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**Environmental influences on the population sex ratio of farmed Pacific oysters in  
Southeast Alaska**

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Resilient Coastal Communities and Economies

### **Question**

How do environmental factors such as water temperature and phytoplankton abundance influence the population sex ratio of farmed Pacific oysters in Southeast Alaska?

### **Hypothesis**

Due to prevailing cold ocean temperatures in Southeast Alaska, Pacific oyster sex ratio will show a male bias. As summer conditions improve—as water temperatures and phytoplankton abundance increase—the percentage of oysters with ova present will increase and more females and hermaphrodites will be seen.

### **Introduction**

This study took place at the NOAA NMFS Alaska Fisheries Science Center in Juneau, Alaska and nearby oyster farm, Salty Lady Seafood Co. Pacific oysters (*Crassostrea gigas*) are an important species for Alaska's growing shellfish aquaculture industry and are farmed throughout the west coast of North America, from Baja California to Alaska. Introduced for farming, Pacific oysters are not native to Alaska, and due to prevailing cold ocean temperatures, they do not naturally reproduce in this region. Oviparous oysters, such as the Pacific oyster, are known to be sequential, protandric hermaphrodites, meaning sex change occurs initially from male to female and does not continually alternate between sexes (Bayne, 2017; Coe, 1932). Due to this characterization, younger oyster populations are expected to favor males, while older populations favor females. Indeed, past studies have shown that sex change favors females as the oysters increase in size and age (Bayne, 2017; Broquard, 2020). Oyster sex determination is also influenced by environmental factors such as temperature and food availability, where warmer water and more abundant phytoplankton yield a female bias in sex ratio (Bayne, 2017; Broquard, 2020; Chávez-Villalba, 2002, 2003; Li, 2000; Santerre, 2013). Additionally, it has been found that there will be a male bias in sex ratio when conditions are poor, or when there is an environmental stressor such as cold temperatures or starvation (Bayne, 2017; Santerre, 2013; Sun, 2023; Quayle, 1969). Most of these past studies have focused on farmed Pacific oyster populations at lower latitudes, primarily along the Atlantic coast of France, where average ocean temperatures are much warmer than in Southeast Alaska (Broquard, 2020; Santerre, 2013). Some research has focused on oyster populations in conditions similar to Southeast Alaska. Namely, Thomas (2011) examined oyster gonadal development in Kachemak Bay, AK and Puget Sound, WA, and Robinson (1992) examined oyster gonadal cycles in Yaquina Bay, OR. While not expressly analyzing oyster sex ratio and its environmental influences, these studies did find that oyster gonadal development and larvae

survival increased with warmer ocean temperatures (Robinson, 1992; Thomas, 2011). Due to an overall lack of knowledge and to inform future hatchery efforts, data on Pacific oyster population sex ratio are needed. Sex ratio data are important as both male and female oysters are needed for a successful hatchery. Important for local hatchery efforts in Southeast Alaska, these data are also important for the wider aquaculture industry. Aquaculture will be an important source of seafood as wild caught and farmed fish efforts plateau and global population increases (FAO, 2020; Langdon, 2023). Therefore, this study aimed to determine environmental factors that influence the population sex ratio of Pacific oysters in Southeast Alaska.

### **Prediction**

Because Southeast Alaska is characterized by cold ocean temperatures, too cold for oysters to naturally reproduce, and because it has been shown that sex ratios of Pacific oysters will favor males when conditions are poor, we predict that the sex ratio of farmed Pacific oysters in this region will experience a male bias. As past studies have observed that a female-skewed sex ratio increases with warmer ocean temperatures and more abundant phytoplankton, we expect to see an increase in the percentage of females seen as the summer progresses, as waters warm and phytoplankton is more abundant. The frequency of oysters with ova present will increase as the summer progresses.

### **Materials**

Water samples for phytoplankton analysis were collected at 1 and 5 m using a Niskin bottle. Samples were stored in a 500 mL Nalgene bottle. Water column temperature was collected using a CastAway CTD. Water samples for phytoplankton analysis were placed in 125 mL glass jars, fixed and concentrated with 0.5 mL of 25% Glutaraldehyde, and viewed under a Leica DMD108 digital microscope using a 1 mL Sedgewick rafter slide and slide cover.

The oysters' shell height and length were measured with a standard ruler. Oysters were shucked using an oyster knife and placed on Petri dishes. Gonad samples of each oyster were taken with transfer pipettes, placed on a microscope slide and viewed under a Nikon eclipse TS100 microscope.

### **Procedure**

#### **Field Sample Collection:**

Water samples for analyzing temperature and phytoplankton were collected every week for eight weeks (June 6th to July 25th, 2023) from Salty Lady Seafood Co., an oyster farm in Juneau, AK. A CastAway CTD was lowered to the seafloor to obtain water column temperature data. Next, whole water samples were collected at a depth of 1m using the Niskin bottle, and these samples were placed in a 500mL Nalgene bottle. 15-20 oysters, aged 2-3 years, were collected from the oyster farm and placed into a pre-labeled Ziploc bag.

#### **Whole Water Plankton Sample Analysis:**

The Nalgene bottle containing the whole water sample at 1m was mixed thoroughly and 100mL of this water was poured into a 125mL glass jar. Using a p1000 micropipette, 0.5 mL of 25% Glutaraldehyde (final concentration 0.25%) was added to the glass jar and mixed. This jar was left undisturbed overnight to allow the phytoplankton to settle to the bottom. Within the next

48 hours, the top 90mL of mixture was carefully removed from the glass jar using a p5000 micropipette so as not to resuspend or suck up the settled phytoplankton. The remaining 10 mL of concentrated sample was then mixed, and 1 mL was removed with a transfer pipette to fill a Sedgewick-Rafter slide counting chamber. Five rows of the counting chamber were scanned using a random row as a starting point, and final tallies of phytoplankton counts were multiplied by four. Phytoplankton were identified to genus level and counted, using Puget Sound Marine Monitoring and Kachemak Bay Research Reserve documentation as taxonomic guides.

#### Oyster Sex Determination:

Each oyster's shell length and shell height (mm) were measured using a standard ruler. Oysters were then shucked with an oyster knife and placed upside down in a Petri dish. A gonadal sample of each oyster was taken by gently piercing the gonad with a transfer pipette, and samples were placed on a microscope slide and viewed under a microscope to determine sex. Males were identified by the presence of activated sperm (movement of flagellum activated by the addition of seawater to the sample) (Figure 1). Females were identified by the presence of eggs, dark ovals with lighter circles inside. Hermaphrodites were identified by the presence of both activated sperm and eggs (Figure 2).



Figure 1. Identified Male, active sperm field is visible

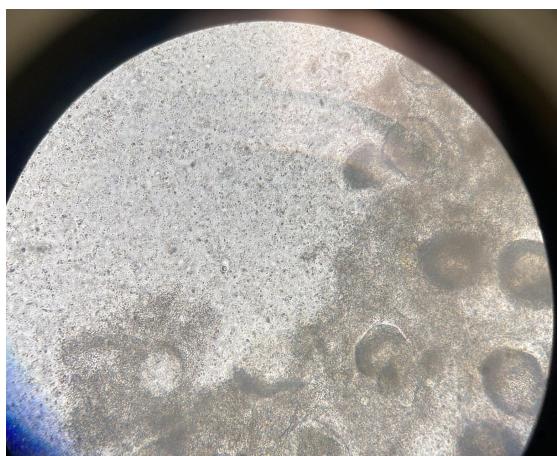


Figure 2. Identified Hermaphrodite, active sperm field is visible in upper left corner, eggs visible as large darker circles in bottom right

## Results

Throughout the eight weeks of the study, we saw a male bias in the Pacific oyster population sex ratio. The frequency of oysters seen with ova present did increase throughout the study. In the first two weeks, the oysters sampled were 100% male. By the last week of the study, the oysters sampled were 53% male (Figure 3). Though there seems to be a positive correlation between frequency of oysters seen with ova present and time, the correlation was not significant ( $p$ -value = 0.325) (Figure 4). There seemed to be a slight positive correlation between percentage of oysters with ova present and an increase in water temperature, but upon running a binomial linear model, the data was not significant ( $p$ -value = 0.446) (Figure 5). There was no correlation between percentage of oysters with ova present and diatom abundance ( $p$ -value = 0.824) (Figure 6). Additionally, conducting an AIC test via the addition of a diatom abundance factor to the model of percentage of oysters with ova present as a factor of water temperature did not yield a better fit (AIC values = 8.409 [binomial 1, frequency of ova present as a factor of water temperature] and 10.454 [binomial 2, addition of diatom abundance factor]).

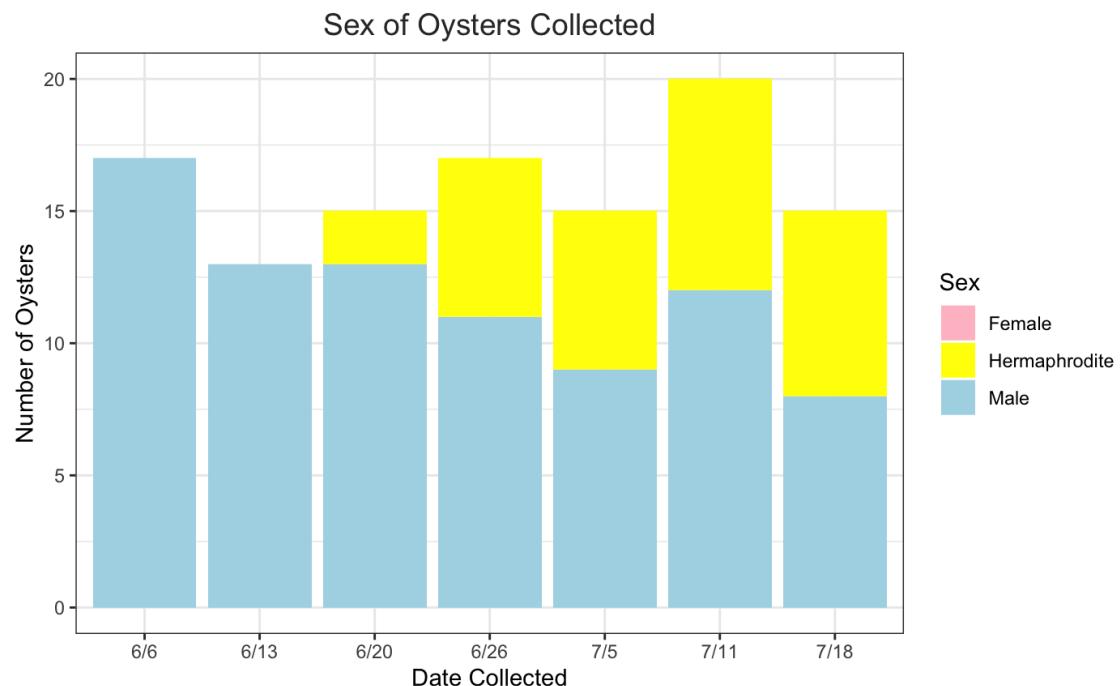


Figure 3. Stacked bar plot representation of the number of oysters collected throughout the study and their sex.

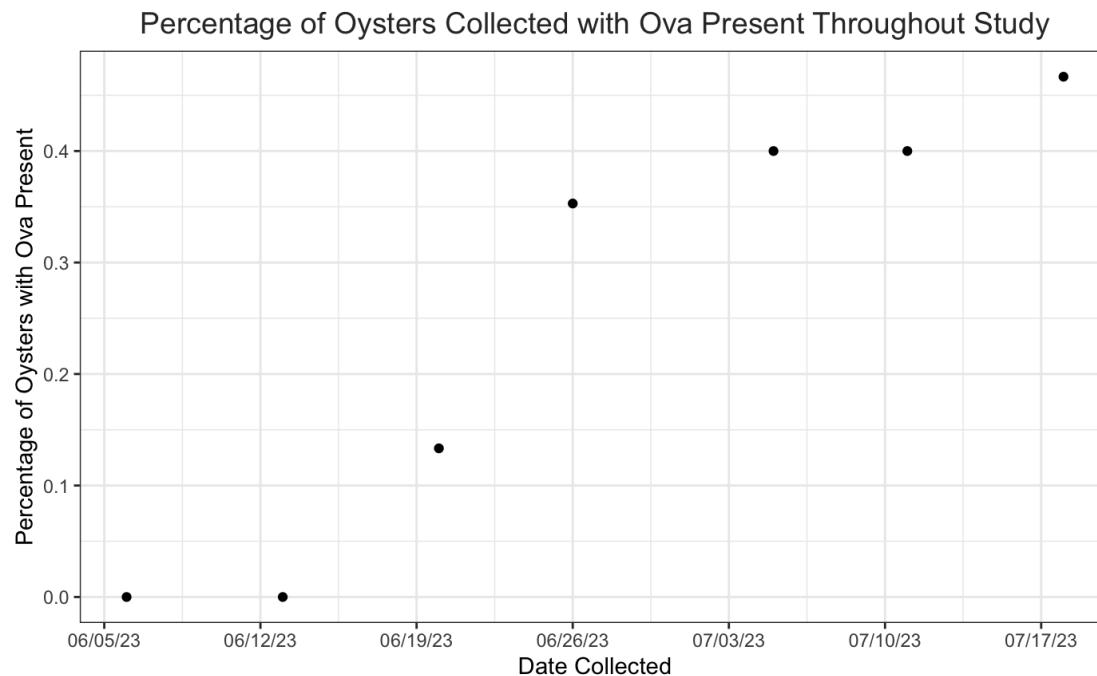


Figure 4. The relationship between percentage of oysters with ova present and time. Though there seems to be a positive correlation, a binomial linear model is not significant ( $p$ -value = 0.325).

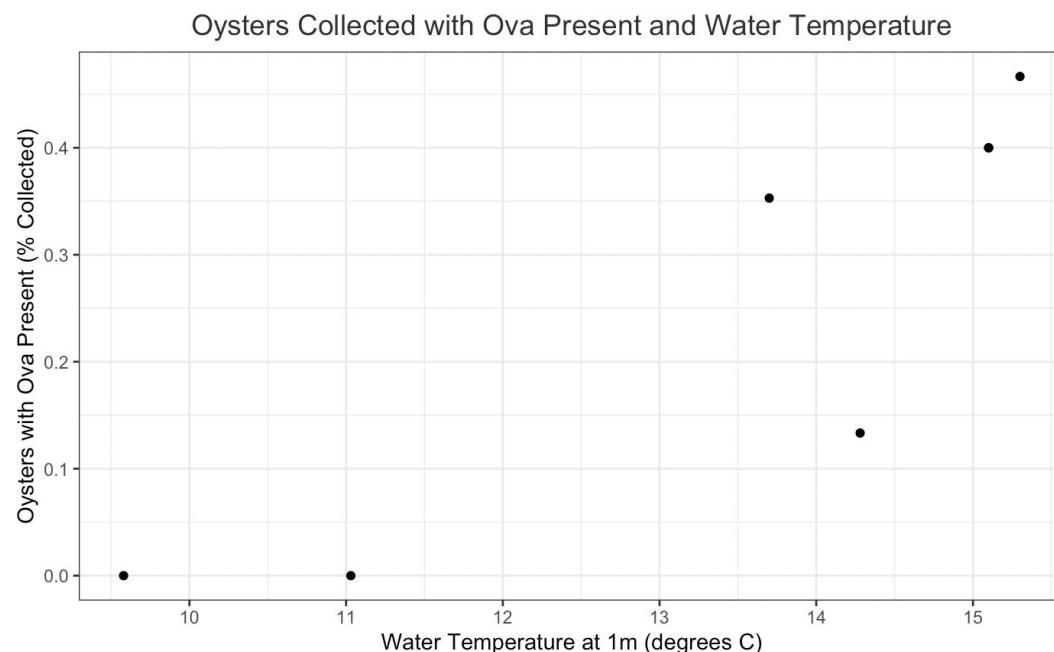


Figure 5. The relationship between percentage of oysters with ova present and water temperature. Though there seems to be a somewhat positive correlation, a binomial linear model is not significant ( $p$ -value = 0.446).

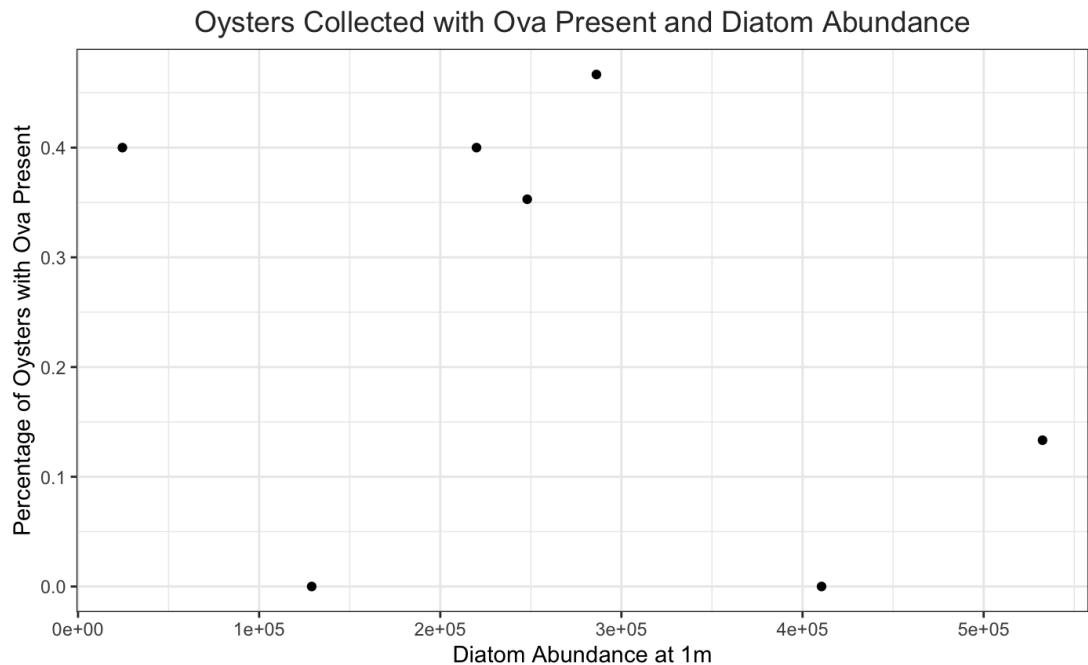


Figure 6. The relationship between percentage of oysters with ova present and diatom abundance. The relationship is not significant ( $p$ -value = 0.686).

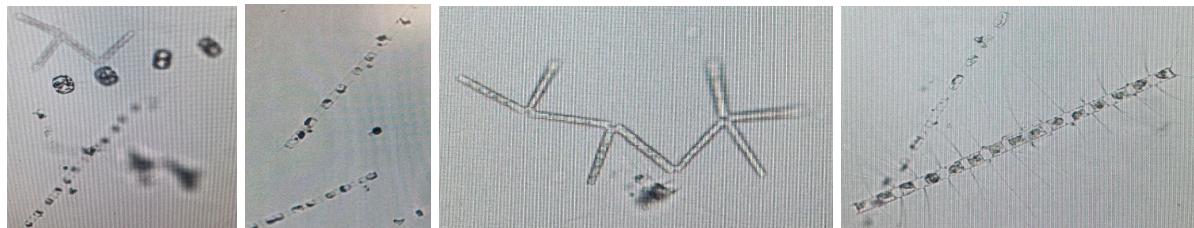


Figure 7. Some of the major diatom genera seen in phytoplankton samples. Genera present in images are Thalassiosira, Skeletonema, Thalassionema, and Chaetoceros.

## Conclusion

This study asked whether environmental factors such as water temperature and phytoplankton abundance influenced the population sex ratio of farmed Pacific oysters from an oyster farm in Southeast Alaska. Based on results showing an increase in the proportion of oysters containing ova as water temperatures increased, though non-significant, the environmental factor of water temperature may influence the population sex ratio of farmed Pacific oysters. Diatom abundance, however, did not seem to influence the population sex ratio. We originally predicted that though the number of oysters collected with ova present would increase throughout the summer months, the population sex ratio would show a male bias due to prevailing cold temperatures. Indeed, a male bias was seen throughout the study. In contrast to our original prediction we saw no female oysters throughout the course of the study. Therefore, the original prediction is somewhat supported—presence of ova increased, but not presence of females. Pacific oysters are known to be sequential hermaphrodites, meaning they may sex change between male and female but are not known to have both sexual gonads at the

time of gonadal maturity. Interestingly, hermaphrodites made up about one fifth of our data set. This number is much higher than has been seen in past studies, as hermaphrodites make up less than 1% of data sets in previous results (Broquard, 2020). The high frequency of hermaphrodites in this study could be due to cold Alaskan waters inhibiting complete sex change or our weekly sampling methods highlighting a time of sex transition. Under ideal conditions, Pacific oysters expel their sperm and absorb their gonads before transitioning to female (Bayne, 2017). It could be that because water temperatures are too cold for Pacific oysters to naturally spawn, they also can't release sperm in the same way before sex transition. Pacific oysters in high latitude and cold environments have not been extensively studied. Because these oysters are an important species for Alaska's growing aquaculture industry and to better inform future hatchery and farming efforts, future research should focus on sex change and sex ratio throughout the year. Additionally, though oysters do not reproduce naturally in the cold seawater environment of Southeast Alaska at this time, natural spawning events may become possible as global ocean temperatures warm, making an understanding of oyster reproduction in these environments imperative. Some error in procedure could have occurred in the first two weeks as sampling methods were refined. For the first two weeks, we assessed gametes from oysters that were doubled and fused together or otherwise unfit for market. While these oysters are suitable for other analyses, it was soon realized that these would be insufficient for a sex ratio analysis. After the first two weeks, sampling methods were refined and we collected adult oysters ages 2-3 years. Though we confirmed hermaphrodites by ensuring there was active sperm in the same sample as ova, we were limited in this area of methodology. Stuart Thomas (2011), who has conducted sex analyses on Pacific oysters in Kachemak Bay, AK and Puget Sound, WA, used histology to determine oyster sex. This is a more accurate methodology, but one to which we did not have access.

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