OBERVATIONS OF GONAD STRUCTURE AND GAMETOGENIC TIMING IN A RECOVERING POPULATION OF *OSTREA LURIDA* (CARPENTER 1864)

by

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A THESIS

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THESIS ABSTRACT

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From January 2012 to December 2012 I collected adult oysters from two intertidal populations on a monthly basis in the Coos Bay estuary, Oregon for histological analysis of their gonads. Gametogenesis and spawning occur seasonally from May through September, when water temperatures exceed 14.5^{o} C, with brooding oysters found from July through September. Oocyte diameters increased significantly from May to June, and from June to July within oyster populations at Haynes Inlet and Coalbank Slough, respectively. Male gametogenesis initiated in May at Haynes Inlet and in June at Coalbank Slough. Dry meat condition values increased significantly during periods of reproduction and decreased following the reproductive season's end. Condition index values for Coalbank Slough were consistently lower than those at Haynes Inlet, suggesting poor nutrition or physiological stress. Salinities below recorded physiological thresholds are believed to be the primary environmental factor influencing the discrepancy in reproductive activity at Coalbank Slough.

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For those who have remained close to my heart, from hundreds of miles away.

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CHAPTER I

GENERAL INTRODUCTION

The Olympia oyster, *Ostrea lurida* (Carpenter 1864), is the only native oyster on the west coast of the United States (Baker, 1995; McGraw, 2009). Commercial exploitation of the species began in 1851 and led to near extirpation in many estuaries (Kirby, 2004). Today, populations persist in estuaries and bays ranging from central British Columbia to Baja California (Dall, 1914; Gillespie, 2009; Polson et al., 2009a). Olympia oysters provide ecological services in the form of habitat for estuarine biota, biofiltration, protective shoreline buffers, and denitrification (Baker, 1995; Kimbro and Grosholtz, 2006; Dinnel et al., 2009; Groth and Rumrill, 2009; McGraw, 2009; Brumbaugh et al., 2010; Beck et al., 2011).

Ostrea lurida belongs to the smaller of the two commercially harvested ostreid genera (the other being *Crassostrea*) (Ahmed, 1975), with average shell lengths of 3.5-4.5 cm, occasionally reaching sizes of 9 cm (Peter-Contesse and Peabody, 2005; Gilespie, 2009). Olympia oysters are typically associated with estuaries or bays with salinities above 24, but are capable of thriving in full strength seawater (Baker, 1995; Peter-Contesse and Peabody, 2005) and tolerating exposure to salinities of 0-5 for up to 4 weeks (Gibson, 1974).

Olympia oysters are protandrous sequential hermaphrodites, maturing first as males then alternating sexes after every spawning event (Coe, 1931a, 1931b, 1932, 1934). Females brood their young from fertilization through to the D-veliger stage of development before releasing them as free swimming larvae (Hori, 1933; Hopkins, 1937). The first published observations of spawning in Olympia oysters were conducted

by Stafford (1913) off the coast of British Columbia. Later studies published by Coe (1931a, 1932b, 1932, 1934) investigated the seasonal gametogenic timing in La Jolla, California. Numerous reproductive studies have been devoted to sister species within the genus *Ostrea*. The most popular, due to its value as a commercial stock, is the European flat oyster *Ostrea edulis* (Orton, 1927, 1931, 1933; Loosanoff and Davis, 1963; Wilson and Simons, 1985; Abellan et al., 1989; Shpigel, 1989; da Silva et al, 2009), however other species have also been investigated, including *O. nomades* (Siddiqui and Ahmed, 2002), *O. stentina* (El Gharsalli and Aloui-Bejaoui, 2011), and *O. chilensis* (Jeffs and Hickman, 2000).

Olympia oyster populations have struggled to recover since the majority of harvesting pressures were removed over 80 years ago (Trimble et al., 2009; White et al. 2009b). Remnant populations, however, still persist throughout its historical range (Dinnel et al. 2009; Blake 2010). Recent studies have identified reproductive limits, inadequate availability of substratum, poor post-recruitment survival, predation, and competition as potential inhibitors to full population recovery (Groth and Rumrill, 2009; White et al., 2009a).

As Olympia oyster populations began their precipitous decline in the late 1800s and early 1900s, efforts to restore natural populations were largely abandoned (Trimble et al., 2009), replaced instead by large-scale commercial mariculture of *Crassostrea gigas* (Thunberg, 1793), a faster growing and more pollution tolerant species (White et al. 2009a). In the last ten years, interest in native oyster restoration has resurfaced. Projects aimed at restoring native oyster populations are currently underway in estuaries within every state along the west coast of the United States (White et al. 2009b; Groth and

Rumrill, 2009; Dinnel et al. 2009; Buhle and Ruesink, 2009; Polson and Zacherl, 2009b) and British Columbia.

The history of *Ostrea lurida* in Coos Bay, Oregon is unique to populations found elsewhere along the west coast of North America. While European settlers found no evidence of extant oyster populations in the bay upon their arrival in the 1850s, fossilized shell deposits discovered along the shoreline of the bay reveal a distinct historical presence. These deposits are thought to be death assemblages and the remains of Native American shell middens, suggesting Olympia oysters were once abundant in Coos Bay as well as an exploited food source for indigenous populations (Baker, 1995). Shells from these locations have been aged at approximately 400 years through the use of radiocarbon dating (Groth and Rumrill, 2009). Groth and Rumrill (2009) have suggested that a tsunami and/or large fire initiated a massive sedimentation event, suffocating oyster beds and contributing to localized extinction within the bay. Olympia oysters are believed to have been inadvertently reintroduced to the bay during the late 20th century. *Crassostrea gigas*, imported to Coos Bay from Willapa Bay, Washington for aquaculture are believed to have carried Olympia oysters as epibionts on their shells as "hitch-hikers" (Baker and Terwilliger, 2000). Olympia oysters soon became re-established across the eastern arm of Coos Bay.

The early life history of Olympia oysters is a topic of great concern to restoration efforts. Reproductive seasons, larval abundances, and settlement success may vary widely in different habitats, highlighting the importance of regional observations and site-specific data (Stafford, 1913; Coe, 1931a; Hopkins, 1937). In Coos Bay, investigations have been conducted investigating seasonal larval abundance (Laura Peteiro, unpublished

data) and seasonal settlement patterns (Sawyer and Young, 2011), however, little information exists on the regional reproductive behaviors and underlying gametogenic mechanisms for this species. In this study I will examine seasonal gametogenesis, dry meat condition indices, and the presence of brooding oysters as indicators of reproductive activity in *Ostrea lurida* and compare reproductive periods at two sites within the bay. I will also investigate possible physical and biological parameters that may influence the timing of regional reproductive events.

CHAPTER II

GAMETOGENIC ANALYSIS OF OLYMPIA OYSTER POPULATIONS IN COOS BAY, OREGON

INTRODUCTION

The Olympia oyster, *Ostrea lurida* (Carpenter, 1864), is the only native oyster species along the west coast of Canada and the United States with a range extending from Sitka, Alaska to Baja, California (Dall, 1914; Baker, 1995). Found predominately in intertidal and subtidal estuarine environments (Cook et al. 2000), *O. lurida* are considered ecosystem engineers for their ability to filter seawater for particulate matter as well as provide food and shelter for a multitude of organisms (Baker, 1995; Beck et al., 2011; Gray and Langdon, 2012). In addition to their ecological value, Olympia oysters are commercially exploited and support highly profitable fisheries (Korringa, 1976). Usually preferring to settle on hard substratum, *O. lurida* is commonly found attached to other oysters, large boulders, rip-rap, gravel/cobble, and wood (Baker, 1995; Groth and Rumrill, 2009).

Ostrea lurida, once native and abundant in Coos Bay, suffered a relatively recent extinction event, followed by a subsequent reintroduction (Baker, 1995; Baker and Terwilliger, 2000; Groth and Rumrill, 2009). In recent years, great attention and resources have been expended to understand the dynamics of re-establishing this ecologically and commercially valuable species within the estuary.

One area of particular concern is seasonal reproductive activity. Olympia oysters are protandrous, sequential hermaphrodites (Coe, 1931a, 1931b, 1932, 1934). Gametogenesis of both sexes takes place within a network of fluid-filled pockets,

otherwise known as gonad follicles, located in the connective tissue between the mantle epithelium and the digestive diverticula (Andrews, 1979). Gonad follicles are often observed with the simultaneous presence of male and female gametes. Juveniles begin developing their gonads in as little as 8 weeks after settlement. Proliferation of male gametes occurs quickly, in some cases producing ripe gonads within as little as 5 months after settlement (Coe, 1931a; Coe, 1932). Through spasmodic contractions of the adductor muscle, tightly packed balls of 250-2000 spermatozoa are released into the water column via the excurrent siphon (Coe, 1931b; Hopkins, 1937). Before male spawning is complete, female gametogenesis proceeds within the same individual oyster alongside the remnants of the previous male phase. Ovulation in female phase oysters is stimulated by the presence of male gametes taken into the incurrent siphon (Coe, 1931a). Ripe oocytes are released into the excurrent chamber where spasmodic contractions and relaxations of the adductor muscle help force them through the gill ostia and into the incurrent chamber of the mother where fertilization occurs (Hopkins, 1937). Depending on environmental conditions, oysters may switch functional sexes 2-3 times in a given breeding season (Coe, 1932).

Female Olympia oysters provide a measure of brood protection to their offspring. Once fertilized, embryos develop within the incurrent chamber for a period of 12 (Coe, 1931a) to 17 days (Stafford, 1913), then are released into the water column as planktotrophic veligers. Hopkins (1937) observed a brood period of approximately 9-14 days with larvae growing at a rate of 12 microns per day. Individuals ranging from 23.5-36.8mm shell length were observed to carry broods from between 70,000 to 350,000 embryos/larvae with an average brood of 215,000 (Hopkins, 1937). Based on

observations of functional guts in brooding *Ostrea circumpicta* larvae (Kang et al., 2004), *Ostrea lurida* larvae are presumed to feed on phytoplankton brought in via the mother's incurrent water flow. Regional differences in brood capacity and average sizes for brooding oysters have yet to be investigated.

Periods of spawning in Olympia oysters have been strongly correlated to seasonal increases in water temperature. Coe (1931a, 1932, 1934) conducted experiments in La Jolla, California in which he followed the gametogenic progress of newly settled *O. lurida* juveniles. By setting out blocks of oysters of known ages and sampling at regular intervals, he characterized the gonad of *O. lurida* and determined that this species alternates its sexuality throughout its lifetime. Additionally, he found that spawning was induced when water temperatures of ~16^{o} C were maintained. Hopkins (1937) collected daily samples of adult oysters from dikes constructed in Puget Sound Washington and observed brooding individuals immediately following a spike in minimum water temperature to ~13^{o} C, indicating this temperature as a spawning trigger. In both of these cases breeding periods varied strongly based on seasonal trends in water parameters. Oysters in La Jolla, California had a spawning season of ~7 months, while reproduction in the seasonally colder waters of Puget Sound, Washington was restricted to a period of 3-4 months. Even further north, Stafford (1913) found that populations of *O. lurida* in British Columbia were limited to a spawning period of only 3 months. The reproductive season of this species appears to be reduced at higher latitudes where water temperatures exceed the spawning threshold for increasingly short periods of time. Laboratory analyses have corroborated field data with spawning temperatures recorded at 14^{o} C (Hori, 1933 Imai et al., 1954). Santos (1992) revealed that oysters may spawn at temperatures from

12-21^{o}C, although oysters spawning at lower temperatures took as many as 8 weeks to produce larvae after the detection of gametogenesis while oysters at 21^{o} C took 2-3.5 weeks and produced significantly more larvae. Seale and Zacherl (2009) suggested that temperature is unlikely to be the sole determinant in spawning periodicity after reporting settlement patterns that did not conform to those expected with the observed temperature regime. This suggests that other environmental parameters must be taken into account for their potential influence on reproduction. Of particular interest to this study are salinity and chlorophyll-a concentrations. Salinity varies widely within estuaries and environments subjected to salinities of 15 or lower demonstrate deleterious effects on their resident oyster populations (Gibson, 1974). The repercussions of salinity for gametogenesis have not yet been explored for *O. lurida*, although Butler (1949) explored this topic with *Crassostrea virginica*. Little is known of the effects of phytoplankton production on the reproduction of *O. lurida* although strong relationships between increases in food availability and reproduction have been demonstrated in *Ostrea edulis* (Cano et al., 1997).

Information on the reproductive cycle of Olympia oyster populations within Coos Bay is limited. Regional data on gametogenesis and spawning is essential for conservation of Olympia oysters and for the design of effective restoration efforts within the estuary. In particular, information pertaining to the timing of spawning, environmental influences and other life-history traits are critical to develop management strategies to foster self-sustaining populations. This project seeks to resolve many gaps in the knowledge about reproduction for this species. First and foremost, I will characterize the reproductive cycle of *O. lurida* within the Coos Bay estuary. In this study, I

determined the timing of initiation, peak production, and cessation of gametogenesis. I then worked to discern which environmental variables influence the regional patterns of reproductive activity observed. I then compared two populations of oysters within Coos Bay to determine how variation in habitat conditions may influence reproduction. Additionally, I observed individuals in the process of brooding larvae to estimate brood size and to determine developmental stages.

METHODS

A total of 960 oysters were studied during 2012. Approximately 240 oysters were used to calculate condition indices and the remaining 720 oysters were used for histological analysis.

Study Sites and Ambient Environmental Conditions

Monthly field collections of *O. lurida* took place from January 2012 until December of that year. Two Coos Bay intertidal habitats were sampled at an elevation of ~.3 m above the mean low tide line. The rocky intertidal habitat of Haynes Inlet (Figure 1) (43°26'32.20"N, 124°13'17.53"W) and the mudflat habitat of Coalbank Slough (Figure 1) (43°21'35.71"N, 124°12'25.60"W) were chosen due to their large populations of Olympia oysters and accessibility. During each monthly sampling, forty large specimens (>30mm Shell length) were collected along a 100 m transect line on the shoreline of Haynes inlet, while habitat constraints limited the transect at the Coalbank Slough mudflat to 50 m. Each sampling event took place along randomly assigned points on a permanent transect line within each habitat. Each month, 40 sampling points were assigned to each transect by randomly choosing a number out of 100 forty times and assigning those numbers to meter marks on the transect line. For each assigned meter

mark and it's corresponding 1 meter interval, a large oyster in the closest proximity to the line was haphazardly sampled. Organisms were transported to the laboratory on ice and left in flowing seawater tables for no more than 24 hours before processing.

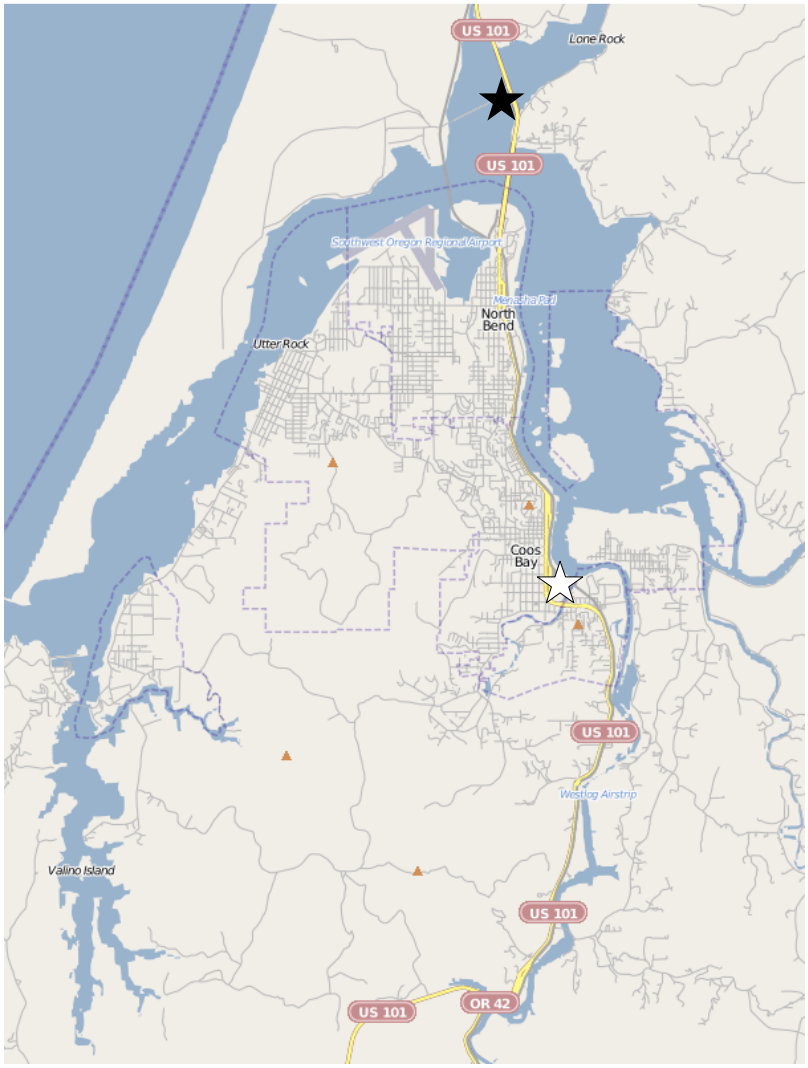

Fig. 1. Map of Coos Bay estuary with study sites marked with stars. The black star corresponds to the Haynes Inlet study site and the white star corresponds to the Coalbank Slough study site.

Monthly measurements of ambient hydrological parameters were recorded on every sampling date and on dates halfway between sampling events from May to September. Temperature and salinity were measured using a YSI 650 MDS probe, and

triplicate water samples were collected in dark nalgene bottles for chlorophyll-a analysis in the lab.

All oysters were measured for shell length (base of umbo to ventral shell margin) using calipers. Each monthly site sample was subdivided from the original 40 into a group of 30 for histological analysis and a group of 10 for a condition index (Grant and Tyler, 1983a). Oysters were randomly assigned groups based on the result of a coin flip.

Histological Analysis

The visceral mass was excised from shucked oysters and placed in Davidson's fixative (2 parts formalin, 3 parts 95% ethanol, 1 part glacial acetic acid, 3 parts RO water) for a period of 24-48 hours. Tissues were transferred to 70% ethanol for storage. After at least 24 hours of storage, tissues were removed from solution and a razor blade was used to excise a ~7mm cross-section of the visceral mass, which included gonad, digestive gland and gill tissue. These sections were transferred to 95% ethanol for ~12 hours. Tissues in 95% ethanol were put through 3 100% ethanol changes for ~2 hours per change to ensure complete dehydration. Tissues were then left to clear in toluene for ~24 hours followed by a transfer into molten paraffin wax for at least 24 hours to complete infiltration. Infiltrated specimens were embedded in paraffin blocks with three specimens to a block. Using a rotary microtome, 7 micrometer ribbons of gonad tissue were sectioned and placed in a warm water bath for stretching. Sections were mounted on slides and left to adhere on a slide warmer for several hours. Two slides per three specimen block were prepared, with at least three sections per mounted ribbon. Using the acidophilic nuclear stain, hematoxylin, and the basophilic cytoplasmic stain, eosin, tissues were dyed to enable visualization of gonad tissues (Coe, 1931a; Galigher and

Kozloff, 1971; da Silva et al., 2009). Slides were mounted using Permount mounting medium and glass cover slips.

Sections were observed under an Olympus BX50 compound light microscope at 40x magnification. Male and female gametes are sometime unevenly distributed throughout the gonad in this species (Coe, 1932; Jim Moore, personal communication), so all tissue sections on both slides were considered during the analysis to minimize the potential for confounded results.

Sex Category

To accommodate hermaphroditism in this species, a sex category scheme was adopted from gametogenic studies of *Ostrea edulis* by da Silva et al. (2009) (Figure 2). The following scale of six sex categories was used:

- Indeterminate (I): Follicles are either collapsed or empty. No residual gamete material can be found in follicles
- Male solely (M): Follicles contain only male gonad material.
- Female solely (F): Follicles contain only female gonad material.
- Hermaphrodite with both sexes equally represented (HBS): Follicles contain approximately half male and half female gonad material.
- Hermaphrodite predominantly male (HPM): Follicles contain predominantly male but also some female gonad material.
- Hermaphrodite predominantly female (HPF): Follicles contain predominantly female but also some male gonad material.

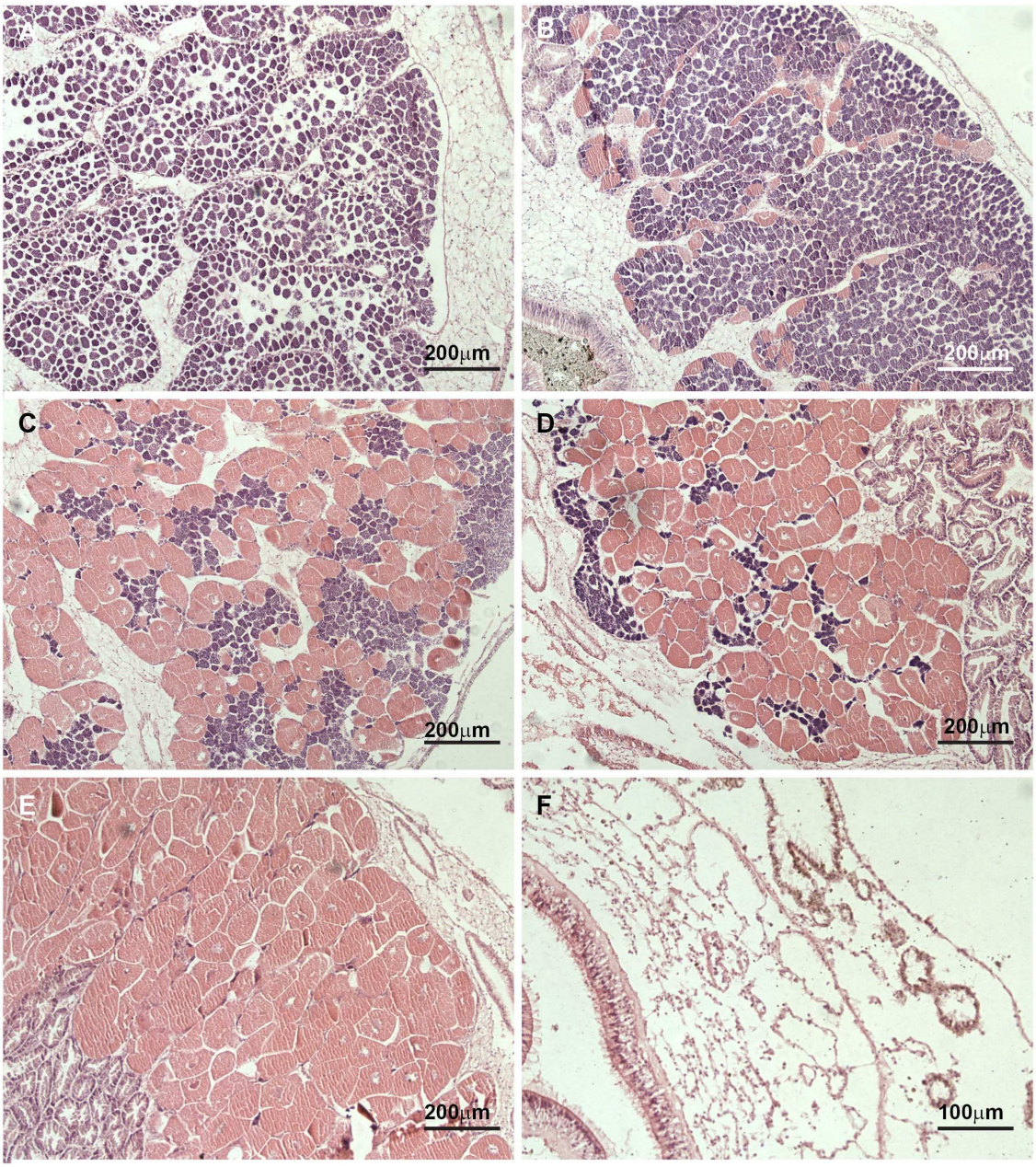

Fig. 2. Micrographs of histological sections of *Ostrea lurida*, showing all six sex categories and various maturity stages. A. Male, ripe gonad. B. Hermaphrodite predominately male, ripe male gonad, partially spawned female gonad. C. Hermaphrodite with both sexes represented equally, ripe male gonad, advanced gametogenesis in female gonad. D. Hermaphrodite predominately female, partially spawned male gonad, advanced gametogenesis in female gonad. E. Female, ripe gonad. F. Indeterminate, no gonad activity.

Maturity Stage

Each individual was assigned a maturity stage number corresponding to its level of gonad development (Figures 3 and 4). This scheme resembles similar constructs used for *O. edulis* (Mann, 1979; da Silva et al, 2009) and *O. stentina* (El Gharsalli and Aloui-Bejaoui, 2011). Hermaphroditic individuals were given two scores, one to delineate male stage and one for female stage:

- Inactive Gonad (Stage 0): No evidence of gamete development. Specimen is either immature, experiencing a resting stage between spawning cycles, or undergoing reproductive failure. This maturity stage is exclusively reserved for the Indeterminate (I) sex category.
- Early Gametogenesis (Stage 1): Notable expansion of gonad follicles. In males, male gamete proliferation is observed. Spermatogonia and spermatocytes observed in abundance, with some spermatids present. In females, the gonad is composed of primarily oogonia with some small oocytes (diameter ~15-30μm).
- Advanced Gametogenesis (Stage 2): Further expansion of gonad follicles. In males, all cell stages in the spermatic series are present. The majority of the follicle is filled with the developing germ line. In females, developing oocytes line the follicle wall with some occupying the follicle lumen (diameter ~30-80μm).
- Ripe Gonad (Stage 3): Follicles have expanded to fill most of the space between the digestive gland and mantle epithelium. In males, the majority of the follicle is devoted to mature sperm balls. In females, ripe oocytes (diameter ~90-110μm (Loosanoff, 1963)) line the follicle walls and fill the follicle lumen.

- Partially Spawned (Stage 4): Follicles are partially empty and have been reduced in size with large amounts of residual mature gametes still present. In males, large amounts of spermatozoa remain while the rest of the spermatic series is absent. In females, large quantities of post-vitellogenic oocytes remain, sometimes alongside residual pre-vitellogenic oocytes and the developing male line of the subsequent sexual phase. Phagocytes may be present at this stage.
- Resorbing (Stage 5): Follicles are greatly reduced in size from the previous stage, with only small amounts of mature gametes remaining. Individuals expressing this stage for one sexual phase may also contain gametes of the subsequent sex phase. Spermatogonia or oogonia may be present in the acinus. Presence of phagocytes is ubiquitous.

Oocyte Diameter

Sections containing female gonad material were photographed under an Olympus BX50 compound microscope at 40x magnification using a Canon PC1192 digital camera. Photographs of two complete gonad sections (one from each slide, randomly selected) were taken for each individual. For each oyster, a total of 100 oocytes, each with apparent nuclei and nucleoli, were measured for diameter (Grant and Tyler, 1983b) using ImageJ image processing software. Average egg sizes within individuals contributed to the determination of the female maturity stages outlined in the previous section.

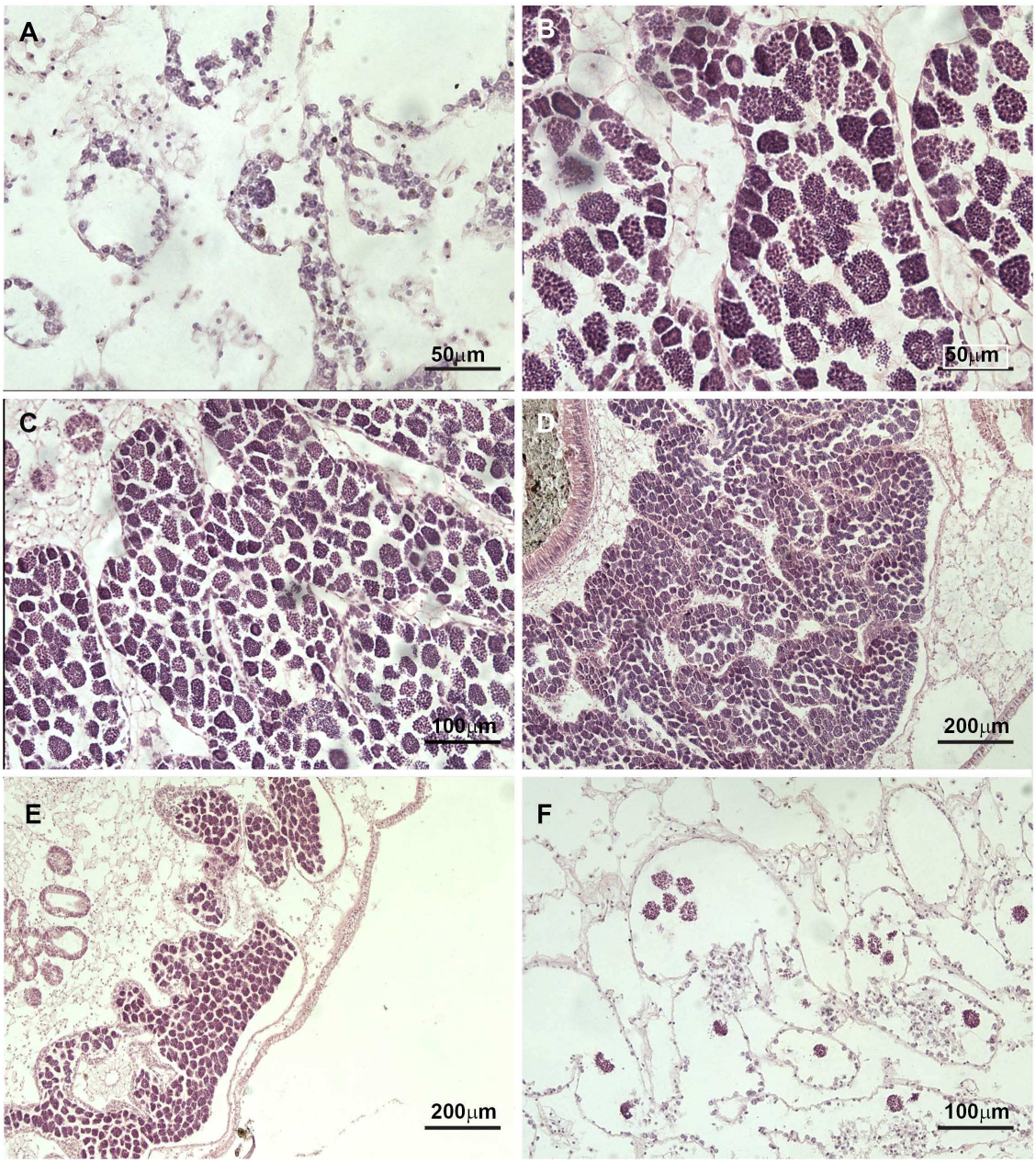

Fig. 3. Micrographs of histological sections of *Ostrea lurida*, showing all five male maturity stages. A. Early gametogenesis. B. Advanced gametogenesis. C. Advance gametogenesis from lower magnification. D. Ripe gonad. E. Partially spawned. F. Resorbing.

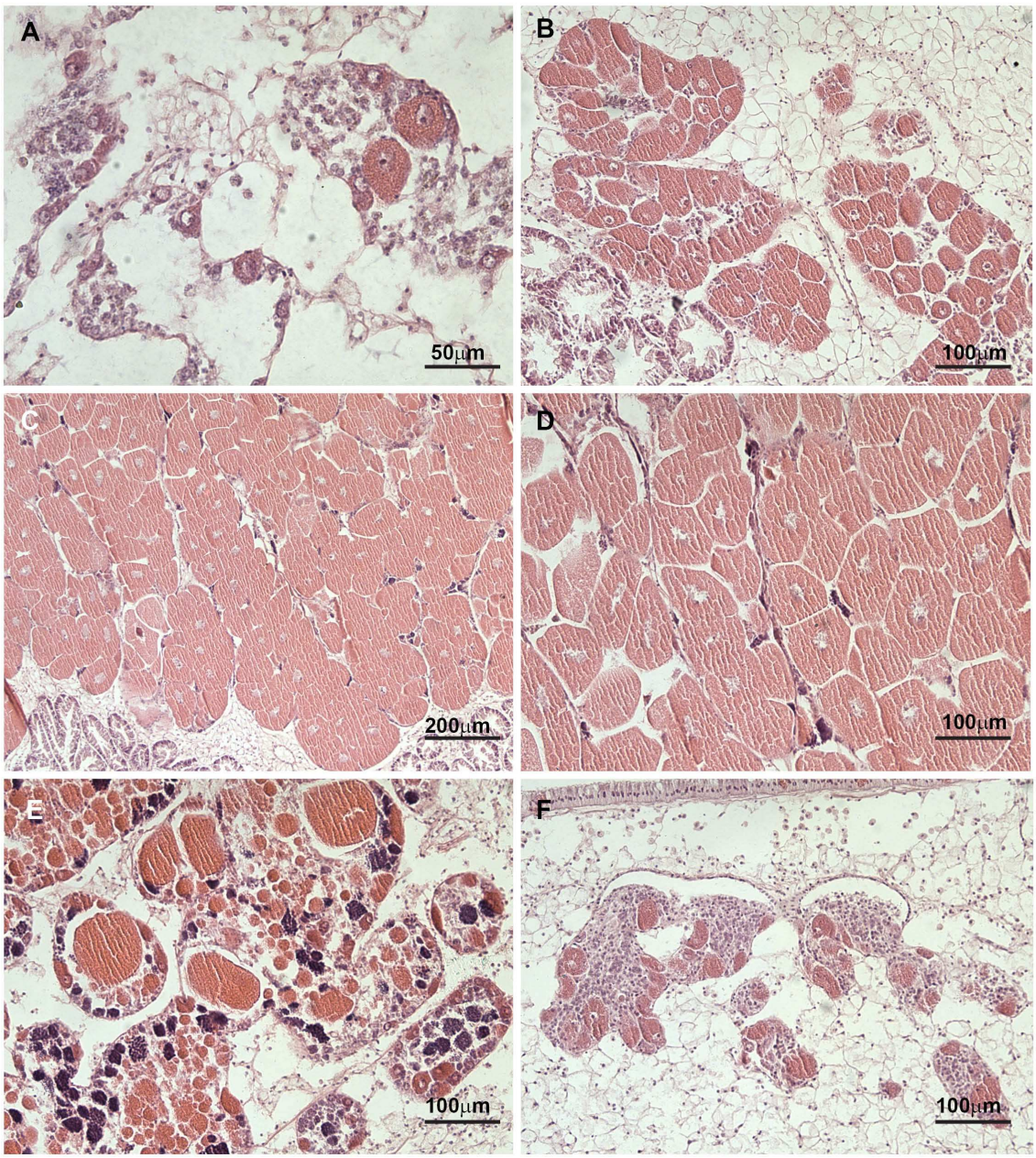

Fig. 4. Micrographs of histological sections of *Ostrea lurida*, showing all five female maturity stages. A. Early gametogenesis. B. Advanced gametogenesis. C. ripe gonad. D. Ripe gonad from lower magnification. E. Partially spawned. F. Resorbing.

Condition Index

A condition index was employed to provide a rough estimate of monthly gametogenesis and spawning activities of *O. lurida* populations (Gibson, 1974). Oysters assigned to condition index analysis were left for 24 hours in running seawater tables to provide an opportunity for feces and pseudofeces to be eliminated (Hawkins and Rowell, 1987). Individuals were thoroughly scrubbed of epibionts, and then kept in seawater until processing to ensure that the shell cavity remained full of fluid before measurement. Whole weights (g) of each oyster were taken after each individual was patted dry with a Kim-wipe. Following storage in a -20^{o}C freezer, the visceral mass of each oyster was excised and dried for 24 hours in a 100^{o}C oven. Dried tissues were then weighed to the nearest milligram. Shells from freshly shucked oysters were patted dry with a Kim-Wipe and weighed (g).

This study employed the use of the condition index proposed by Lawrence and Scott (1982) and later modified by Hawkins and Rowell (1987), wherein dry soft tissue weight (g) is held as a function of internal shell cavity capacity (g) (Equation 1). This method has been popular within studies of many Ostreid genera (Crosby and Gale, 1990; Cano, 1997; Abbe and Albright, 2003).

Eq. 1. Condition index formula proposed by Lawrence and Scott (1982)

$$CI = \frac{\text{dry soft tissue wt (g)} \times 1000}{\text{internal shell cavity capacity (g)}}$$

Statistical Analysis

An ANOVA test was used to determine if the mean monthly condition index values for the 12 month period are significantly different. ANCOVA was run with the dry flesh weight as the dependent variable, shell length as the dependent variable, and sample month as the factor. This analysis allowed for the tracking of increases in flesh weight, while simultaneously compensating for the effect shell length and allometric growth. Tukey HSD pairwise comparisons were run to discern significant differences in condition between months. Pearson correlation coefficients were calculated between each environmental parameter and the monthly condition index measurements. Multiple regression analysis was also used to determine the combined predictive capability of temperature, salinity, and chlorophyll-a on condition index. A Kruskal Wallis test was used to determine if mean oocyte sizes were significantly different across the 12 month period. Steel-Dwass pairwise comparisons were employed to determine significant increases or decreases in diameters between months. Wilcoxon tests with bonferroni adjusted p-values compared sample sites for each given sample date. Contingency table analysis was conducted to determine significant differences between maturity stage between sample sites as well as sex category between sample sites over the course of the 2012 sampling period.

RESULTS

Ambient Environmental Parameters

Temperature fluctuated seasonally in Haynes Inlet and Coalbank Slough (Figure 5). The highest temperatures in Haynes Inlet were recorded in June (18.66^{o} C) and the lowest in March (8.38^{o} C). Coalbank Slough's highest temperature was recorded in July

(19.67^{o} C) while its lowest temperature was detected in January (7.15^{o} C). During 2012, temperature differences between sites were typically <1^{o} C for any given sampling date. However, between July and September, water temperatures at Coalbank Slough were consistently 1.5-2^{o} C above those of Haynes Inlet. This result is not surprising, as the upper estuary is less subject to cold oceanic water influxes than mid estuary sites (Gibson, 1974; Kimbro et al., 2009).

Salinity demonstrated a seasonal sinusoidal pattern, with low spring values slowly transitioning to high autumn values (Figure 5) at both sites. In Haynes Inlet, salinity ranged from 30.79 in September, to as low as 12.5 in December. For Coalbank Slough, salinities failed to reach the highs of Haynes Inlet, but demonstrated a September peak of 28.45, while its lowest salinity value of 4.5 occurred during May and December. Haynes Inlet recorded consistently higher salinity values than Coalbank Slough. Differences in monthly salinity values ranged from 2.3 to 12.3 between sites.

Due to equipment malfunction, Chlorophyll-a values were not collected for January through March of 2012. Chlorophyll-a values rose steadily during the summer months at both sites (Haynes: 8.615 μg/L in August; Coalbank: 9.59 in June) and showed marked decline during the winter season (Haynes: 2.283 μg/L in December; Coalbank: 0.966 μg/L in December) (Figure 5). Chlorophyll exhibited large fluctuations during summer.

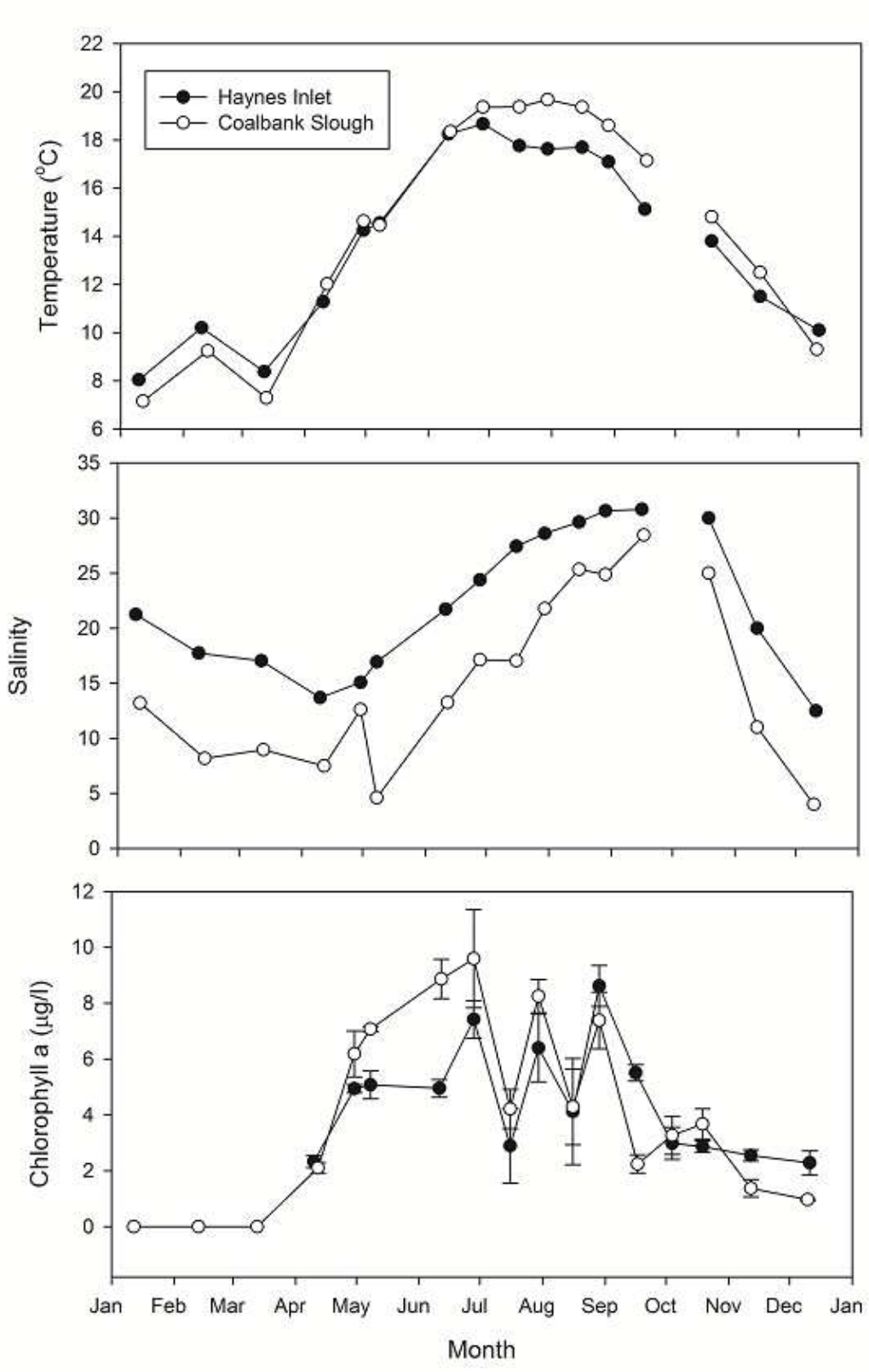

Fig. 5. Recorded Temperature, Salinity and Chlorophyll-a values (with SE bars) for Haynes Inlet and Coalbank Slough.

Seasonal Shifts in Sex Categories

Table 1 and Figure 6 show the percentages and distributions of sex category observations in 2012, combining results from both Coalbank Slough and Haynes Inlet. During the period between January and May, female (F) and predominately female hermaphrodites (HPF) far outweighed all other categories detected, ranging from 43-58% and 18-30% respectively. Combined, males (M) and predominately male hermaphrodites (HPM) comprised an average of 10% of each month's sample for this period. The male to female ratio began to reverse in June, with increasing proportions of male- dominated gonads. Males and predominately male gonads peaked in abundance in August, constituting a combined 73% of the sample. During the months of July and August, F individuals disappeared entirely. Male (M) and HPM proportions fell steadily into the winter months, restoring the male to female ratios observed during January through May. Hermaphrodites with both sexes represented equally (HBS) accounted for no more than ten percent of each months sample with the exception of June at 16%. Shell size was not a significant predictor of sex category was not significant (Kruskal Wallis; $\chi^2 = 2.7370$; df = 5; p = 0.7405), indicating that sex was not determined by oyster size. Contingency table analysis revealed significant differences in sex category ratios between the two sites ($\chi^2 = 23.996$; df = 5; p = 0.0002), most notably in the number of indeterminate (I) individuals. During the 2012 sampling period, there was a marked difference in the number of indeterminate individuals. With the exception of April, Coalbank Slough samples contained at least one individual with empty follicles with no gonad material (Figure 8). Indeterminate individuals peaked in abundance between May and September with as many as 5 individuals (20% of monthly sample) detected in June

and August, coinciding with water temperatures above 15^{o} C. In contrast, Haynes Inlet contained only sporadic and isolated indeterminate individuals and no occurrences during the peak observed in Coalbank Slough (Figure 8).

Table 1. Distribution (%) of sex categories across samples sites during the 2012 sampling period.

Sex Category	Haynes Inlet	Coalbank Slough
Indeterminate	1.1	7.2
Male	12.8	14.7
Hermaphrodite Predominately Male	24.4	15.6
Hermaphrodite Both Sexes Equally Represented	7.2	8.1
Hermaphrodite Predominately Female	25.3	24.4
Female	29.2	30

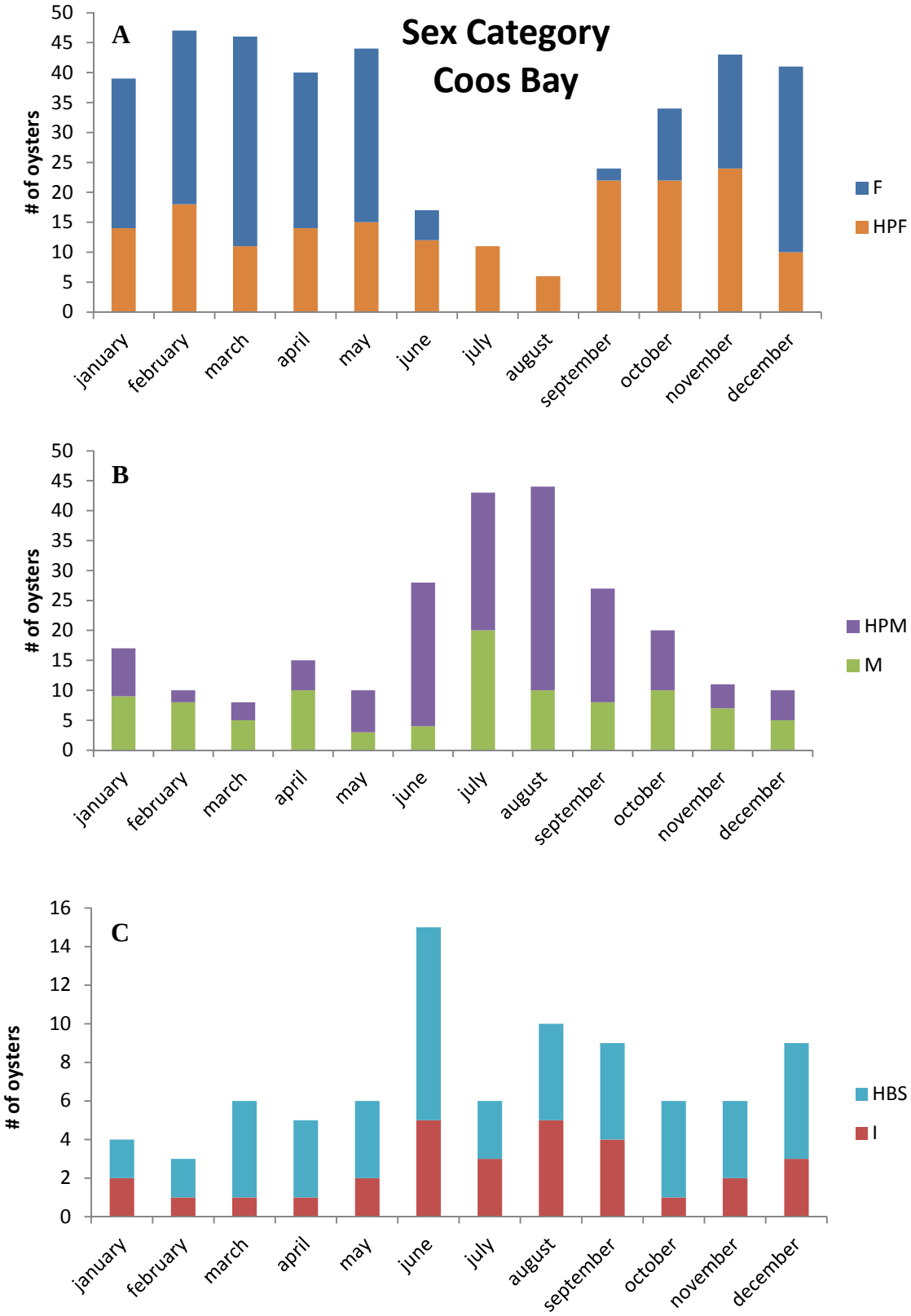

Fig. 6. Monthly abundances of all six sex categories from both Haynes Inlet and Coalbank Slough during the 2012 sampling period. N = 60.

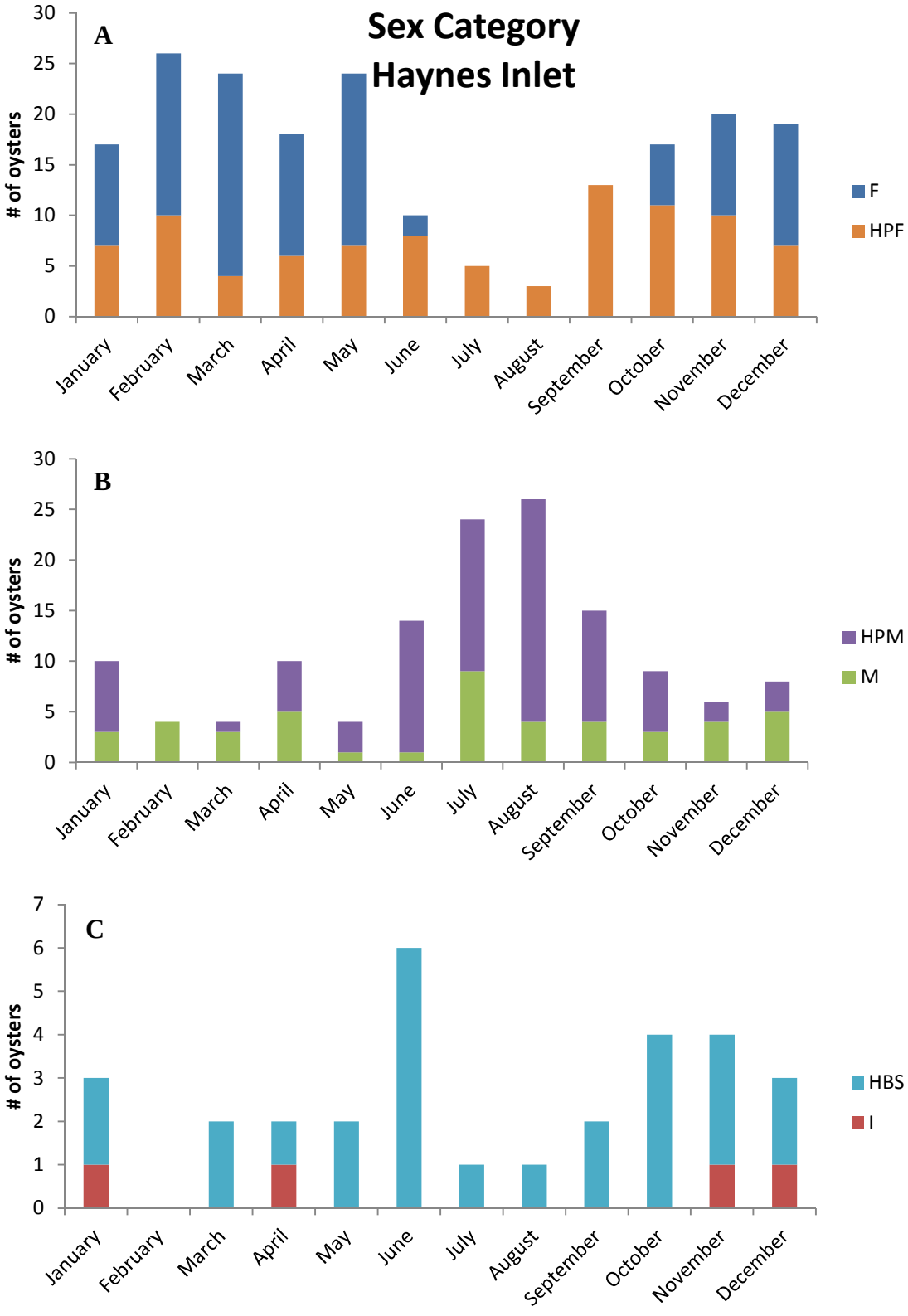

Fig. 7. Monthly abundances of all six sex categories from Haynes Inlet during the 2012 sampling period. N = 30.

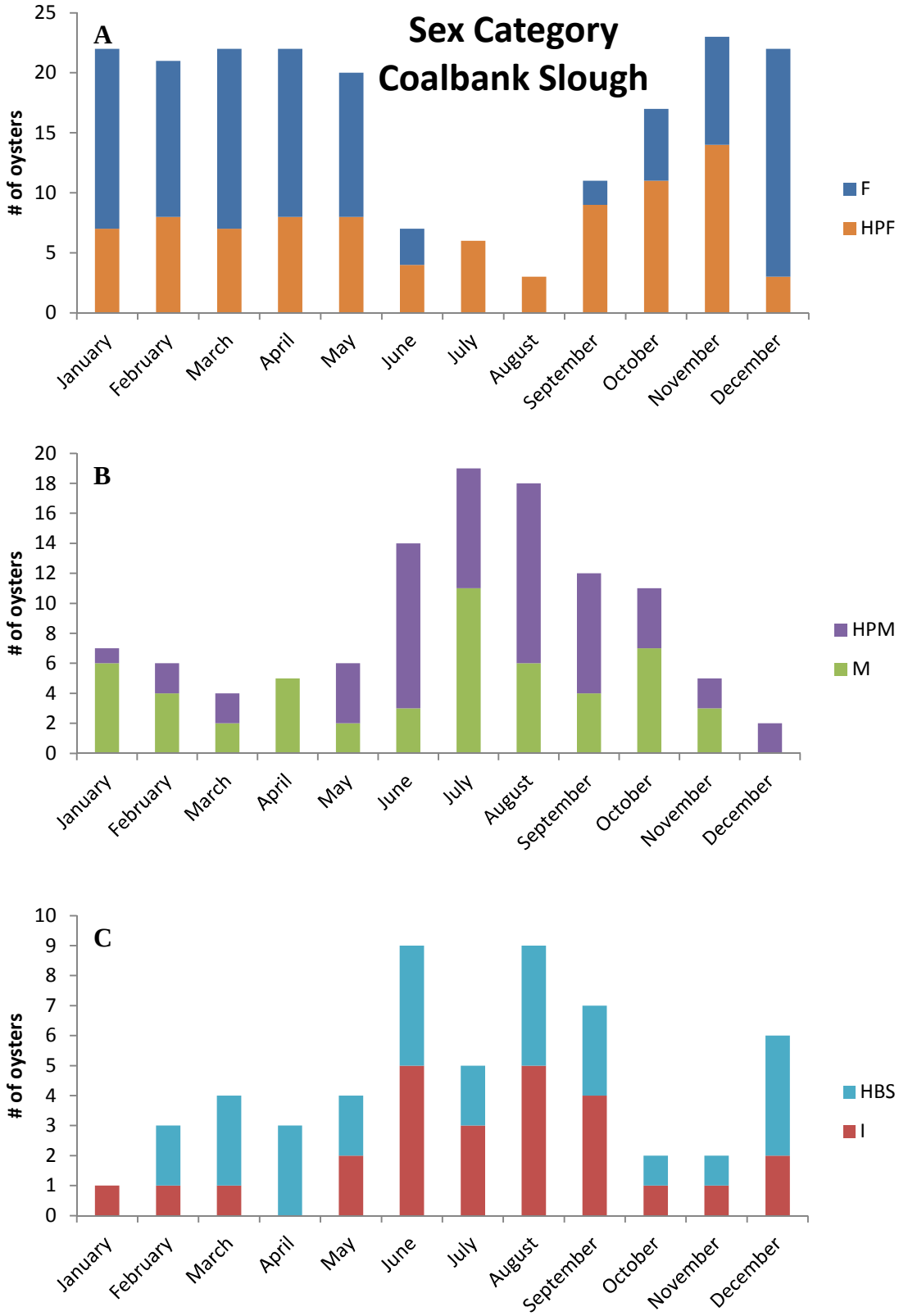

Fig. 8. Monthly abundances of all six sex categories from Coalbank Slough during the 2012 sampling period. N = 30.

Maturity Stage

Male and Female oysters both demonstrated a unimodal summer peak in advanced gametogenesis (Stage 2) and ripeness (Stage 3) during the summer (Figures 9 and 12), when water temperatures exceeded 15^{o} C. Below this temperature, gametogenesis ceased with partially spawned (Stage 4) and resorbing (Stage 5) gonads dominating. During the winter, it was common to find follicles containing large quantities of unspawned, residual gametes from the previous reproductive season.

Male Phase

Cumulatively, male oysters analyzed during 2012 showed predominately Stage 4 or Stage 5 gonads from January to April, with intermittent, isolated detections of early gametogenesis (Stage 1) (Figure 9). During the winter months, in the absence of gametogenic activity, residual spermatozoa persisted in the follicle lumen and gonoducts of many oysters. Stage 1 males began to appear in larger numbers when water temperatures exceeded 15^{o} C in May, peaking in abundance in June (25% of males sampled). Stage 2 males also reached their greatest abundance in June (37% males sampled), followed by a peak in ripe Stage 3 individuals in July (38% of males sampled). Stage 1 through Stage 3 individuals gradually declined until October, when they cumulatively constituted less than 8% of males sampled, disappearing entirely in December. Shell length showed no significant relationship with male phase (Kruskal Wallis; $\chi^2 = 5.8269$; df = 4; p = 0.2125).

Contingency table analysis revealed significant differences in male phase presence between the two sites ($\chi^2 = 16.495$; df = 4; p = 0.0024). Stage 5 oysters made up nearly 50% of the sample in Coalbank Slough (Figure 11), far outweighing the totals

from Haynes Inlet. A greater proportion of Stage 3 and Stage 2 individuals were observed in Haynes Inlet, although fewer Stage 1 individuals were also observed at this site (Figure 10). It is also interesting that the timing of stage appearances was noticeably different between sites. While Stage 1 males appeared in Haynes Inlet in high numbers in May, Coalbank Slough exhibited a one month delay, as it did not exhibit Stage 1 males until the month of June.

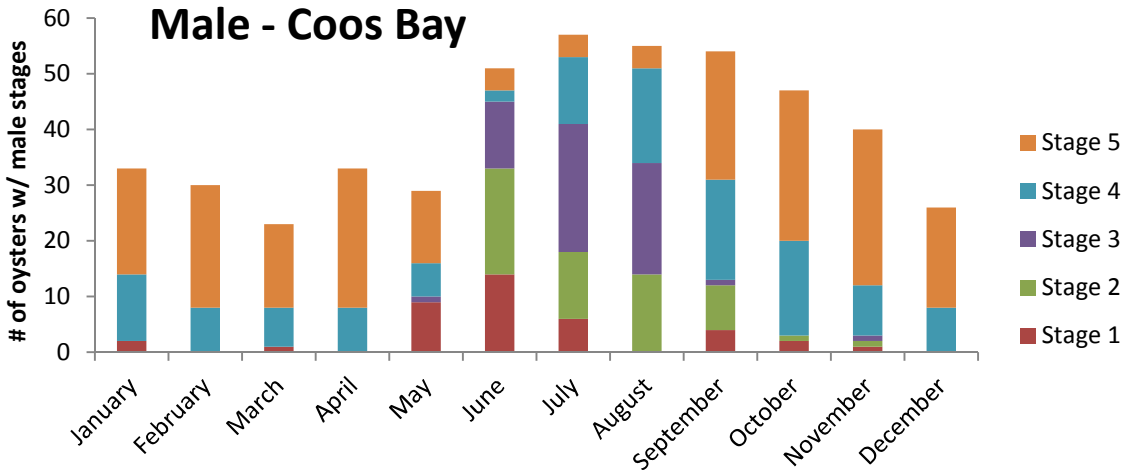

Fig. 9. Monthly abundances of five male maturity stages (excluding Stage 0) from Haynes Inlet and Coalbank Slough during the 2012 sampling period. N = 60

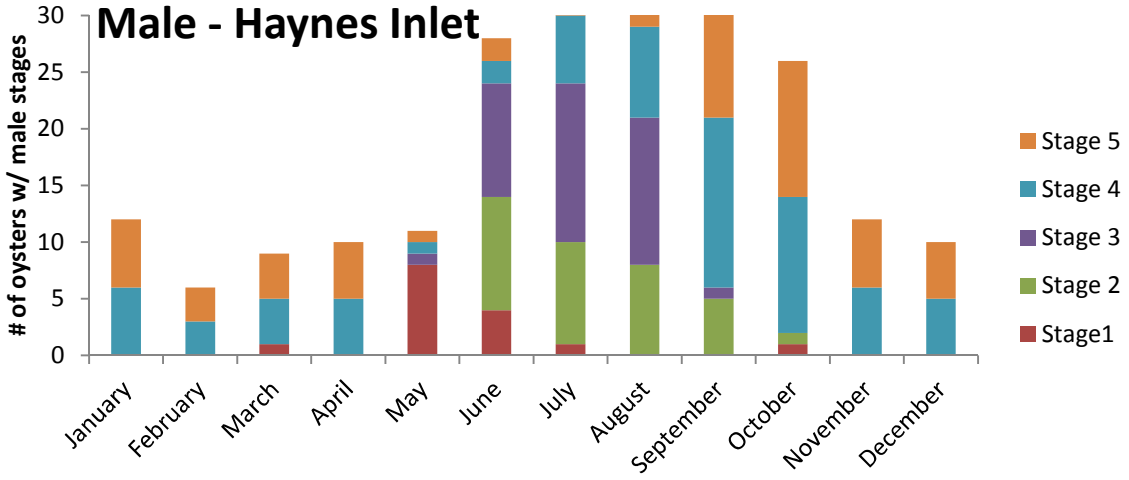

Fig. 10. Monthly abundances of five male maturity stages (excluding Stage 0) from Haynes Inlet during the 2012 sampling period. N= 30

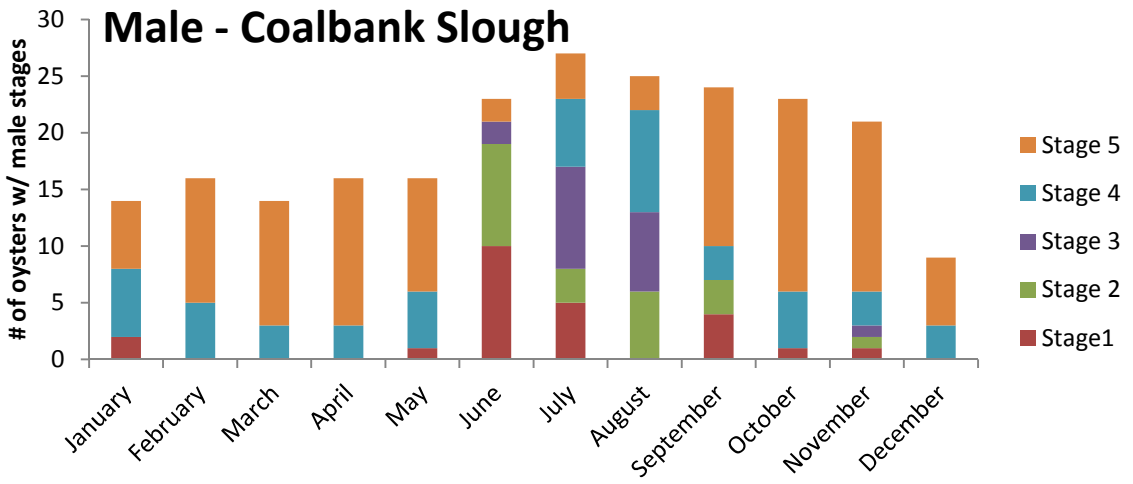

Fig. 11. Monthly abundances of five male maturity stages (excluding Stage 0) from Coalbank Slough during the 2012 sampling period.

Female Phase

Cumulatively, in Coos Bay, low levels of Stage 1 or Stage 2 individuals persisted throughout the year, although egg size and follicle volume were not large enough to signal substantial gametogenic activity (Figure 12). May had the highest number of Stage 1 females (40% of May sample and 44% of females sampled), with developing oogonia and uniformly small oocytes scattered throughout the follicles. This spike was followed by a peak in Stage 2 individuals during the month of June (45% of females and 38% of sample). In July, the highest number of ripe individuals was detected, constituting 13% of the females sampled. As with the male gametes, substantial residual gamete carryover was observed outside of the breeding season. Scattered oocytes of varying sizes remained attached to the shrinking follicle walls of spawned individuals (Figure 4E). Shell length did not have a significant relationship with female maturity stage (Kruskal Wallis; χ^2 = 1.7626; df = 4; p = 0.7793).

Contingency table analysis revealed no significant difference between prevalence of female maturity stages between sites (χ^2 = 4.388; df = 4; p = 0.3560). As with the male

Maturity Stages, female gametogenesis appeared to experience a delay in Coalbank Slough. Ripe females could be found from June to September in Haynes Inlet (Figure 13), while ripe individuals in Coalbank Slough were limited to the months of July and August (Figure 14).

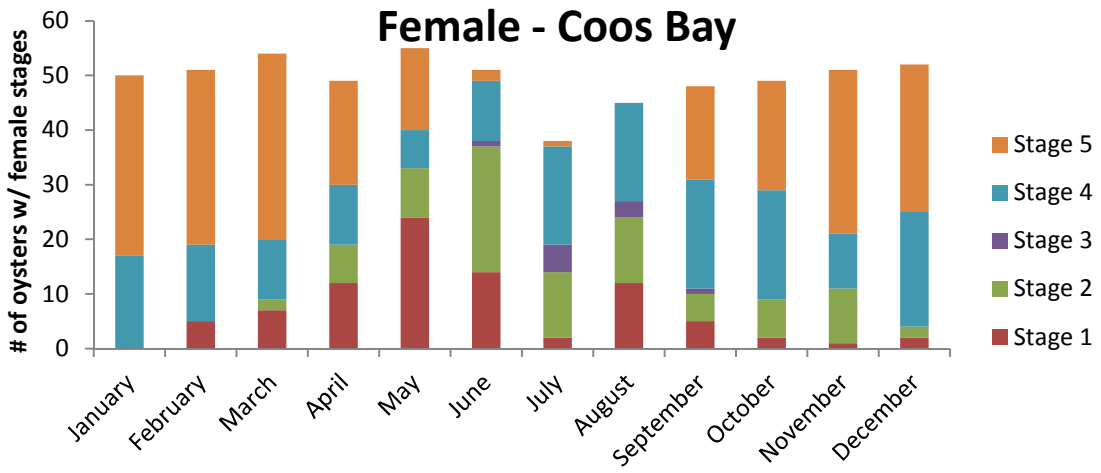

Fig. 12. Monthly abundances of five female maturity stages (excluding Stage 0) from Haynes Inlet and Coalbank Slough during the 2012 sampling period. N = 60

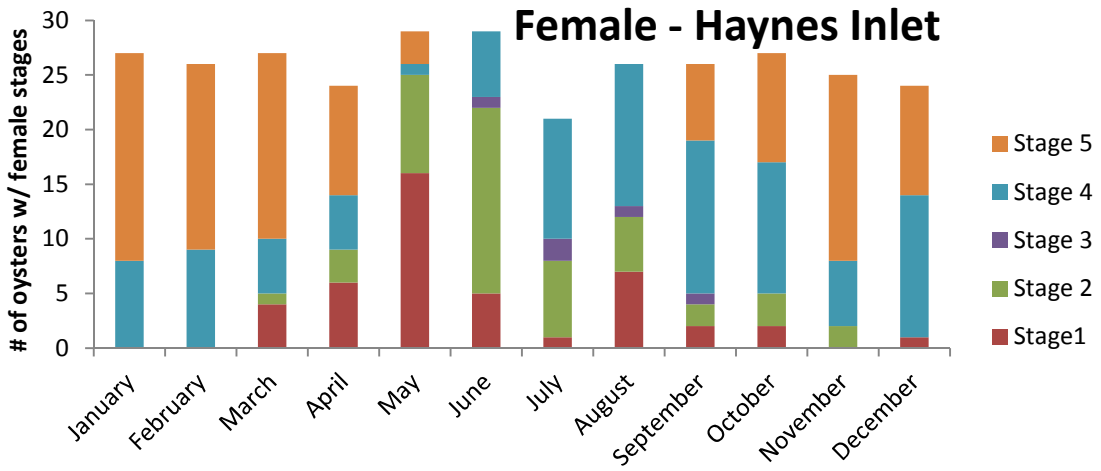

Fig. 13. Monthly abundances of five female maturity stages (excluding Stage 0) from Haynes Inlet during the 2012 sampling period. N =30

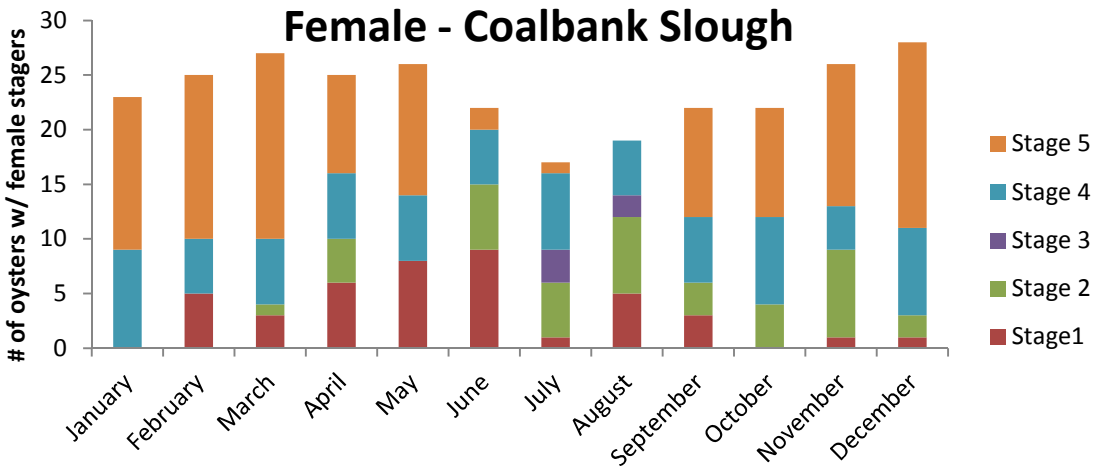

Fig. 14. Monthly abundances of five female maturity stages (excluding Stage 0) from Coalbank Slough during the 2012 sampling period. N = 30

Oocyte Diameter

Figure 15 illustrates the monthly variation in oocyte diameter for both Haynes Inlet and Coalbank Slough. Both sites exhibited relatively low values during the winter months and large increases in egg diameter during periods of elevated water temperature, chlorophyll-a, and salinity.

In Haynes Inlet, egg diameters varied significantly over the seasons (Kruskal Wallis; $\chi^2 = 34.8728$; df = 11; p = 0.0003; Figure 15). From January through May, egg sizes declined gradually and consistently. From May to June a significant increase in egg diameter was detected (Steel-Dwass pairwise comparison; z = 3.84271; p = 0.0068), followed by another gradual decline and leveling out from September through December. In Coalbank Slough, egg diameter varied significantly over the 2012 sampling period (Kruskal Wallis; $\chi^2 = 64.2224$; df = 11; p < 0.0001; Figure 15). From January to June, average oocyte diameter gradually declined, with the lowest mean value observed in May (Average = 25 μm SD= 6.06). Pairwise comparisons reveal that between June and July, a significant spike in egg diameter occurred (Steel-Dwass; z = 3.84271; p = 0.0004)

followed by a significant drop from July to September (z = -4.1864; p = 0.0017) and then a significant increase from September to October (z = 3.53428; p = 0.0210).

Month by month comparisons of egg diameter between sites showed that Coalbank slough had significantly lower egg diameters than Haynes Inlet during the month of June (Wilcoxon; χ^2 = 14.3759; df = 1; p < 0.0001).

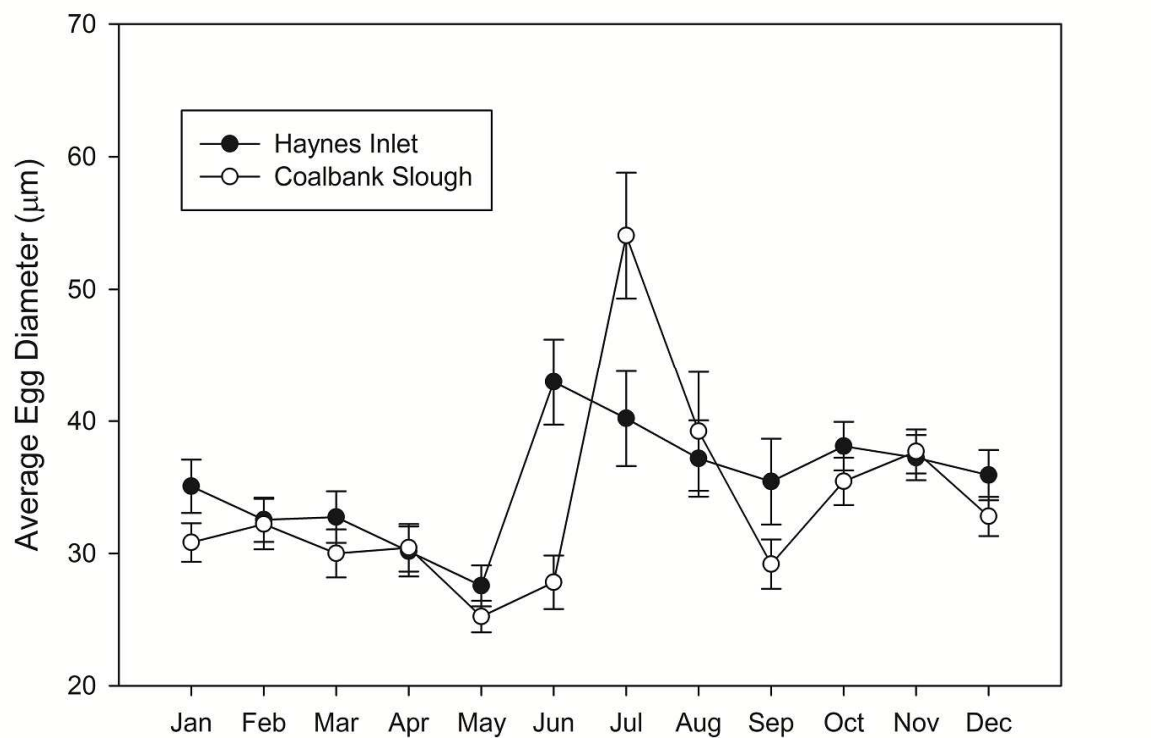

Fig. 15. Mean monthly changes in oocyte diameters with SE bars (N = 100 oocytes per month)

Condition Index

In Haynes Inlet, condition index varied significantly over the 12 month period (ANCOVA; F = 2.5791; p = 0.0058; Figure 16). Condition index demonstrated a significant negative relationship with shell length and was included as a covariate in the analysis. Shell length did not significantly differ between months at Haynes Inlet (ANOVA; F = 1.4054; p = 0.1809). From January through March, monthly index vales averaged 7.62 (SD = 1.38), demonstrating an upward trend. A decline in oyster condition

indices occurred between the months of March and May followed by a steady rise in the index values leading to a significant increase between the months of May and July (Tukey HSD; p = .0365). Average index values were highest during the months of July and August. A steady and significant decline in values followed the August peak into November (Tukey HSD; p = 0.0324).

In Coalbank Slough, oyster condition also varied significantly over the 2012 sampling period (ANCOVA; F = 2.5791; p = 0.0048). Shell length again demonstrated a significant negative correlation to condition index values and was factored into the analysis. Shell length did not significantly differ between months at Coalbank Slough (ANOVA; F = 1.7265 p = 0.077). From March to June, a significant decrease in values was detected (Tukey HSD; p = .0310). A significant increase in values was detected between June and September (Tukey HSD; p = .0366), followed by a gradual decrease in values through December. Coalbank Slough demonstrated consistently lower average condition index values when compared to Haynes Inlet, and the values in Haynes Inlet were 9% to 57% higher for any given month sampled. There appears to be striking similarity in the patterns of mean index variation for these two sites. While these patterns were in close alignment for the months from January through May, Coalbank Slough falls behind Haynes Inlet in June, experiencing a prolonged phase of low condition. Despite the one month delay, patterns in index variation for Coalbank Slough continue to follow those set by Haynes Inlet.

Monthly temperature values showed a significant positive linear correlation with Haynes Inlet condition values (R = 0.037087; n= 119; p = 0.0359), however demonstrated a significant negative linear relationship with Coalbank Slough condition

values (R = .034832; n = 120; p = 0.0412). Salinity values demonstrated a significant positive relationship with condition index in Haynes Inlet (R = 0.120361; n = 119; p < 0.0001), but not in Coalbank Slough. Chlorophyll-a measurements showed a significant inverse relationship with condition index in Coalbank slough (R = .048168; n = 90 p = 0.0377), but this relationship was not present in Haynes Inlet. Using data from April through December, multiple regression analysis identified temperature, salinity and chlorophyll-a as significant predictors of condition index at Haynes Inlet (R = 24.1175; n = 90; p<0.0001), Temperature and chlorophyll-a identified as significant predictors of condition at Coalbank Slough (R = 0.116999; n = 90; p = 0.0045).

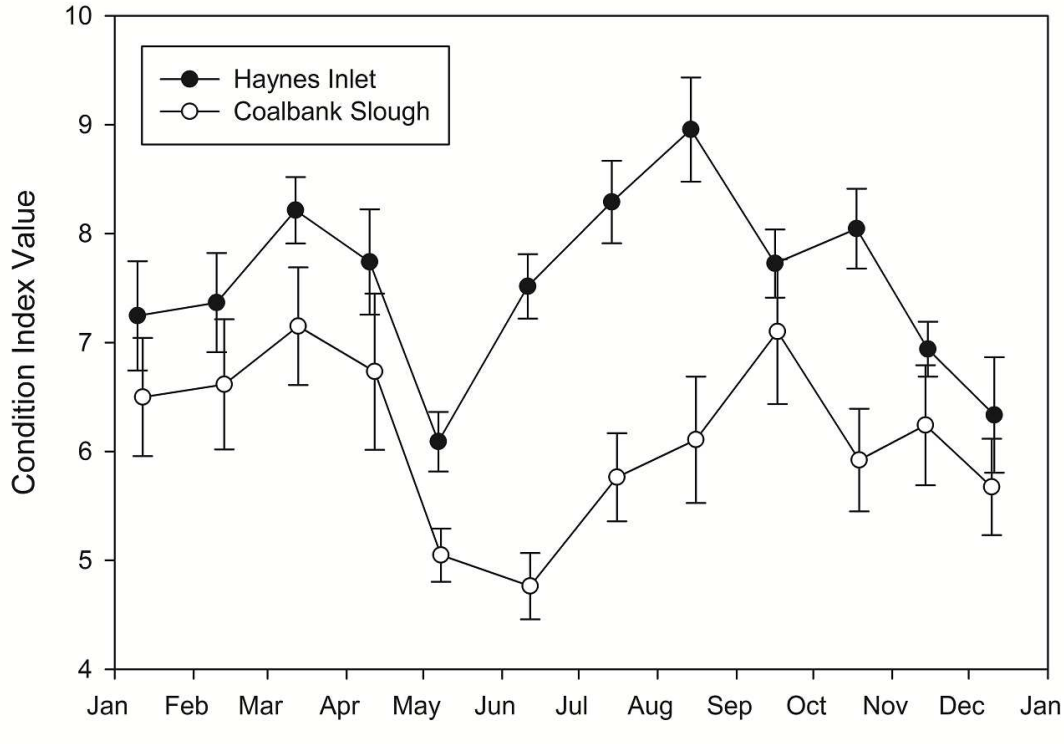

Fig. 16. Mean condition index values with SE bars for Haynes Inlet and Coalbank Slough.

Brooding of Embryos and Larvae

Brooding oysters were found in Haynes Inlet in July, August and September. Larvae observed in July had reached the 4-cell stage of development indicating that fertilization had occurred within the previous 24 hours (Hori, 1933; Strathmann, 1987). Brooders in subsequent months contained shelled veliger stage larvae with rounded shell hinges and no pigmentation, otherwise known as “white sick” (Hopkins, 1937). This stage of development is typically reached after 4-5 days of development (Hori, 1933; Strathmann, 1987)

In Coalbank Slough, brooding oysters were detected in July and September. Embryos found in July had reached the blastula/gastrula stage of development indicating fertilization within the prior 24-48 hours (Hori, 1933; Strathmann, 1987). Brooders in subsequent months held shelled veligers also in “white sick” stage.

Estimated brood counts measured between 79,500 and 316,500 embryos and larvae, falling within the values reported previously in the literature for this species (Hopkins, 1937).

DISCUSSION

The summer peak in oyster reproduction is congruent with that reported in previous literature for reproductive patterns of other *Ostrea lurida* populations (Stafford, 1913; Coe, 1931a; Hopkins, 1937; Seale and Zacherl, 2009). The spawning season commenced in late June to early July and continued through mid to late September with a peak in July/August. This 3-4 month spawning period is similar to the observed interval that Hopkins (1937) described in Puget Sound, Washington.

Male maturity stages showed a distinct onset of gametogenesis. Large-scale gametogenesis initiated when seawater temperatures in Coos Bay reached ~14.5^{o} C. Seawater temperatures in the estuary jumped from ~14.5^{o} to ~18^{o} C from May to June, which corresponded to a marked increase in gametogenic activity. Critical spawning temperatures for *O. lurida* have been recorded between 12.5^{o} and 16^{o} C (Coe, 1931b; Hori, 1933 Hopkins, 1937; Imai et al. 1954; Santos et al., 1992) so it is conceivable, considering when ripe males were present, that oysters had begun to spawn shortly after the June sampling period. The diminished presence of developing and ripe males during the month of September suggests an end of the reproductive period. Female maturity stages were somewhat ambiguous when used to detect the onset of female gametogenesis because Stage 1 and Stage 2 individuals persisted through the winter months. It is unlikely that females were gametogenically active during the period since temperatures (7-12^{o} C) were below those previously cited for reproductive activity in this species (Coe, 1931b; Hori, 1933; Hopkins, 1937; Imai et al., 1954; Santos et al., 1992). In January through May, it was rare to find oocytes greater than 70 μm in diameter and no oocyte was found with a diameter greater than 80 μm, indicating an absence of ripe (Stage 3) females. Oysters identified as Stage 1 or Stage 2 were most likely female oysters that began gametogenesis shortly before temperatures dropped below the critical threshold, leaving their gametogenic progress in stasis through the winter season (Coe, 1932).

The appearance of ripe (Stage 3) female maturity stages is clarified when considered alongside the significant rises in observed oocyte diameters, in June for Haynes Inlet and in July for Coalbank Slough. These diameter increases coincided with the peak in advanced and ripe female maturity stages observed during those months. In

Coalbank Slough, the decrease from July to September is most likely attributable to the spawning of mature eggs during the reproductive season (Ren et al., 2003; Castanos et al., 2009; Kim et al., 2010; El Gharsalli and Aloui-Bejaoui, 2011); however, the significant rise into October suggests that female gametogenesis was beginning anew during that period.

The presence of brooding oysters suggests that reproduction begins in early to mid July and ceases toward early to mid September with temperatures ranging from 15 to 19^{o} C at both sites. High chlorophyll-a concentrations during the summer indicate that food was relatively plentiful during the reproductive period as well, which is consistent with studies linking food availability to reproductive output in oysters (Cano et al., 1997).

Hopkins (1937) observed a lunar periodicity in *Ostrea lurida* spawning cycles in populations occupying oyster dykes in Puget Sound, Washington. Oysters preferentially spawned shortly after neap tides during his study. Although I did not test for this periodicity, my observations of embryonic and larval development in brooding oysters lend support to his hypothesis. Embryos and larvae within and between sites were found at similar stages of development, an indication that they had been produced from one event or multiple events in a short period of time. Brooders observed in July were found at 4-cell and blastula stages of development, which Hori (1933) observed after 1 day of development. Larvae found in August and September were found at shelled veliger stages known as “white sick”, a larval stage occurring after 4-5 days of development (Hori, 1933). An examination of tide charts in 2012 (NOAA Tides and Currents) and the developmental stages of the larvae, suggests a spawning event 0-5 days after the neap tide prior to the collection date. While my sampling size for this inference is too low to be

statistically viable, the preliminary data indicate that this subject warrants further investigation.

In Olympia oysters, the rates and timing of gametogenesis are thought to be dependent on temperature, stored reserves, and food availability (Mann, 1979; Wilson and Simons, 1985; Shpigel, 1989; Santos et al., 1992). Indeed, observed spawning temperatures for this study were consistent with those previously established for *Ostrea lurida* with a threshold temperature of ~14.5^{o} C observed for spawning. Phytoplankton was also in its highest abundance during the summer, indicating that nutritional conditions were conducive to spawning activity.

Temperature and food abundance failed to explain the differences in gametogenic activity observed between sites. In May, while temperature and chlorophyll-a measurements were relatively equivalent (~14.5^{o} C), oysters in Haynes Inlet experienced high rates of early and advanced male/female gametogenesis (Stage 1 and Stage 2), Coalbank Slough oysters demonstrated repressed levels of gametogenesis, with few instances of Stage 1 individuals and no sign of Stage 2 males or females. June saw marked increases in the number of Stage 2 and Stage 3 oysters for this upper estuary location, however, the site failed to produce the same proportions of ripe females until July. Egg diameters did not increase significantly until July in contrast to oysters in Haynes Inlet, where they increased in June.

A likely contributing factor to these observations is the low salinity regime experienced by oysters in Coalbank Slough. Salinities below 15 have been found to be extremely stressful for this species, inducing 90% mortality in populations kept for six weeks at a salinity of 10 (Gibson, 1974). While no evidence of freshwater exposure was

observed during my study, sub-15 salinity measurements persisted throughout the winter and early spring (Figure 5). Ninety percent of *Crassostrea virginica*, in environments experiencing chronically low salinities and frequent freshwater exposure, underwent a two- month delay in gametogenesis when compared to oysters from more consistently saline estuarine environments (Butler, 1949). Advanced gametogenesis was not observed in oysters from Coalbank Slough until salinity was higher than 13, suggesting a threshold for gametogenic activity. Salinity is thought to impact gametogenesis indirectly. Hopkins (1936) found that oysters exposed to periods of low salinity close their valves or cease pumping water over their gills to prevent physiological damage. As a result, the ability to feed is greatly reduced, forcing individuals to rely on stored reserves to survive. This depressed feeding rate would also explain why oysters in Coalbank Slough consistently had lower condition index values than those in Haynes Inlet despite being exposed to equivalent, if not greater chlorophyll-a concentrations.

Salinity can also be offered as an explanation for other findings. Butler (1949) found that 33% of his oyster populations in low salinities were of indeterminate sexes, while he noted a distinct absence of indeterminate sex categories in high salinity environments. While only 7% of Coalbank Slough oysters were found to be indeterminate in my study, <1% of oysters from Haynes Inlet were observed in the same condition, suggesting that these sites confer differing levels of fitness to their oyster populations. Oysters may exhibit a reduced tolerance for low salinities at higher temperatures (Gibson, 1974), which may explain the peaks in indeterminate individuals for Coalbank Slough during the summer months.

Rises and declines in the condition index from May to December coincided with the presence and absence of ripe maturity stages and increasing and decreasing oocyte diameters, providing a providing a rough illustration of seasonal reproductive activity (Ren et al., 2003; Castanos et al., 2009; Kim et al., 2010; El Gharsalli and Aloui-Bejaoui, 2011). Relatively higher temperature and food availability may have contributed to the upward trend in oyster condition throughout the summer; however, the pronounced drop in condition experienced by oysters at both sites in May is harder to explain. Santos et al. (1992) showed that condition indexes decrease steadily in oysters transplanted to higher temperature environments from lower temperature ones. However, this decrease in condition was attributed to spawning activity, which was not detected until July in the present study. Phytoplankton concentrations appeared to be increasing at the time of the condition drop, which suggests that food limitation did not spur the loss of body mass. Because this event coincided with a 2-3^{o} C increase in water temperature and lower seasonal salinity at each site, it is possible that elevated metabolic activity from heightened body temperatures and increased physiological stress from lower salt concentrations spurred a rapid consumption of nutritional reserves. It should also be noted that the lowest average egg diameters were recorded during the period of very low condition index at each site and the beginning of the summer rise in condition index values coincided with the steep increase of egg diameter.

This study focused exclusively on intertidal oyster populations. While subtidal populations have been observed by SCUBA divers in the shipping channel of Isthmus Slough in Coos Bay (Baker 1995), they were not investigated in this study. There is some evidence to suggest that characteristics of reproduction in subtidal populations may differ

from those of intertidal populations. For example, intertidal and subtidal populations of *Crassostrea virginica* were compared to assess discrepancies in gametogenic activity (Brousseau, 1995). In that study, intertidal oysters exhibited only one reproductive period, whereas subtidal populations exhibited two periods, with the second being arrested and held over the winter. However, the timing of the onset of gametogenesis in both the subtidal and intertidal populations was similar. Spawning was delayed by one month for some intertidal populations and an extended spawning period was also observed.

Sawyer and Young (2011) observed *Ostrea lurida* settlement from September to December in Coos Bay. They noted a distinct peak in settler abundance in the two week period before October 5th. Accounting for an approximately 10 day brood period and 30 day planktonic larval duration (Hopkins, 1937), a peak in gametogenesis and spawning should be observed during mid to late August. Observations in 2012 of brooding individuals and large quantities of ripe males and females during August suggest a similar settlement pattern likely occurred in 2012 as it did in the 2010 season. The detection of ripe individuals and brooders in mid-July through mid-September 2012 also is also consistent with the two month window of increased settlement observed during the 2010 season from mid-September through mid-November.

Stafford (1913) cautioned that his studies of reproductive timing in *Ostrea lurida* should not be used to draw sweeping conclusions about the exact dates of spawning. Nearly one hundred years later, the same limitations can be applied to my observations in Coos Bay. With a limited dataset spanning only two and one reproductive season respectively, neither his study, nor my study could be used to reliably estimate the exact

timing of reproductive events. Periodic reproductive failure has been observed and documented in this species (Laura Peteiro, unpublished data) and the underlying causes are still unclear. However, what these studies do provide is a framework for estimating reproductive timing based on the environmental parameters needed for spawning. Measurements of temperature, salinity, and food availability can be used to better inform management strategies and restoration efforts.

Beck et al. (2011) noted that oyster reefs in the Pacific Northwest are either in poor condition (90-99% lost) or functionally extinct (>99% lost). Restoration efforts continue for this species in many west-coast estuaries (Peter-Contesse and Peabody, 2006; McGraw, 2009), including a major effort to restore Olympia oysters in Coos Bay (Groth and Rumrill, 2009). Optimal sites for depositing shell hash and other settlement substrata, to promote oyster colonization, are frequently sought by management professionals (Cohen and Zabin, 2009; Brumbaugh and Cohen, 2009; McGraw, 2009). This research suggests that not all estuarine locations are well suited for restoration efforts, even when large populations of oysters are present in those locations. To maximize output of young oysters, restoration efforts should target environments with seasonally high temperature and food regimes as well as consistently elevated salinities. Avoiding habitats that consistently fall below the physiologically tolerable thresholds for this species will maximize reproductive output and overall oyster health.

CHAPTER III

GENERAL DISCUSSION

The goal of my thesis was to elucidate temporal variation in patterns of Olympia oyster reproduction within the Coos Bay estuary. Annual sampling at two intertidal sites revealed that ripe males and females were present in June through September of 2012. Additionally, brooding oysters were also observed from July through September. The absence of brooding oysters in June does not provide definitive proof that spawning did not take place during that month, as detection rates of brooders during periods in which ripe gonads were observed was 0-10% per sample. These data suggest that intertidal oysters in Coos Bay have a spawning period of 3-4 months. Coe (1931a, 1932) observed a 7 month long spawning period off the coast of La Jolla, California with spawning commencing as early as April and continuing through November. My results are more similar to the observations of Hopkins (1937) who observed spawning periods of approximately 3-4 months in Puget Sound, Washington.

Coe (1931a) suggested that an average temperature of 16^{o} C serves as a critical threshold for *Ostrea lurida* spawning. Subsequent field and laboratory studies have suggested critical temperatures of 12-14^{o} C (Hori, 1933; Hopkins, 1937; Imai et al., 1954; Santos et al., 1992). Gametogenesis was detected in May when water temperatures rose to 14.5^{o} C and continued through September until water temperatures fell below this threshold. Oysters sampled from Coos Bay in 2012 did not demonstrate evidence of spawning until July, when water temperatures were greater than 17^{o}C. Curiously, water temperatures in June were just as high, if not higher than temperatures in July. In spite of the increased temperatures, oysters collected in June, while exhibiting ripe gonads, did

not demonstrate brooding. Temperatures in Coos Bay appear to have exceeded all reported spawning temperatures for at least one month before any spawning activity was recorded. While it is possible that spawning events in June were not detected in this study, my observations suggest that additional factors may play a role in reproductive timing. Seale and Zacherl (2009) did not observe the critical temperature of 16^{o} C put forth by Coe (1931a) in their investigations in estuaries in close proximity to Coe's study site. They suggested that other environmental variables, like salinity, in conjunction with temperature may contribute to reproductive timing. While my study measured temperature, salinity, and chlorophyll-a, these variables failed to explain much of the variability in reproductive activity. Many ambient environmental parameters, such as dissolved oxygen or pH, were not measured in my study and should be investigated in future reproductive investigations for this species.

Significant differences in the dry-meat condition index were observed between months within both sites. Oysters at both Haynes Inlet and Coalbank Slough experienced significant increases in meat condition from May to August and June to September, respectively. Values peaked during these times and significantly decreased during the winter months, signaling the end of the reproductive season. A significant drop in condition values occurred between March and May. It is unclear what caused such a dramatic decline at both sites outside of the spawning season.

Site comparisons in Coos Bay revealed considerable differences in reproductive activity. Oysters at the upper estuary site, Coalbank Slough, demonstrated a marked retardation in gametogenic timing when compared to oysters at the mid-estuarine site, Haynes Inlet. While a discernible rise in male gametogenesis took place in May at

Haynes Inlet, a similar rise was not recorded in Coalbank Slough until June. In addition, egg diameters increased significantly from May to June at Haynes Inlet, yet a comparable rise at Coalbank Slough was observed one month later. Examinations of condition indices taken from oysters reveal that dry meat increases associated with gametogenic activity occur in Coalbank Slough one month after increases in Haynes Inlet. While no previous observation of gametogenic delays have been reported for this species, Butler (1949) found that *Crassostrea virginica* exposed to prolonged periods of salinity below their physiological tolerances delayed gametogenesis by up to two months. Gibson (1974) demonstrated that salinities lower than 15 were physiologically stressful for Olympia oysters and extended exposure to these salinities caused massive die-offs. At the Coalbank Slough study site, salinities below 15 were observed for 8 months of the year, suggesting that the resident oyster population was under considerable stress. Interestingly, gametogenesis was detected in this population only when salinities above 13 were observed.

Preliminary data collected from brooding oysters suggest that spawning events are tied to lunar periodicity. Hopkins (1937) observed spawning events shortly after spring and neap tidal phases by assessing the developmental stage of brooding embryos/larvae and using that data to infer fertilization time. For each month's sample, within and between collection sites, brooding embryos were all of similar developmental stages, suggesting a synchronized spawning event shortly before observation. By assessing the developmental timing of embryos and larvae (Hori, 1933; Hopkins, 1937; Strathmann, 1987) and consulting tide charts (NOAA Tides and Currents), I found that spawning commenced 0-5 days after minimum neap tides.

Lack of hard substrata has been identified as the limiting factor for recruitment in several Oregon and Washington estuaries (Trimble et al., 2009, Groth and Rumrill, 2009; White et al. 2009b). Outplanting of *Crassostrea gigas* shells into areas previously devoid of hard substrata is a common practice for restoration projects (Cohen and Zabin, 2009; Brumbaugh and Cohen, 2009; McGraw, 2009) and serves to create new settlement habitat for native oysters. When choosing sites for restoration projects within Coos Bay, managers employing these strategies must take into account the environmental constraints that could limit reproduction. Temperature, salinity, and food availability are all factors that exert considerable control over gametogenesis, spawning, and how effectively a population will contribute new generations of oysters.

Based on my findings, I would recommend selecting sites for restoration that experience seasonally high temperature and chlorophyll-a levels. While adult oysters may tolerate wide ranges of salinity, reproduction is inhibited in upper estuarine sites that consistently experience salinities of 15 or lower. For this reason, habitats beset by periodic freshwater inundation and/or salinities below the physiological threshold for *O. lurida*, should be avoided for outplanting projects. Sites in the mid-estuary, such as Haynes Inlet, and perhaps westward toward the more saline regions of Coos Bay, would provide maximum seasonal reproductive activity and output.

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