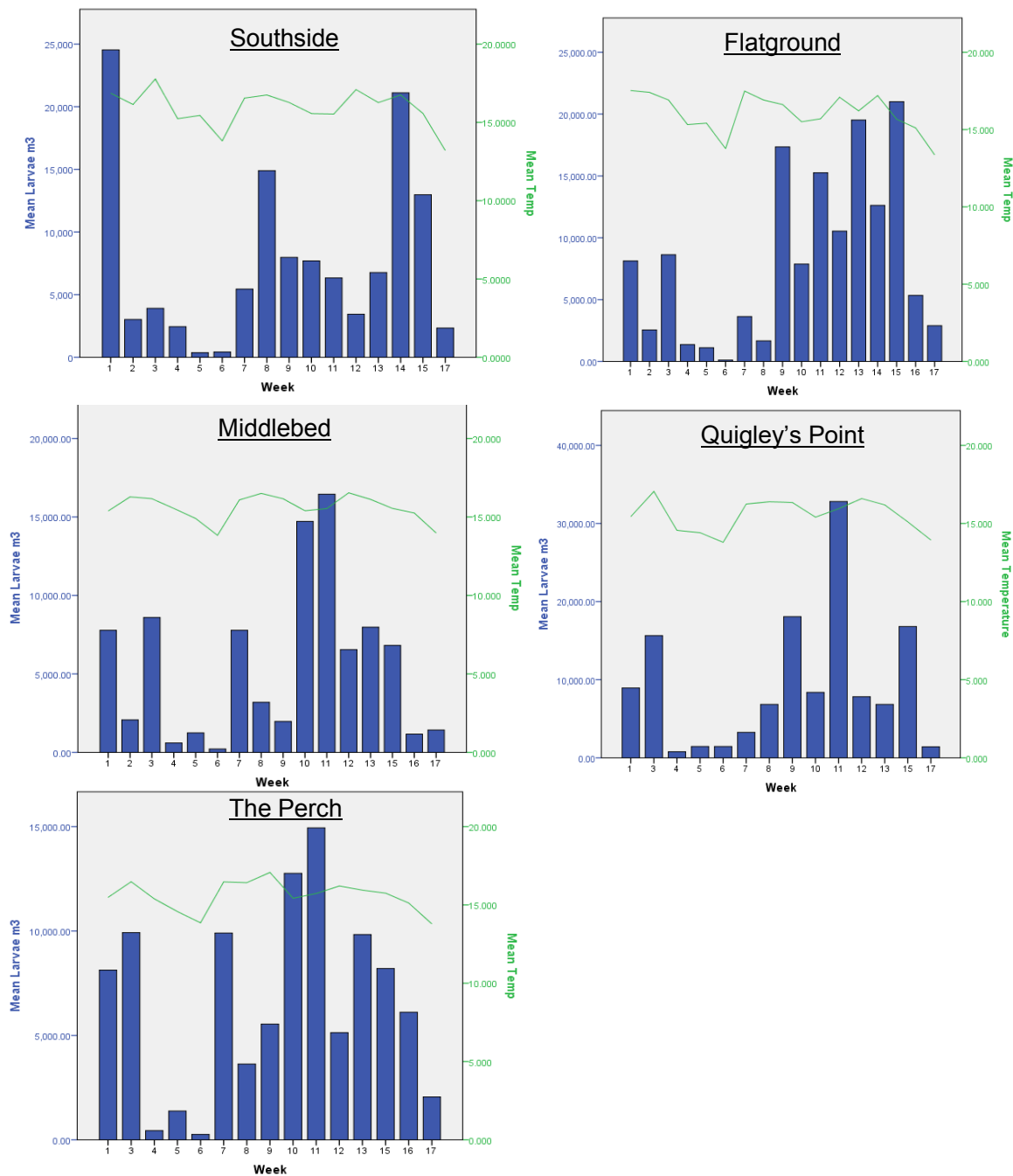


FIGURE 8, DISTRIBUTION GRAPHS OF MEAN LARVAE M³ IN EACH BED PER SPAWNING SEASON.

Figure 8, highlights the variation of mean larval counts per m³ at weekly intervals for each sampled site alongside the average weekly temperature of each site. The Southside Graph (top left) had a high count of 24531.0819 per m³ during week 1 and a low count of 3911.4837 per m³ during week 3, however, as temperature varies there is also a variation in mean larval counts per m³ for the same week. This is again shown in week 6 of the Flatground graph which had a mean temperature of 13.77°C and a mean larval count of 103.75 per m³ which then increase to 3631.09 per m³ at 17.49°C during week 7. However, this does not be the case for the Middlebed, Quigley's Point or the Perch graphs which show that the highest mean larval count did not occur alongside the highest mean temperature value. The Friedman repeated measure test found no significance between the larval counts with temperature at each bed.

Larval Counts and Salinity

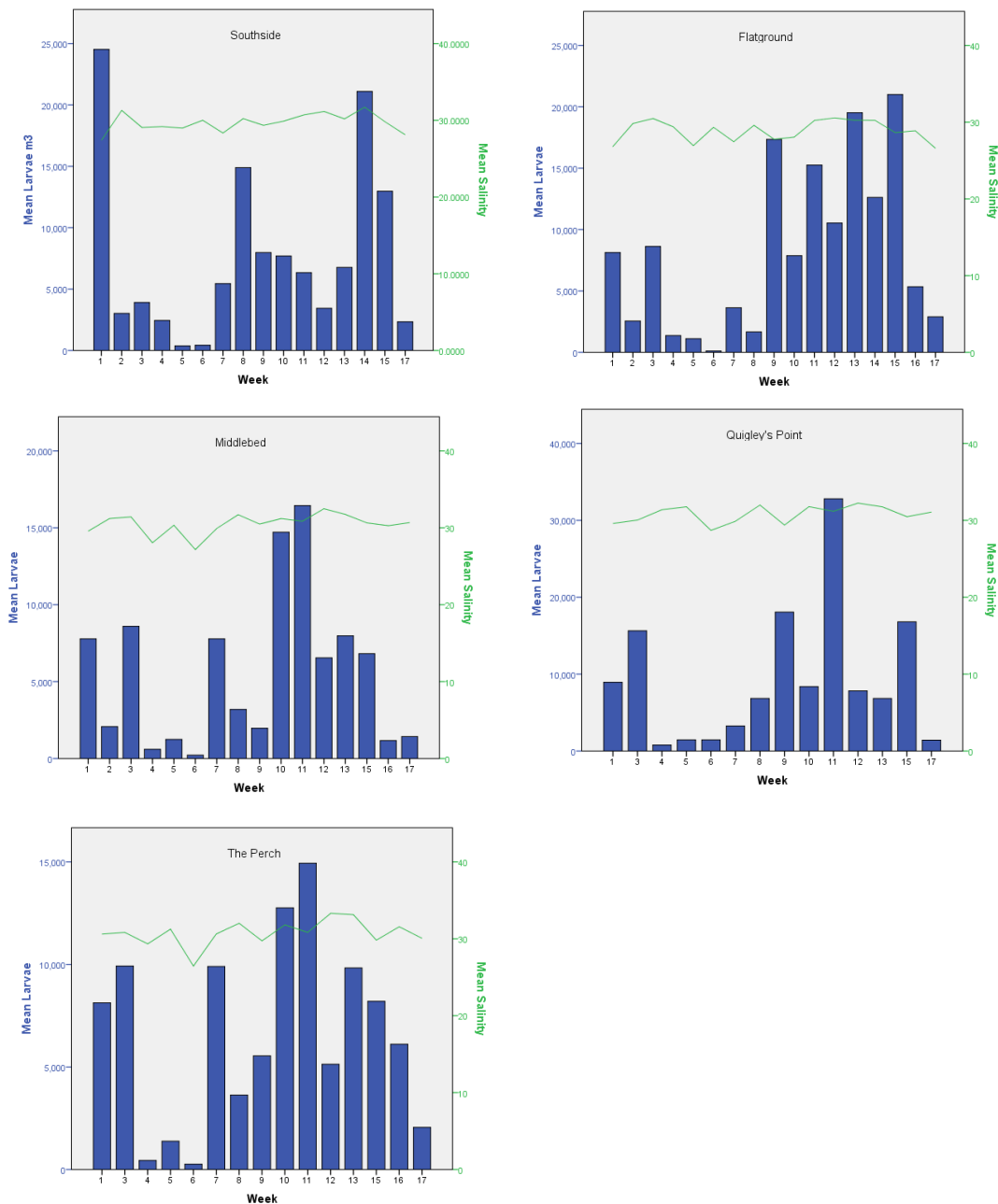


FIGURE 9, THE DISTRIBUTION OF LARVAE COUNTS AND SALINITY ACROSS EACH BED.

Figure 9 highlights the correlation between larval count and salinity at weekly intervals across each bed. In the perch graph (Bottom left) as salinity changes each week the larval count for the same week also changes in correlation. This can be seen in week 13 where salinity was at 31.739 with a larval count of 7975.43m³ which then fell to 6817.56m³ in week 15 with a decrease in salinity to 30.652. The results of the Friedman test found a significant relationship below 0.01 for Southside, Flatground, Quigley's Point and The Perch. Results were not applicable for Middlebed. However, a Friedman test carried out on the average size of larval and salinity had found that Southside and Middlebed were both significant at the 0.01 level.

Gonad Stage with Time and Location Variance

The results of a Chi-Square test indicated that there are very strong associations ($P < 0.01$) between maturity and weeks throughout each of the five beds. A one-way ANOVA was also carried out to explore the relationship between the gonad stage variance at weekly intervals and found that there was no significant relationship between both variables with a p value of 1. This was also the case for an ANOVA test between gonad stages at each bed location with a p value of 1.

Discussion

This investigation was carried out to explore the effects of the abiotic factors, temperature and salinity on the spawning cycle of *O. edulis* within Lough Foyle during spawning season 2016. Hauton et al, (1998), Hawkins and Hutchinson, (1990, 1992) suggested that at a physiological level bivalve species are sensitive to temperature and salinity fluctuations. This was found in the results of the Friedman test between gonad stages and salinity. These findings were coherent with previous studies (Davis and Ansell, 1962 Loosanoff and Davis, 1968 and Newkirk et al, 1977) which also found that the development level of larvae increased with increasing salinity. It was also stated that healthy larval growth can occur at salinity values as low as 15-20‰ (Global Invasive Species Database, 2018). Korringa (1940) has suggested that temperature is a critical factor for settlement success of oyster larvae and this is also found in O'Connor et al (2007) which stated about previous laboratory studies had found that when temperatures are factorially tested alongside other abiotic factors such as salinity or food availability the results conclude that temperature has the greatest effect on the development time of marine species (Hoegh-Guldberg & Pearse, 1995). The Global Invasive Species Database (2018) states that temperature can influence the sex of *O. edulis* during spawning as when the temperature reaches 16°C the species changes from male to female around every three to four years, however, when at temperatures of 20°C the sexual changes occurs annually. Colder temperatures cause the species to revert into the male form. Depending on water temperatures there can be two sexual phase changes per season.

The present study did not find any significance in the Friedman repeated measures test between the gonad stages and each oyster bed which was also tested using the ANOVA analysis test which had the same result. These findings coincide with other studies by Hauser and Ward (1998), Palumbu (1996), Ward et al (1994) and Launey et al (2002), which found that marine species such as the *O. edulis* have less geographical differentiation than other species. Korringa (1976) suggests that this could be due to the aquaculture potential of the *O. edulis* which allows the species to thrive in many locations globally. This large global distribution can be seen in ranges along the European Atlantic coastline, the Mediterranean as well as the Black Sea (Launey et al, 2002) Habitats.org.uk (2018) however explains that during an increase of sea water temperatures there is a coinciding intense oyster settlement. Davis and Calabrese (1969) found that at higher temperatures more oyster spat had settled and with an increase in temperature there was an increase in the growth of spat. Bayne (1969), stated that oysters prefer to settle in areas where larvae have previously settled, with further investigation by Woolmer et al (2011) and Kennedy and Roberts (1999) who found that *O. edulis* settlement was most popular in areas of cultch and dead oyster shells.

The Horn Point Oyster Hatchery (2018) states that this species is heavily influenced by environmental cues to begin their ripening process in the early spring due to increases in temperature alongside changes in the biomass of phytoplankton or changes in salinity levels, this coincides with McGonigle et al, (2016) who suggest that after the period of good conditioning in the spring, successful spawning should occur with appropriate temperature and food availability. The results of the Friedman test found a significant relationship between

each gonad stage and weekly intervals. This correlates with the weekly intervals as they fall within the known spawning season of *O. edulis* between June and September. According to The Horn Point Oyster Hatchery within the University of Maryland (2018) oysters can begin to spawn within their first year and have reached their prime by their third year. In season, the release of the gonad from one oyster encourages the surrounding male and females to release their gonad and therefore the beginning of a mass spawning event. (*Horn Point Oyster Hatchery, 2018*).

Although there was a significant relationship between gonad stages and weekly intervals this was not the case for the brooded larval count and temperature although a significance was found between the brooded larval count and salinity in most of beds. Previous studies have found that with increased salinity there was an increase in larval growth (*Davis and Ansell, 1962 and Newkirk et al, 1977*). This was again mentioned by Matozzo and Marin (2011) who found that salinity can impact numerous metabolic and physiological strictures in marine organisms. Crisp et al, (2017) also found that salinity has an influence on the survival of larvae within the early stages. Despite the result of no significance between larval counts and temperature many studies have found that temperature has a direct link on larval dispersal and survival. Nozawa and Harrison (2007) found that at higher temperatures there was a more positive effect on larvae settlement. Verween et al, (2007), found that survival during the larval stages is heavily temperature and salinity dependent however, Robert et al (1998) found that these abiotic factors were not separate influential factors but in fact when combined the changes in these abiotic factors had a significant effect on the larval growth. Although *O. edulis* has a varied resistance to environmental factors, the spawning initiation is heavily temperature dependent although salinity levels may be a limiting factor (*Verween et al, 2007*).

The relationship between length and weight of *O. edulis* correlates as length increases, weight also increases. McGonigle et al, (2016) suggests that length and weight correlate with larval counts due to the larger the size of oyster the more larvae can be obtained within the mantle. Valero, (2006) stated that growth rates are dependent on hydrodynamic activity, food availability, location and age. This is strengthened in studies carried out by Partridge (1981) and Larsson and Jonsson (2006). Walne, (1974), highlighted a relationship between age/size and the quantity of brooded larvae. This is again due to the size of the mantle cavity. This explains why older and larger oysters are key in the species recruitment process each year (*McGonigle et al, 2016*). Although McGonigle et al, (2016) stated that larval counts are related to mantle cavity size, Hofman et al, (1988) suggested that this may not be true in the older *O. edulis* species due to reproductive senility which reproduces the reproductive effort and metabolic effort within the species. For this study samples above the harvestable size (50mm) were retained to allow smaller and younger species to grow and reach sexual maturity. However, the results have shown that spawning had occurred at a range of various lengths and weights (52mm-97mm, 27.5g-120g) across all beds throughout the season with 68.7% of oysters within the white sick stage with a mean length of 67.2mm and a mean weight of 62.5g. the distribution highlights the lack of oysters above 100g in weight and 95mm in length which is due to the removal of large oysters during the commercial fishing season each year.

Limitations

The limitations to this study include the availability of length and weight data of stages such as Developing, Ripe and Spent gonad stages as there was no data collected for each of these stages which therefore affects the study of the effects of morphological features on spawning activity.

Another limitation would be the scarce availability of temperature and salinity data which came from a CTD instrument taken at each site however the use of digital data loggers at each site

with daily intervals would have been more beneficial to give an insight into the temperature and salinity at the exact level the oysters are exposed to.

During statistical analysis testing a two-way ANOVA was tested between the gonad stages and weekly intervals, however, it was found that there were not enough replicates at the lowest level for analysis to occur and a result was not obtained. Therefore, in future studies more data is needed for example samples taken at daily intervals instead of weekly or even more data taken from previous years.

For future research it would be beneficial to acquire more annual data to allow for more comparisons to be made on the effects of abiotic factors across many years or seasons. Different factors such as substrate type, disease, mortality, water quality and the commercial fishing rate during each season would allow a wider exploration into the main influencers of the spawning cycle of *O. edulis*. Few studies have also researched the effects of hydrodynamic activity on spawning cycles of bivalves (*Newell et al, 2001 and Cranford et al, 2003.*). There are significant gaps in research in the effects of location on the spawning cycle of *O. edulis* which may be due to their aquaculture potential but it is not yet significantly clear. It would also be of interest to further research an aspect of the null model created by O'Connor et al, (2007) to investigate the influence of water temperature on the dispersal of marine larvae.

Conclusion

From the investigation of the effects of abiotic factors on the spawning cycle of *O. edulis* it is key to highlight that there is indeed a correlating relationship and this was strengthened through the previous studies mentioned. This investigation was successful in determining the significant relationship between salinity and the spawning activity whilst little results determined fully the effects of temperature on the spawning activity. However, there was a significant relationship found between gonad stages at weekly intervals during a known spawning season with a small variation in water temperatures therefore implying that these water temperatures were a factor in successful spawning.

The study found that bed variance and the gonad stages of *O. edulis* had no significant relationship which therefore accepted the null hypothesis ' H_0^3 : There is no inter bed variability within Lough Foyle impacting the spawning activity of *Ostrea Edulis*.' However, H_0^1 and H_0^2 is rejected due to the significance between salinity, gonad stages and brooded larval counts whilst a significant relationship was highlighted between gonad stages and weekly intervals during a known spawning season. More research is needed within this study to further investigate the impacts of abiotic factors on spawning activity and the recommendations for future studies have been highlighted.

Acknowledgments

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