

JAPANESE OYSTER DRILLS (*OCINEBRELLUS INORNATUS*): EXPLORING PREY SIZE AND SPECIES PREFERENCE IN THE NETHERLANDS

FINAL REPORT

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SUMMARY

Dutch oyster cultivation has experienced its share of challenges throughout history, including the effects of climate change, overfishing, and the downfall of the native European oyster (*Ostrea edulis*). These challenges are especially significant within the Eastern Scheldt, dubbed the shellfish centre of the country. Since 2007, the Dutch oyster industry faced a new challenge for production: the introduction and expansion of the Japanese oyster drill (*Ocenebrellus inornatus*), a predatory marine snail that preys on market-size oysters and their spat. Currently, there are established populations of the Japanese oyster drill in Yerseke and Gorishoek (in the Eastern Scheldt), which have grown significantly in the recent years. Oyster farmers are already experiencing significant economic losses for local operations and the contamination of other commercial stocks and natural reefs in the Netherlands, and now worry that progress for native oyster population increase will also be impacted. There has been limited success in terms of the management, mitigation, and elimination of Japanese oyster drills in the Eastern Scheldt; furthermore, there is still limited knowledge on their feeding behavior and prey preference. This study addressed two topics of interest: the potential impact of adult Japanese oyster drills on commercially grown oysters, and on European oyster cultivation and stock recovery. Building on past studies done within the Saline Production Project in the HZ University of Applied Sciences, experiment trials were conducted to determine whether Japanese oyster drills preferred native European oysters over introduced Pacific oysters, and which size class was most vulnerable to adult drill predation.

The results from the size preference experiments indicated that there was no significant difference in predation between the distinct size classes, although the smaller oysters (smaller than 6.6 cm) were generally more targeted than larger oysters (larger than 6.6 cm). This suggests that all life stages are equally impacted by the presence and growth of the oyster drill population in the Netherlands. These results are especially relevant for farmers that practice bottom culture techniques, as this culture provides greater opportunities for contact with adult Japanese oyster drills. The results from the species preference experiments indicated that there was no significant difference in predation between the introduced Pacific and the native European flat oyster. This suggests that both species groups are impacted by the Japanese oyster drill population, emphasizing the growing concern for the recovery and cultivation of native oyster stocks in the Netherlands. The results are especially relevant amidst the numerous “unofficial” sightings for oyster drill population increase in additional areas in the Netherlands, including near Lake Grevenlingenmeer, where native oysters are currently most abundant.

For future research, the size preference feeding experiments should be repeated with an extended study duration and increased predator abundance, thus increasing the total feeding rate and the likelihood for adaptation of drills to experimental conditions. Additional feeding experiments should also be conducted to explore the effects of predator size, predator density, and their interactions on prey size choice. On the other hand, species preference experiments should be repeated with larger oyster samples and increased prey abundance, thus improving the ability to distinguish prey preference between species groups. Furthermore, all future feeding experiments should consider field trials rather than lab trials, thereby reducing any behavioral effects specific to captivity. Overall, this research supports recommendations to avoid bottom culture practices and to invest in off-bottom techniques, as well as to prevent secondary introductions of oyster drills in areas where native oysters are abundant. Since there is still limited knowledge on the feeding behavior of the oyster drill and prey preference, the research also supports monitoring of local oyster farms by their owners. Through regular monitoring, local farmers may be able to expand on current knowledge on the Japanese oyster drill, and support future research on drill management and mitigation.

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1. INTRODUCTION

1.1 BIOLOGICAL INVASIONS IN CONTEXT OF OYSTER CULTIVATION

Aquaculture, or the cultivation of aquatic animals, plants, and algae in freshwater, brackish and saltwater, accounts for a growing percentage of global food production (FAO, 2014). Oysters are a large part of aquaculture and are considered nutrient-rich and sustainable food options, presenting a viable alternative to other forms of animal protein amidst the declining rates of catch for common fisheries (FAO, 2016). Due to its favorable location by the North Sea, the cultivation of oysters is a profitable industry within the Netherlands, particularly in the Eastern Scheldt. In 2013 alone, it was recorded that the total income for oyster production exceeded 6 million euro in revenue (FAO, 2016).

However, Dutch oyster cultivation has also experienced its share of challenges, including extreme weather patterns, overexploitation, native species decline, and the introduction of new pathogens and predators (Troost, 2009). Overall, native European flat oyster (*Ostrea edulis*) stocks have declined significantly since the 1970s, while introduced Pacific oyster (*Crassostrea gigas*) stocks have expanded and naturalized across the Eastern Scheldt (Troost, 2009). Although native oyster stocks are now in recovery, Dutch oyster cultivation is faced with the new challenge of biological invasions. Since 2007, the Japanese oyster drill (*Ocenebrellus inornatus*) has been identified as an invasive species in the Eastern Scheldt, affecting both native and Pacific oyster stocks (Buhle & Ruesink, 2009). These snails drill holes through oysters' shells and flesh, while also preying on juvenile oysters and spats (Walker, 1981; Federighi, 1931). The expansion of the Japanese oyster drill population is now a major concern for oyster farmers around the Netherlands, as they are already experiencing significant economic losses (e.g. decrease in market size shellfish and spats) and the contamination of other shellfish hub, and are now worrying that the progress towards native European oyster stock increase will be lost (e.g. in Lake Grevenlingenmeer) (Van den Brink & Wijsman, 2012; Didden & Gittenberger, 2013; Smaal, Kamermans, & Strietman, 2016).

1.2 AIM AND MAIN QUESTION

A study has already been done in the HZ University of Applied Sciences, as part of the Saline Production Project, on the topic of Japanese oyster drills. The Saline Production Project (known in Dutch as Zilte Productie) was a project created to develop innovative solutions for challenges surrounding the aquaculture sector, in order to maximize productivity for shellfish farmers in the Eastern Scheldt, and to provide useful information and tools for shellfish farmers (Oosterwal, 2016). This specific study was done to observe the foraging behavior and to note the food preference of Japanese oyster drills, specifically between

Pacific oysters and blue mussels of different sizes. The main purpose of the study was to provide information on which population was most vulnerable to drill predation; the final results concluded that *O. inornatus* preyed upon oyster samples smaller than 5cm and mussel samples smaller than 3 cm (Oosterwal, 2016).

Current literature focuses mostly on the oyster drills' preference between Olympia and Pacific oysters in the United States, hence, there are still many questions surrounding Japanese oyster drills' preference between European and Pacific oysters in the Netherlands (Buhle & Ruesink, 2009). Although the study done by Oosterwal (2016) concluded that Japanese oyster drills preferred Pacific oysters over blue mussels from the Eastern Scheldt, there were no experiments done using the native European flat oyster. Furthermore, the feeding behavior of drills was only observed for a limited duration of time (i.e. 4 days); consequently, the total feeding rate of sampled drills was fairly low. The current study will, hence, fill in the missing gaps in the literature, and address the two main topics of interest: the potential impact of adult Japanese oyster drills on commercially grown oysters, and on native European oyster cultivation and stock recovery.

Based on the previous studies, as well as the theories provided in the Background section, this study addressed the following research questions:

1. Do adult Japanese oyster drills prefer preying on native European flat oysters over introduced Pacific oysters?
2. Which life stage of the oyster is most vulnerable to predation from adult Japanese oyster drills?

1.2.1 Sub-questions

1. Do adult Japanese oyster drills prefer preying on native European flat oysters over introduced Pacific oysters?
 - a. Which oyster species (i.e. *O. edulis*, *C. gigas*) does an adult Japanese oyster drill prefer to eat or prey on? How many oysters from each species were drilled?
2. Which life stage of the oyster is most vulnerable to predation from adult Japanese oyster drills?
 - a. Which life stage (i.e. spat, juvenile, or adult) of the Pacific oyster does the Japanese oyster drill prefer to eat?
 - b. What size do oysters have to be, in order to avoid predation from oyster drills in bottom culture?
 - c. How thick are the shells of the sample oysters that were preyed upon?
 - d. Is the location of drilling by oyster drills dependent on the shell thickness at that location?

1.2.2 Hypotheses (Expected Results)

1. Do adult Japanese oyster drills prefer preying on native European flat oysters over introduced Pacific oysters?
 - a. Adult oyster drills will prey more on the introduced *C. gigas*, since it is their natural prey

2. Which life stage of the oyster is most vulnerable to predation from adult Japanese oyster drills?
 - a. The spat and juveniles of both oyster species will be most susceptible to predation by the adult oyster drills, since they are smaller and have thinner shells. Hence, they should reach the adult or market-size (i.e. larger than 6.6 cm) to avoid predation.

2. BACKGROUND

2.1 OYSTERS AND OYSTER CULTIVATION IN THE NETHERLANDS

2.1.1 Life Cycle of Bivalves

The life cycle of most intertidal bivalves (such as oysters) includes: a planktonic larval stage, and a benthic juvenile and adult stage. Once individuals are sexually mature, they release their eggs and sperm into the water column for fertilization. These gametes have a limited lifespan and diffuse easily; fertilized eggs then develop into larvae within two days. To balance high rates of larvae mortality, bivalves release large amounts of gametes resulting in large amounts of larvae. Many species also spawn synchronously and live in dense aggregations to maximize success in fertilization (Troost, 2009). Spawning is triggered by environmental cues or cycles, such as lunar and tidal patterns or seasonal temperatures (Troost, 2009).

After a certain period of time, the larvae shift from obtaining food from energy reserves to active foraging (Troost, 2009). Three weeks after fertilization, the larvae settle on a suitable substrate and undergo metamorphosis into the benthic juvenile stage. If a suitable substrate is not found, larvae may postpone settlement and metamorphosis, or even move to another suitable location (Troost, 2009). Benthic juveniles (or spats) then proceed to their growth stage, and recruit into sexually mature adults (Troost, 2009). It is important to note that during all life stages, bivalves are vulnerable to predation by other benthic animals, and by oyster drills. In early stages, they are preyed on by small crabs and shrimps. In later stages, they are preyed on by crabs, starfish, and birds (Troost, 2009). Consequently, predation affects bivalve survival and growth into later life stages.

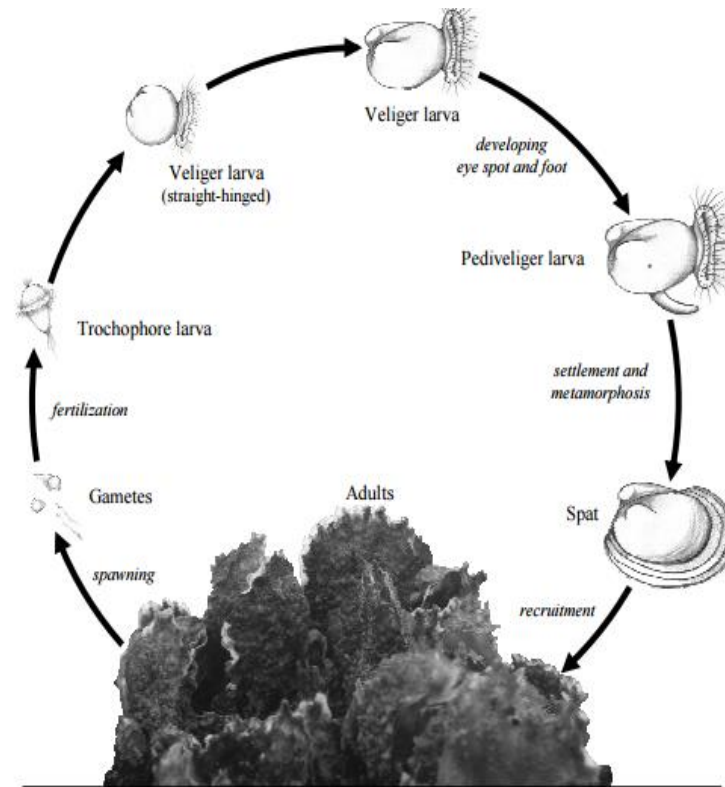


Figure 1: Life cycle of benthic bivalves, such as oysters (Troost, 2009)

2.1.2 Decline and Recovery of the native European oyster

From the 1870s until the 1970s, Dutch oyster cultivation concentrated on the native European flat oyster (*Ostrea edulis*). However, a combination of severe winters, intermixing of stocks, overfishing, habitat change, and the accidental introduction of parasites led to the downfall of the native oyster in Dutch waters (Troost, 2009). In 1962, the extreme winter caused the European stock to drop from 120 million to 4 million; as a result, Dutch oyster farmers began to import native oysters and spat from surrounding European countries (e.g. France, Ireland, Norway) until 1977 (Troost, 2009). In 1940, European oyster stocks were also severely depleted in the Wadden Sea due to habitat change and overexploitation (Troost, 2009). In 1980, the Bonamia parasite (*Bonamia ostrea*) was introduced in the Eastern Scheldt through contaminated strains from France, inducing high mortality rates in native stocks, and the end of native oyster cultivation in the area (Troost, 2009).

Small stocks of the European native oyster continue to be cultured in Lake Grevelingenmeer (in the Grevenlingen estuary), however, after noting that the population was coping despite the Bonamia disease (Troost, 2009; Smaal, et al., 2015). In 2014, a small European oyster bed was also found in the northern and eastern part of the Eastern Scheldt, and inshore in the Voordelta (Smaal, et al., 2015). As there is still evidence of native oysters thriving in their former distribution range in the Delta area of the Netherlands (e.g. the Grevenlingen estuary and the Eastern Scheldt estuary), researchers predict that native oyster populations could still rebound, and are now making efforts towards population recovery and expansion (Smaal, et al., 2015).

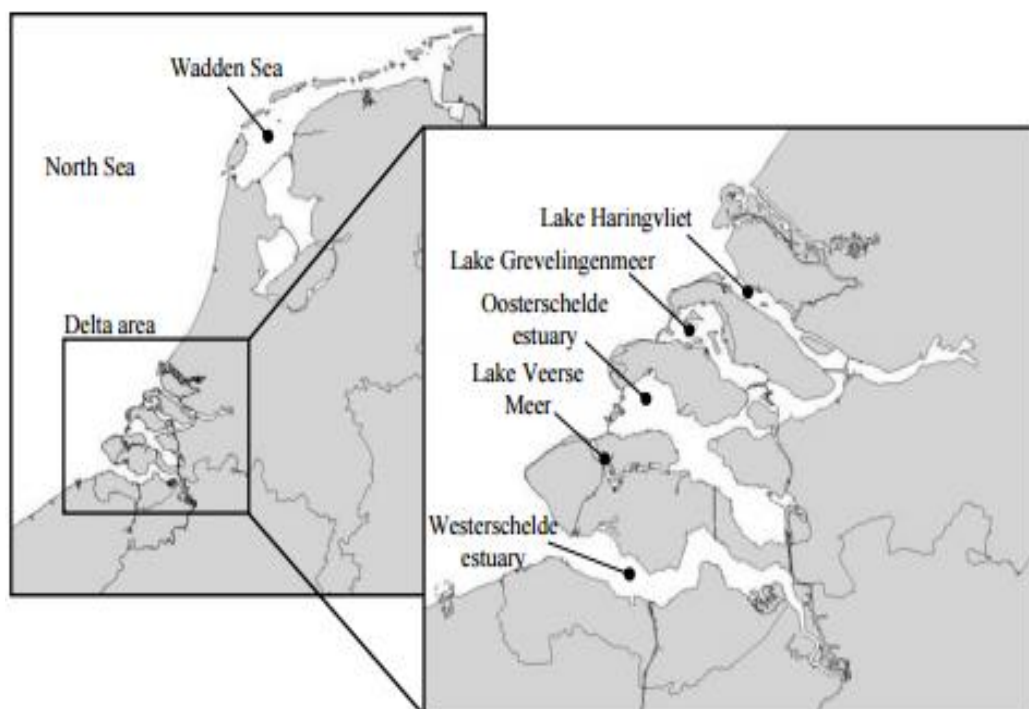


Figure 2: Map indicating the location of Lake Grevelingenmeer (within the Grevelingen estuary), the Netherlands (Troost, 2009)

2.1.3 Expansion of the Pacific oyster

The intentional movement of bivalves, crossing both national and ecosystem boundaries, for economic purposes has been done throughout human history. This includes the translocation of species from hatcheries, or from wild to new fishery sites. Ultimately, the purpose of these movements is to develop a sustainable food supply, or to start a new culture (Brenner, et al., 2014). Stock transfers can also be done to replenish a local supply or to enhance wild native stocks, due to poor spat settlement or the inability to produce a consistent supply of bivalve seeds (Brenner, et al., 2014).

Due to the downfall of the native European oysters, Dutch oyster farmers began to search for profitable alternatives, such as the Pacific oyster (Troost, 2009). The Pacific oyster (*C.gigas*) was introduced in the Eastern Scheldt estuary in 1964, when the shellfish industry seeded the area with spat originating from British Columbia (Canada); more introductions of *C.gigas* spat and adult oysters soon followed (Walles, 2015). Although it was originally thought that *C. gigas* would not be able to survive and spawn in colder Dutch waters, *C. gigas* stocks in the Eastern Scheldt expanded and naturalized, while native stocks (e.g. blue mussel *Mytilus edulis* or edible cockle *Cerastoderma edule*) declined or remained stable (Troost, 2009). Oyster reefs of *C. gigas* now occupy about 9 km²(about 8%) of the lower intertidal area of the Eastern Scheldt (Walles, 2015).

2.2 OYSTER DRILLS AND THEIR INTRODUCTION IN THE NETHERLANDS

2.2.1 Taxonomic Information

The Japanese oyster drill (also known as the Japanese oyster snail) is a predatory marine gastropod, from the family Muricidae (Lützen, et al., 2012; Afonso, 2011). The species was originally identified as *Murex inornatus* in 1851, but has since been renamed as *Ocenebrellus inornatus*. Other scientific names for the Japanese oyster drill include *Ocenebra inornata*, *Pteropurpura inornata*, *Cerastona inornata*, or *Ocenebra japonica*. For this study, the scientific name *Ocenebrellus inornatus* will be used (Lützen, et al., 2012; Afonso, 2011).

2.2.2 Features

All individuals from the genus *Ocenebrellus* share common features of a ventrally sealed siphonal canal and axial ribs on the whorls of their shells. *Ocenebrellus inornatus* have shells with five whorls, with the last whorl containing 4-12 axial ribs, coming to points in the apical edge of the body whorl; there can also be spiral ridges seen on the shell (Amano & Vermeij, 1998; Fey, van den Brink, Wijsman, & Bos, 2010). The aperture of the shell is oval with a thick outer lip (Fey, van den Brink, Wijsman, & Bos, 2010). These shells can reach a height of almost 50mm, although individuals may only reach 40mm (Amano & Vermeij, 1998). All individuals from the family *Muricidae* also share the common feature of a labral tooth, however, *Ocenebrellus inornatus* has been found with and without the labral tooth in their introduced areas (Amano & Vermeij, 1998).

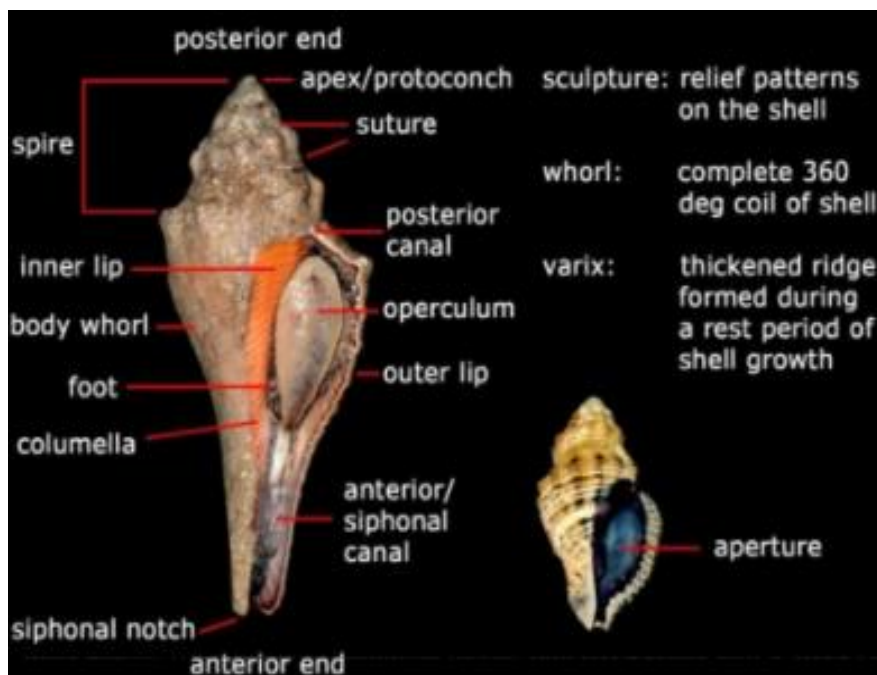


Figure 3: Diagram depicting features of *Ocenebrellus inornatus* (Keur, 2017)

This species can be yellow, white, beige, brown, orange, and also striped (Amano & Vermeij, 1998; Fey, van den Brink, Wijsman, & Bos, 2010). *Ocenebrellus inornatus* can also display physical signs of sexual dimorphism, in that female individuals are slightly larger than males as adults (Martel, et al., 2004). Overall, this species has a wide range of phenotypic variation, which increases the challenge for researchers and farmers to properly identify and classify individuals.

2.2.3 Life cycle

The life cycle of muricid gastropods, like the *Ocenebrellus inornatus*, does not contain a free-swimming larval stage or a planktonic stage. Oyster drills have separate sexes and group together to mate during the spawning season (around April to June); eggs are then fertilized internally (Martel, et al., 2004). Introduced populations can also extend their reproduction period, spawning again in autumn (Buhle, Margolis, & Ruesink, 2004). The females produce up to 40 yellow egg capsules that are slightly larger than a grain of rice, each producing 10-15 embryos. Hatched larvae turn into fully-developed juveniles after three weeks, and settle directly on the seafloor (Lützen, et al., 2012; Buhle, Margolis, & Ruesink, 2004; Martel, et al., 2004).



Figure 4: Egg capsules of the Japanese oyster drill (to size) (Babaran, 2017)

Juveniles are only 2mm after hatching, and grow at a rate of 2mm per month. Individuals reach adulthood at the size of 27mm, and can reproduce after 1-2 years (Buhle, Margolis, & Ruesink, 2004). In general, the adult drill survival rate is only 10% annually; however, healthy adults are able to live for an average of 10 years (Buhle, Margolis, & Ruesink, 2004; Titselaar & Oonk, 2014).

2.2.4 Introduction and Performance in Dutch Waters

Japanese oyster drills are naturally found in Northern China through Korea, and in all seas around Japan to Sakhalin and the Kurile Islands; this overlaps the natural distribution of the Pacific oyster and the presence of natural Pacific oyster reefs (Lützen, et al., 2012; Didderen & Gittenberger, 2013). Due to their lack of a free-swimming larval stage, their natural ability to disperse is limited. Long-distance dispersal, hence, is a product of transportation by human-mediated vectors (e.g. human transportation, boat fouling, oyster transfers, and transfers of material from infected areas into new areas) (Lützen, et al., 2012; Didderen & Gittenberger, 2013, Wolff & Reise, 2002).

The Japanese oyster drill was introduced to Europe through the French Atlantic coast around 1995; the source population was most likely from oysters imported from the United States, which were also infested by the species. The oyster drills have since been found on

European flat oysters in Denmark (2006-2010) and on Pacific oysters in Portugal (2005-2008) (Didderen & Gittenberger, 2013). In 2007, the species was first reported in the southwest of the Netherlands (i.e. the Eastern Scheldt) (Afonso, 2011). It is speculated that Japanese oyster drills were introduced in the Eastern Scheldt together with imported Pacific oysters from British Columbia, where the drills were well-established (Lützen, et al., 2012). During 2008-2009, Japanese oyster drill populations in the Eastern Scheldt increased at the original site at Yerseke (<1 km) and in the next locality at Gorishoek (1 km); these populations are now established in the littoral and sublittoral zone (Didderen & Gittenberger, 2013). Their typical habitat in the littoral zone are boulders and near Pacific oyster reefs, while their habitat in the sublittoral zone is near the Pacific oyster reefs (Didderen & Gittenberger, 2013). In general, the species is present in the more sheltered Pacific oyster reefs in the southeastern part of the Eastern Scheldt (Didderen & Gittenberger, 2013).

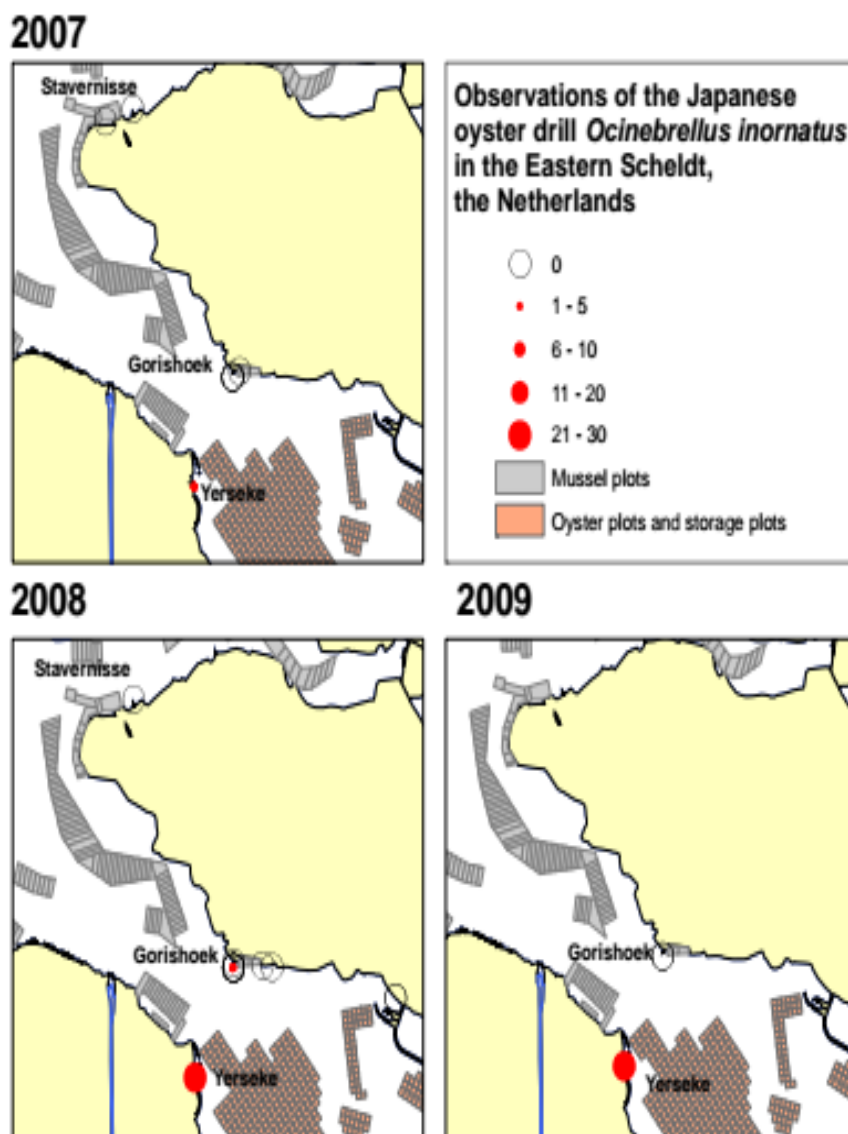


Figure 5: Map of distribution and growth of Japanese oyster drills observed in the Eastern Scheldt, in the Netherlands (Fey, van den Brink, Wijsman, & Bos, 2010)

2.3 CURRENT KNOWLEDGE ON FEEDING BEHAVIOUR AND PREFERENCE OF OYSTER DRILLS

2.3.1 Feeding behavior

Gastropods, such as the Japanese oyster drill, have chemoreceptive mechanisms for detecting prey. Drills respond to the substances in the effluent of their prey and associated chemical cues, and move towards areas where they receive the strongest signals for healthy individuals. Oyster drills feed on their prey by drilling a hole through their shell; this feeding process involves several steps. Once they find a suitable individual, the drills will probe the shell with their propodium (or foot) and proboscis. The initial probing will take up to 30 minutes, until the drill has settled and extends its accessory boring organ (ABO) on the chosen site (Carricker, 1981; Carefoot, n.d.). The rasping of the hole is done by the radula, which is able to turn to 180°. The ABO then secretes sulfuric acid to soften the shell for drilling by the radula. After the hole is drilled, the drills secrete digestive enzymes on their prey; the proboscis then extends and extracts the digested tissues for consumption (Boersma, et al., 2006; Carricker, 1981; Carefoot, n.d.). Predation on oysters by *O. inornatus*, hence, is very identifiable by the 2mm hole left on the shell (Fey, van den Brink, Wijsman, & Bos, 2010).

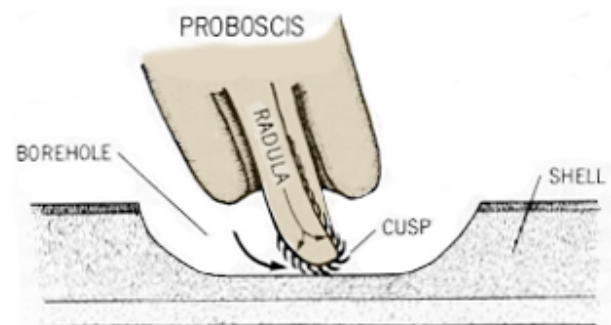


Figure 6: Diagram of the accessory boring organ (ABO) for feeding of the oyster drill (Keur, 2017)

The duration of the feeding process depends on the size of their prey, as well as the size of the oyster drill (Boersma, et al., 2006). In a study done in Denmark, it was found that Japanese oyster drills took 5-7 days to consume an adult clam or mussel, and 2 weeks to eat an adult Pacific oyster (Lützen, et al., 2012). In general, their ability to feed is limited by their ability to drill into their prey; hence, it is theorized that shell thickness is the main determinant for individual performance. In a study done in Connecticut on the impact of prey shell thickness and oyster drill feeding, it was

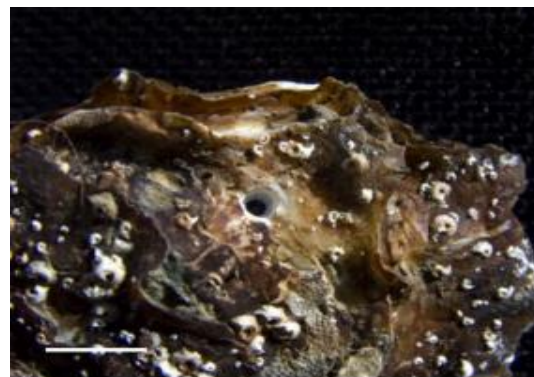


Figure 7: Evidence of predation of oyster by oyster drills (Lützen, et al., 2012)

found that drills fed on smaller Eastern oysters with thinner shells, 86 % of the time during trial feeding experiments (Lord & Whitlatch, 2013). In a risk assessment done on oyster drills in Dutch waters, it was found that it took an adult *O. inornatus* one day to drill into a Pacific oyster that was 2.5 cm long, and around two weeks for a Pacific oyster that was 5 cm long (Fey, van den Brink, Wijsman, & Bos, 2010). These theories support observations that oyster drills prey more heavily on oyster spat and juvenile oysters, whose shell thicknesses are less in comparison to fully-developed oysters. In theory, oyster drills can consume up to 3 small oysters in a week (Boersma, et al., 2006).

The total feeding rates of oyster drills can be attributed to many factors, including temperature change. In the study done in Connecticut, it was found that Eastern oyster drills had a 60% increase in total feeding rate during late summer, fall, and even early winter, when ambient and water temperatures were still fairly high (Lord & Whitlatch, 2013). The results from annual and seasonal temperature alteration experiments indicated that there was a strong linear relationship between temperature and feeding. As such, projected trends for climate change (i.e. increase of 2°C in global temperature) could result in a significant increase in drill feeding rates, prolonging their feeding and growth season (Lord & Whitlatch, 2013).

2.3.2 Natural Prey and Prey Preference

In general, Japanese oyster drills consume oysters and oyster epifauna, such as barnacles (Buhle & Ruesink, 2009). Although the Pacific oyster is their natural prey (as it coexists in their natural range for distribution), the Japanese oyster drill can switch to other species in its absence or presence; their food preference, hence, is not an adaptive coevolution. Many studies have concluded that the drills were able to survive on a diet of solely common mussels, and a combination of *Olympia* and Pacific oysters (Buhle & Ruesink, 2009; Lützen, et al., 2012). Prey availability also influences the foraging behaviour of oyster drills; when one prey species declines, individuals will look for another species to prey upon (Carricker, 1981). This behavior was seen in a study done in the United States, with *Olympia* and Pacific oysters. All oyster drill species attacked Pacific oysters rather than *Olympia* oysters, as they were less abundant in the natural system (Buhle & Ruesink, 2009). The study also concluded that the sampled oyster drills preferred small oysters for both species, supporting the theory that shell thickness is also a major factor in determining prey preference (Buhle & Ruesink, 2009).

Table 1 summarizes the information collected on oyster drills, from various past feeding experiments, or in commonly cited literature. It is important to note that due to the differences in experimental protocols and conditions, results may differ between studies. In other cases, there were no experiment protocols recorded or sample groups classified.

Table 1: Summary of results from various past feeding experiments or past studies done on oyster drill species

Reference	Location	Experiment Type	Sample Groups	Drill Feeding Rate	Drill Prey Preference
Buhle & Ruesink (2009)	Willapa Bay, Washington (United States)	Lab 5 weeks, 20 replicates	One Japanese drill per replicate; 10 Pacific oysters (9-76 mm), 2-6 small Pacific and Olympia oysters (15 mm)		Small oysters over larger oysters (both species); Pacific oysters over native Olympia oysters
Boersma, et al. (2006)			Japanese oyster drills; Pacific and Olympia oysters	3 small oysters per week	
Lützen, et al. (2012)	Limfjord (Denmark)	Lab and field 4 weeks	Japanese oyster drills; Various prey species	2 days to drill through mussel; 5-7 days to consume a clam or mussel; 2 weeks to eat an adult Pacific oyster	
Fey, van den Brink, Wijsman, & Bos (2010)	Netherlands		Japanese oyster drills Pacific oysters	One day to drill into a Pacific oyster (2.5 cm) Two weeks to drill through a Pacific oyster (5 cm)	
Brown & Richardson (1987)	Louisiana (United States)	Lab 3-4 days per trial	Southern oyster drills (<30mm or >30mm) with one, three, 5 drills per replicate; mussels (<2g), Virginia oysters (<36g)		Small snails fed on small mussels, but had less success on large mussels, or solitary oyster with thicker shells; Total prey eaten increased when drills fed in groups
Heimbigner (2012); Joseph (2004)	Willapa Bay, Washington (United States)		Atlantic and Asian oyster drills	One oyster every 3 days	
Lord & Whitlatch (2013)	Connecticut (United States)	Lab 4-9 months	One Eastern oyster drill per replicate; 3 small Eastern oysters (2cm), 3 large Eastern oysters (5cm) per replicate	60% increase in feeding rate during warmer months (late summer, fall, early winter)	Drills preyed on oysters with thinner shells 86% of time; Preyed on smaller oysters (1 g) in colder months and larger oysters (10 g) in warmer months

2.4 IMPACT OF OYSTER DRILLS ON OYSTER CULTIVATION AND RECOVERY OF *O. EDULIS*

2.4.1 *Oyster drills as an invasive species*

The impact of an invasive species on a fishery is determined by its geographic range, density, and per capita effect (i.e. the mean percent mortality per individual). Understanding the impact of a species, hence, requires in-depth knowledge of the direct and indirect interactions between native and exotic species, harvesting, and surrounding human activities (Buhle & Ruesink, 2009). As mentioned before, Japanese oyster drills are generalist species, meaning that they have a wide variety of prey species in the sea-bottom. This makes the drills very successful in the Eastern Scheldt, known as one of the most productive areas for shellfish and related fauna in the Netherlands (Buhle & Ruesink, 2009). Due to the fact that the Japanese oyster drill is an introduced species to the Netherlands, it has very few predators in Dutch waters (Lützen, et al., 2012).

Although the Japanese oyster drill usually survives and reproduces in the warmer sea temperatures of northeastern Asia, the Japanese oyster drill has survived multiple cold spells (in 2009-2011) and colder winter temperatures in the Eastern Scheldt (0 to -1°C) (Lützen, et al., 2012; Amano & Vermeij, 1998). Additionally, introduced populations of this species experience a second reproduction period, giving them higher resistance to seasonal fluctuations and a greater probability to spawn (Buhle, Margolis, & Ruesink, 2004). In 2010, the risk assessment on the Japanese oyster drill in the Eastern Scheldt reported that the Japanese oyster drill population would cause an economic loss of up to 50% for European and Pacific oyster growers, the destruction of natural Pacific oyster beds, the disruption of restoration efforts for the European oyster beds, and competition for native oyster drill species (Fey, van den Brink, Wijsman, & Bos, 2010).

Figure 8 illustrates the more recent situation in 2016-2017, depicting the estimated stock populations for the Pacific and native European flat oyster in the Netherlands (Smaal, Kamermans, & Strietman, 2016). Since 2007 (i.e. when the Japanese oyster drill was identified as an invasive species in the Eastern Scheldt), the native oyster population has stabilized and has even increased slightly (i.e. slight population increase in 2012, although numbers are still relatively low compared to Pacific oyster stocks). On the other hand, the Pacific oyster population has undergone two significant declines in 2007 and in 2013, and has been projected to decline even further around 2016-2017. Although the projected decline of Pacific oyster stocks could be attributed to multiple factors, such as disease and climate change, the rapid growth of the Japanese oyster drill population is likely to be most culpable.

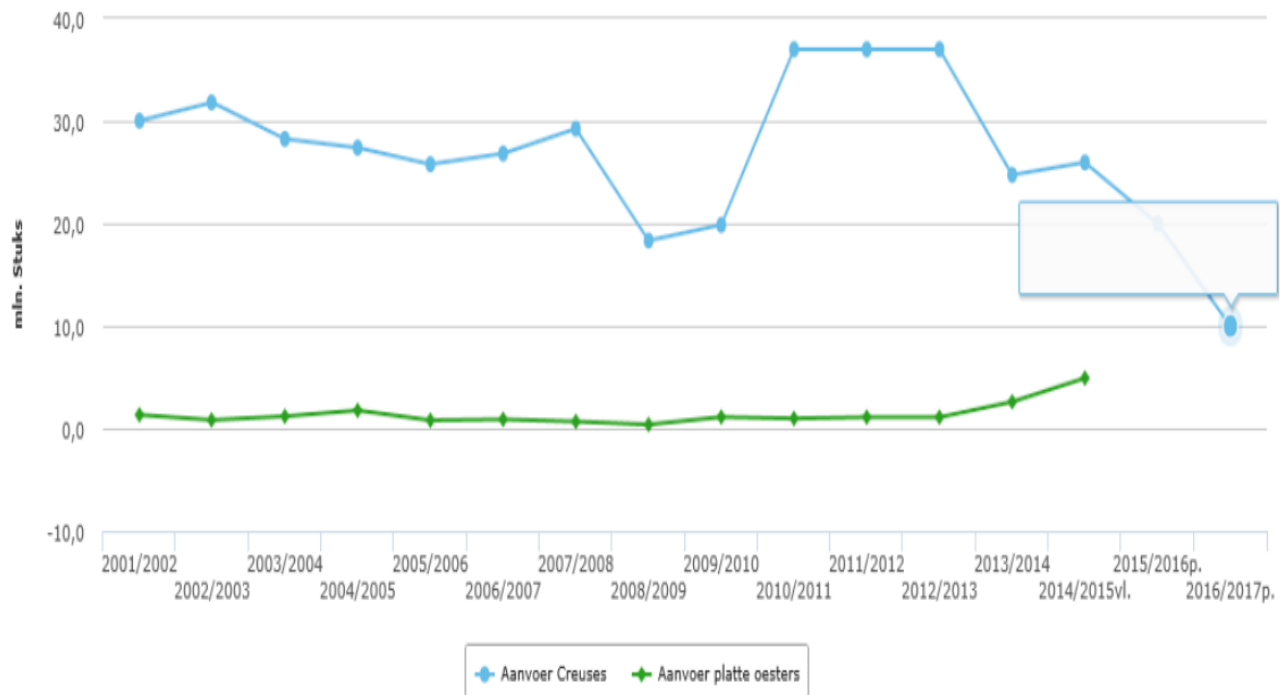


Figure 8: Estimated stock population (in millions) for the Pacific and European flat oyster in the Netherlands, from 2001-2017 (Smaal, Kamermans, & Strietman, 2016)

2.4.2 Potential impact of oyster drill expansion in the Netherlands

The 2012 risk assessment stated that there were no additional reports for Japanese oyster drills in other parts of the Eastern Scheldt or in other areas in the Netherlands (Lützen, et al., 2012), however, their increased distribution at Yerseke and Gorishoek suggests that further dispersal will follow (Lützen, et al., 2012). Since the species survives and reproduces well in the Eastern Scheldt, it could also perform well in other areas in the Netherlands. Despite the lack of “official” reports for additional dispersals, there have been numerous recent sightings by local researchers and farmers for Japanese oyster drill population increase, that claim otherwise (Smaal, Kamermans, & Strietman, 2016). If these claims are true, it is likely due to material, especially Pacific oyster stocks and other surface materials, being transported from contaminated areas into new areas (Didderen & Gittenberger, 2013).

Permits are needed to transfer shellfish from the Eastern Scheldt to the Dutch Wadden Sea under the Dutch Nature Protection Law, a law enforced as an effort to prevent contamination of cultured and natural shellfish stocks (Fey, van den Brink, Wijsman, & Bos, 2010). However, mussel transfers from the Danish and German Wadden Sea to the Dutch Wadden Sea do not require a permit. Furthermore, illegal, unregistered, or indirect transfers of shellfish from the Eastern Scheldt have been observed throughout the recent years. This further supports the fact that secondary introductions of the Japanese oyster drill have already occurred (Fey, van den Brink, Wijsman, & Bos, 2010). This presents a major threat for oyster cultivation in areas where Pacific oyster stocks are abundant, and in Lake Grevelingenmeer, where native oyster stocks are recovering (Troost, 2009).

2.4.3 Potential impact of oyster drills on European oyster cultivation and stock recovery

As mentioned earlier, efforts have recently been made to recover the depleted native oyster stocks in Lake Grevelingenmeer (Troost, 2009), resulting in a slight increase in the native stock population in the Netherlands around 2012 (Figure 8). As this species is already susceptible to high mortality rates and the *Bonamia* parasite, the potential impact of oyster drill expansion will hinder or completely destroy the progress made to recover and increase the native oyster population in the Netherlands (Troost, 2009, Smaal, Kamermans, & Strietman, 2016). In a study done on the impact of the Japanese oyster drill on the recovery of the Olympia oyster, researchers posed the concern that the smaller size of native Olympia oysters to Pacific oysters would make them more susceptible to drill predation. Building on the theory that drill predation is influenced by shell thickness, it was also inferred that there could be an advantage for Pacific oysters due to their thicker shells (i.e. less likely to be preyed upon), while adults of the native species would still be vulnerable (Buhle & Ruesink, 2009). The experimental trials found that although smaller oysters were preferred, drills also preferred Pacific oysters over the native Olympia oysters (Buhle & Ruesink, 2009). Since this study has not been done for a long duration of time (i.e. only 5 weeks) or on different oyster species, these concerns are still plausible for the European flat oysters in the Eastern Scheldt and elsewhere in the Netherlands.

2.4.4 Current strategies for management, mitigation, and elimination

Throughout the years, many strategies have been tested to address the oyster drill introduction and dispersal in the Eastern Scheldt. The common methods include manual removal, freshwater treatment, and chemical treatment (Didderen & Gittenberger, 2013). In regards to manual removal, there have been debates on which life stage (i.e. eggs or adults) should be targeted. Since Japanese oyster drills have high mortality rates, it would be more effective to target fecundity during the breeding season by removing egg capsules from affected areas (Buhle, Margolis, & Ruesink, 2004; Didderen & Gittenberger, 2013). Unfortunately, egg capsules are hard to find, can be improperly identified, and are often costly to remove (e.g. time and human resources); as a result, this strategy is deemed unsuccessful. In regards to freshwater treatment, it is theorized that immersing oysters in fresh water before transportation will cause oyster drills to detach from oysters (Mueller & Hoffman, 1999). Although very cost-effective, this method is very time-consuming; it was found that it could take more than 24 hours of exposure to 100% freshwater to affect acclimatized Japanese oyster drills in the Eastern Scheldt, if at all (Fey, van den Brink, Wijsman, & Bos, 2010). In terms of chemical treatment, Dutch fisheries have used an anti-fouling paint containing tributyltin (TBT) for a certain period of time. Although this was very effective against the Japanese oyster drills (i.e. the toxic substance injured or completely eliminated individuals), the use of TBT also greatly reduced the populations of native muricid species in the Eastern Scheldt. As a result, authorities have banned the substance in the Netherlands (Faasse & Ligthart, 2009).

Since mitigation and elimination strategies are currently unfeasible, the most appropriate course of action is management. Truthfully, there are limited options to manage established populations, however, it is possible to prevent secondary introductions of the species to other economically-important shellfish areas. For example, oyster farmers are advised to prevent transfers of oysters and hard materials from contaminated areas (i.e. where the Japanese oyster drill has established) (Didderen & Gittenberger, 2013). Since 2006, random samples have also been taken and checked for invasive species in all areas where shellfish and spat transfers are made to the Eastern Scheldt (Lützen, et al., 2012). Management strategies for Japanese oyster drills can also be implemented into the actual culture techniques for shellfish growers. Oyster growers in Japan and the Netherlands have implemented off-bottom techniques, including the use of trellises and off-bottom baskets and bags, that prevent shellfish contact to the bottom-dwelling drills and force the drills to feed on economically unimportant shellfish (Lützen, et al., 2012; BST, 2009). In the United States, authorities have also promoted the transition to hatchery-produced seed, thus preventing possible dispersals of the Japanese oyster drill from infested seed shipments (Fey, van den Brink, Wijsman, & Bos, 2010).

There are new solutions that continue to be investigated in Wallapa Bay, testing alternative methods to mitigate oyster drill populations. However, tested methods in controlled lab settings have not proven to be fully effective when transferred into the field (Heimbigner, 2012). For example, it was found that sample oyster drills secreted chemicals or hormones to signal when they have been injured in controlled lab systems, causing other individuals to evade the area and to cease feeding; however, the same results were not transferred to surveying results in the field (Heimbigner, 2012). Methods are also being explored to capture oyster drills before they mate and release egg capsules; these methods include the use of a glue-like substance on preyed barnacles, manual traps and traps involving pheromones, and the use of absorbent fiber. Unfortunately, these methods are currently expensive, labor-intensive, or require further lab and field trials (Heimbigner, 2012).

3. METHODOLOGY

3.1 EXPERIMENTAL CONTROLS AND CONDITIONS

3.1.1 Collection of sample oysters and oyster drills

Although the study was focused on the impacts of Japanese oyster drills on oysters from the Eastern Scheldt, the Pacific oysters used for the trial experiment and Experiment 1 were hand-picked from a natural oyster bed in the town of Vlissingen, in the Western Scheldt, due to time limitations. All the oyster drills were hand-picked by local oyster farmers in the Eastern Scheldt. It is important to note that the initial size of the Pacific and native spat samples used for Experiment 2 were smaller in comparison to the other oysters within the same size class, in the other feeding experiments. This is due to the fact that they were collected from hatcheries, and adhered to a standardized size.

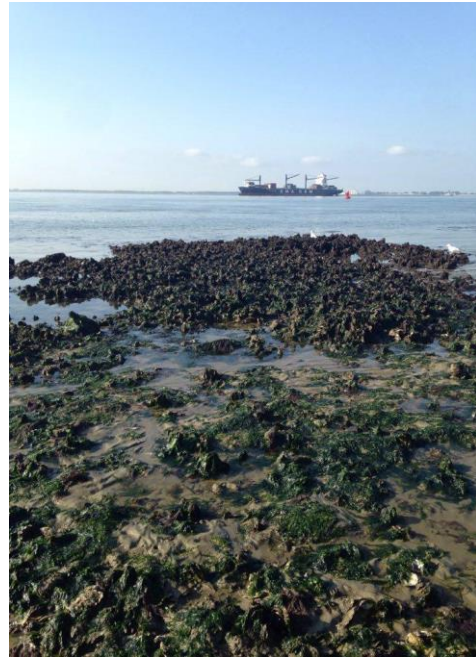


Figure 9: Site of oyster sample collection in Vlissingen, Western Scheldt (Babaran, 2017)

3.1.2 Temperature & Water Quality

As found in the study done by Oosterwal (2016), decreasing temperatures (i.e. less than 10 °C) resulted in decreased foraging or ceased foraging by Japanese oyster drills. As such, it was recommended that subsequent experiments should be done in controlled circumstances (i.e. indoors with set temperatures) or during the warmer months outside. The feeding experiments were done outdoors, where temperatures were at least 10°C. Similar to the study done by Keur (2017), the water temperature was set at 15°C with the use of a cooler, to reduce the influence created by fluctuating temperatures. Garden hose tubes with a 12mm diameter were connected to the cooler, and 2 pumps with a discharge of 10 L/min were used for the flow through. An air pump was installed for all experimental and storage boxes to provide aeration and sufficient oxygen levels. The water for all boxes was also refreshed every 3 days to maintain adequate water quality. Temperature and DO was monitored twice daily, using a YSI meter.

3.1.2 Experimental Substrate and Light Penetration

As found in the study done by Keur (2017), the Japanese oyster drill was most comfortable in captivity when the oyster shell substrate was used. Hence, oyster shells were used as the substrate for the experimental box, to imitate the natural environment of Japanese oyster drills most closely. A supply was taken from a previous study done on *C. gigas*. Similarly, oyster drills are usually found in deeper waters, hence, they were expected to be more accustomed to low light penetration. Hence, a tarp was used to cover the experimental containers throughout the study.

3.1.3 Starving and storage of oyster drills

Similar to the study done by Oosterwal (2016), the Japanese oyster drills used for the experiment were starved before the feeding experiments, in order to control the initial condition of sample oyster drills. The oyster drills were starved for 4 days in a separate storage container, and then placed in the experimental setup. Drills not in use for experiments were kept in a separate storage container (with aeration and stable temperature), and fed oysters to ensure their survival.

3.1.4 Feeding and storage of oysters



Figure 10: Columns with *Tetraselmis suecica* algae grown

Similar to the study done by Keur (2017), all the shellfish were kept in a flow-through basin (with aeration) before experiments. All shellfish were fed 1 L of *Tetraselmis suecica* algae, daily. The algae was regrown using 2ml/ liter of NHN medium. This process controlled the condition of the prey used for the feeding experiments, and ensured that they were all healthy and suitable for consumption by the oyster drills.

3.2 PREY AND PREDATOR SIZES AND LIFE STAGES

The length of all sample *O. inornatus* were measured prior to the experiments using a vernier caliper, from the posterior end to the anterior end. After measurement, all the *O. inornatus* were marked with different colors of nail polish, to clearly identify which size group each individual belongs to. From the study done by Amano & Vermeij (1998), an adult oyster drill reached a maximum shell length of 50mm. Hence, this study used shell lengths to depict the two life stages for the Japanese oyster drill: 20-27.5mm for juvenile oyster drills, and 27.6-50 mm for adult oyster drills. For this study, only the adult oyster drills were used.

The length of all sample *O. edulis* and *C. gigas* were also measured using a vernier caliper, from the posterior end to the anterior end. After measurement, all the oysters were marked with different colors of nail polish to clearly identify which size group and which oyster species each individual belongs to. From the study done by Oosterwal (2016), different shell lengths were already designated to the appropriate life stage, and were thus used in this study: 1-3.6 cm for oyster spats, 3.61- 6.6 cm for juvenile oysters, 6.61-10.6 cm for adult oysters. As mentioned previously, the oysters from Experiment 2 were slightly smaller than the oysters designated to the same size classes. This is due to the fact that they were obtained from a hatchery with standardized sizes.

3.3 EXPERIMENTAL SETUP



Figure 11: Experimental set-up with replicates, with flow through system, coolers, and aeration (Babaran, 2017)

This study used the same experimental setup as the study done by Oosterwal (2016). A curverbox was used with the dimensions of 77cm x 56.5cm x 30.5cm. By placing a transverse plank in the middle of the curverbox, the box was split into two rooms that acted as replicates. A total of two boxes were used to provide four replicates. Each box had the necessary flow-through system, and aeration.

3.3.1 Pre-trial Findings Using Drilled Shells

Before the experimental trials, 31 drilled oyster shell valves (from juvenile and adult oysters) were analyzed to determine whether there was a pattern for the location of drilling, and the shell thickness at the location of drilling. The results from the pre-trial findings were used as the basis for data analysis on shell thickness and the location of drilling. Specifically, the pre-trial tested whether it was possible to measure shell thickness using the configured vernier caliper (Figure 11) on three different sites on the shell (i.e. around the tip of the shell, at the middle section of the shell, and near the hinge of the shell), as well as on the lid and cup of the shell (i.e. the individual valves of the shell).

3.3.2 Trial Experiment on Drill Behavior in Captivity

Since there is limited information about the foraging behavior of *O. inornatus*, especially in captivity, a trial experiment was done to observe their behavior within the experimental setup. Specifically, the drill's behavior to controlled light availability and a 4 day starvation period were observed. One curver box was split into two replicates: both sides were covered with a tarp to limit light exposure. All other aspects of each replicate mimicked the original setup. Five oyster drills were placed into each room, and left to their own devices. After 4 days of starvation, 15 oysters of different size classes (spat, juvenile, adult) were put inside each replicate, to see if the oyster drill would eat in captivity and the duration of time needed until feeding behavior manifested in the drills. The oysters were monitored every 3 days over a week to check for signs of predation; any signs of foraging were noted. The results from the trial experiment were used to improve the experimental set-up and time duration for Experiment 1 and 2.

3.4 EXPERIMENT PROTOCOL & PROCESSING

The experiment protocol, as well as the parameters for analysis were based on the previous study done by Oosterwal (2016), as well the study done by Buhle & Ruesink (2009).

3.4.1 Experiment 1: Size preference for *C. gigas*

For Experiment 1, 10 oyster drills were used for each replicate. The drills were offered 15 Pacific oysters in total, 5 from each assigned age class, which were spread randomly around the experimental box. Since it was uncertain whether the drills would feed immediately, the study was done over the course of 9 days, and monitored every 3 days for signs of predation and probing. The prey were fed as needed, while the oyster drills were left to prey on the oysters.

After the experiment was done, the oysters were processed and dissected immediately. All predated and untouched oysters were grouped, measured for shell length, and identified for size class. They were then dissected so that the shells were removed. The drill holes were first counted on each valve (i.e. the cup or lid of the shell), and then the thickness of each valve was measured using a vernier caliper. Three measurements were made for three locations along the shell: at the tip of the shell, the middle of the shell, and near the hinge of the shell. The overall location of drilling was also noted (i.e. none, tip, middle, hinge).

3.4.2 Experiment 2: Species preference for *C. gigas* and *O. edulis*

For Experiment 2, 15 oyster drills were used for each replicate. The drills were offered 10 oysters in total, 5 from each species of the spat size class, which were spread randomly around the experimental box. Since it was uncertain whether the drills would feed immediately, the study was done over the course of 9 days, and monitored every 3 days for signs of predation and probing. The prey were fed as needed, while the oyster drills were left to prey on the oysters.

After the experiment was done, the oysters were processed and dissected immediately. All predated and untouched oysters were grouped, measured for shell length, and the species was identified. They were then dissected so that the shells were removed. The drill holes were first counted on each valve (i.e. the cup or lid of the shell), and then the thickness of each valve was measured using a vernier caliper. One measurement was made for three locations along the shell: at the tip of the shell, the middle of the shell, and near the hinge of the shell.



Figure 12: Vernier caliper configured to measure shell thickness (Babaran, 2017)

3.4.3 Disclaimer

1. Only adult oyster drills were used for these experiments, since they are predicted to be the biggest threat to oysters (i.e. they are predicted to be stronger, and to be capable of greater damage for oysters).
2. Although Oosterwal (2016) and Keur (2017) both starved the oyster drills for one week before feeding trials, this study reduced the starvation period to 4 days to prevent cannibalism in the group of drills, which was observed in previous studies.
3. Spat oysters were used in Experiment 2, rather than other size classes, due to time limitations and resource availability from local oyster hatcheries. Although juveniles would have been easier to analyze and to measure (especially for shell thickness and the location of drilling), the use of spat samples were still consistent with previous theories that oyster drills prefer smaller individuals to larger individuals.
4. Although it is advisable that the experiments run over 2 weeks, the duration of each experiment was cut down to 9 days. This was done to meet predicted time limitations.
5. The total number of drills used in Experiment 2 increased due to the low feeding activity observed in both the trial experiment and Experiment 1, which used fewer adult drills. This was done to get more accurate and conclusive results regarding prey species preference.

3.5 DATA ANALYSIS

Data analysis for this study was done using the IBM SPSS Statistics 23 software and the Microsoft Excel 2016 software. Mean values for the total number of holes drilled on each valve and shell thickness were calculated using the replicate measurements for each experiment, and compared between oyster species, size classes, valves, and shell locations. Data was analyzed using the standard analysis of variance (ANOVA) techniques or the Welch's ANOVA, depending on whether assumptions for equal variance is met. Further analyses was done using the appropriate post-hoc tests, i.e. Tukey or Games- Howell tests. Homogeneity of Variances was tested using Levene's test. Normality was tested using a Q-Q plot. If Equal Variance Assumption was not met, classic ANOVA tests and Tukey post hoc tests were not performed. The Welch's ANOVA and Games-Howell post hoc test were done when the Levene's test was significant. In all tests, the level of significance was $p= 0.05$.

4. RESULTS

4.1 PRE-TRIAL FINDINGS (DRILLED SHELLS)

From the 31 Pacific oyster samples examined, it was found that more adult shells (68-100mm) were drilled than juvenile shells (50-67 mm). The drill holes were primarily found in the middle and near the hinge of the shell (18 and 13 drill holes counted, respectively). Grouping the drilled adult and juvenile shells, it was found that both size classes were targeted primarily in the middle of the shell, and least in the tip of the shell (Figure A-1).

In terms of shell thickness, it was found that the drilled adult shells were thicker than the drilled juvenile shells in all sections. The thinnest section of the adult shells (i.e. middle section) coincided with the most targeted shell location for drilling. However, the thinnest section of the juvenile shells (i.e. tip) did not coincide with the most targeted shell location for drilling (Figure A-2).

From the pre-trial findings, there was not conclusive evidence that supported the theory that shell thickness was a significant factor that influenced prey preference. Similarly, due to the lack of drilled spat shells or native oyster shells, no conclusions could be made regarding prey size or prey species preference.

4.2 TRIAL EXPERIMENT

In general, the trial experiment confirmed that the oyster drills were able to live and feed in captivity within the experimental set-up. The first signs of probing from the oyster drills were found after 3 days in the experimental set-up, although only in Replicate 1. At the end of the trial experiments (i.e. 7 days), only 2 oysters in total were drilled with the rest of the oyster samples untouched; the adult oyster was 92mm in length and the oyster spat was 30 mm in length. During the experimental trial, 2 oyster drills (one from each replicate) died. These deaths, as well as the low total predation, may be accounted for by the malfunctions in the cooler and aeration system, as well as the extreme heat recorded during the study period. Data analysis for this experiment was limited due to the lack of predation, as well as the limited number of active predators in each replicate. As a result, the number of oyster drills used in each replicate was increased to 10 for Experiment 1.



Figures 13 & 14: Photos of the two drilled oyster samples from Replicate 1 (Babaran, 2017)

4.3 EXPERIMENT 1

4.3.1 Size preference (Number of boreholes observed)

Out of the total 60 Pacific oyster samples used in Experiment 1, only 13 oysters were drilled, for a total of 14 boreholes recorded after 9 days of monitoring. Examining Figure A-3, it can be seen that the juvenile samples were the most targeted with 6 boreholes, followed closely by the spat samples with 5 boreholes. The adult samples were the least targeted with 3 boreholes. Only one sample in the juvenile size class had more than one borehole drilled (i.e. 2 boreholes found).

To test whether the boreholes recorded between each size class was significantly different, the mean number of boreholes were compared and the ANOVA test was used (Table B-1, Table B-2). The ANOVA test indicated that the number of boreholes drilled between size classes was not significantly different ($p>0.05$).

4.3.2 Location of drilling

As seen in Figure A-4, Pacific oyster samples from all size classes were drilled through in the middle and tip of the shell. However, with the juvenile oysters, there were samples that were also drilled through at the hinge of the shell. Overall, the majority of samples from each size class were not drilled through (i.e. no predation).

To further examine the preferred location for drilling, the valves for each oyster sample were examined individually, with Valve 1 representing the cup of the shell, and Valve 2 representing the lid of the shell. Figure A-5 shows that almost all the boreholes were found on Valve 2, with one exception on a spat sample.

4.3.3 Shell thickness

Examining the specific locations for drilling from the previous section, the shell thickness was compared across all size classes. Figure A-6 and Table B-3 indicate that across all size classes, the hinge was the thickest part of the shell, followed by the middle and the tip. The shells of all adult oysters were thicker than the juvenile oysters in most locations, while the shells of the juvenile oysters were thicker than the spats in all locations (Figure A-6, Table B-4). Between Valve 1 (the cup) and Valve 2 (the lid), shell thickness was slightly greater for Valve 1, for oysters of all size classes (Figure A-6, Table B-5). Overall, there was a high standard deviation for all shell thickness measurements (Figure A-6).

ANOVA tests and Games-Howell post hoc tests were used to check whether mean shell thickness between each size class and between each shell location was significantly different. The Welch's ANOVA test indicated that there was a significant difference in mean shell thickness between size classes ($p<0.05$) (Table B-6). The results from the post-hoc test indicated that mean shell thickness between adult and spat samples, as well as juvenile and

spat samples were significantly different (Table B-7). Mean shell thickness between the juvenile and adult samples was not significantly different (Table B-7). The Welch's ANOVA test indicated that there was a significant difference in mean shell thickness between shell locations ($p < 0.05$) (Table B-8). The results from the post-hoc test indicated that mean shell thickness between the hinge and middle of the shell, as well as the hinge and tip of the shell were significantly different (Table B-9). Mean shell thickness between the middle and tip of the shell was not significantly different (Table B-9). A Welch's ANOVA test was also used to check whether mean shell thickness between Valve 1 and Valve 2 was significantly different; the results found that it was significantly different ($p < 0.05$) (Table B-10).

4.4 EXPERIMENT 2

4.4.1 *Species preference (Number of boreholes observed)*

Out of the total 40 Pacific oyster and native European flat oyster spat samples used in Experiment 2, 26 oysters were drilled (13 from each species group), for a total of 29 boreholes recorded after 9 days of monitoring. Examining Figure A-7, it can be seen that the native oysters were more targeted with 15 boreholes, compared to the 14 boreholes on the Pacific oysters. There were two native samples that were drilled twice, and only one Pacific sample that was drilled twice.

To test whether the boreholes recorded between each species group was significantly different, the mean number of boreholes were compared and the Welch's ANOVA test was used (Table B-11, Table B-12). The Welch's ANOVA test indicated that the number of boreholes drilled between species groups was not significantly different ($p > 0.05$).

4.4.2 *Shell Thickness*

Comparing shell thickness between the drilled and undrilled samples, Figure A-8 and Table B-13 indicated that the shell thickness of undrilled samples was greater. Between the native and Pacific species, Figure A-8 and Table B-14 indicated that the shell thickness of Pacific spat samples was greater. Overall, there was a high standard deviation for all shell thickness measurements (Figure A-8).

ANOVA tests were used to check whether mean shell thickness between the drilled and undrilled samples, and between the Pacific and native species groups, was significantly different. Both Welch's ANOVA tests indicated that there was not a significant difference in mean shell thickness ($p > 0.05$) (Table B-15, Table B-16).

5. DISCUSSION

5.1 SIZE PREFERENCE

As seen in Figure A-3 and Table B-2, the number of boreholes drilled between the size classes of the Pacific oyster was not significantly different, contradicting past studies that suggested that oyster drills would be more inclined to feed on oyster spat or juveniles (Fey, van den Brink, Wijsman, & Bos, 2010). However, it is also important to note that juveniles and spat (i.e. samples that were 1-6.6 cm) were overall more targeted than the adult samples (6.61-10.6 cm). This could suggest that although the number of boreholes drilled between each distinct size class was not significantly different, there could still be a difference with the number of boreholes drilled between the smaller oysters compared to the larger market-size oysters. One factor that may have influenced the results regarding size preference is the actual feeding behavior of oyster drills. In a study done on the prey size choice of the southern oyster drill, it was found that larger drills would choose their prey based on whether they were foraging as a single individual or in a group (i.e. feeding in a group of either all large drills, or a mixture of large and small drills) (Robinson & Richardson, 1988). For example, larger drills preferred larger prey in the situation of group feeding; this was due to the fact that larger oysters had longer handling times and a higher rate of decomposition before consumption (Brown & Richardson, 1988). Essentially, group feeding would be more profitable for larger drills, as it allowed them to increase the size and selection of their prey, as well as the total amount of individuals they preyed upon (Brown & Richardson, 1988). The possible effects of predator density can be supported by field surveys of oyster drills in Willapa Bay, where densities of up to 800 individuals per square meter were found to be capable of large-scale damage on natural oyster reefs (Joseph, 2004). In Experiment 1, there was a relatively low predator density (i.e. 10 adult oysters per replicate) and limited occurrences where the drills preyed on the same oyster. It is possible that group feeding behavior was not initiated throughout the study, and hence, the adult drills were choosing their prey and prey size in terms of their potential gains as single individuals. This could explain why the sampled drills targeted more of the smaller oysters, and why the total feeding rate was relatively low (i.e. it is relatively easier and faster to fully consume a smaller oyster than a larger oyster within a given time period).

Nevertheless, the low amount of boreholes drilled throughout Experiment 1 supported the fact that the final results were also partially inaccurate. The oyster samples used for the trials were all healthy individuals and the natural prey species of the Japanese oyster drill (Lützen, et al., 2012; Didderen & Gittenberger, 2013). In theory, the adult oyster drill should have been able to drill through at least 3 small oysters within a week (Boersma, et al., 2006). However, less than half of the oyster samples were drilled over the course of 9 days (i.e. only 13 oysters in total out of 60 oysters). Since there was only 1 oyster drill death and 29 visibly healthy oyster drills throughout Experiment 1, it is evident that there was an unusually low total feeding rate. The most plausible reason for the low predation is the time required for the oyster drills to adjust to captivity before feeding. From previous feeding experiments (Oosterwal, 2016), it was also noted that there were limited results from 4 day trials, due to

low predation from sample oyster drills. Although there was still a low total feeding rate throughout the 9 day trials, there were some oyster drills attached to new oyster samples on the final monitoring day. However, these drills had not yet created boreholes. This suggests that at 9 days, more drills could have just completed their adjustment to the conditions within the experimental setup, and were only beginning to conduct their initial probing and selection for prey. If this is true, then it may explain the seemingly low feeding rate throughout Experiment 1. It is also important to note that during the trials for Replicate 3 and 4, there was a prominent rain and windstorm that affected the experimental set-up and the cooling/heating system, resulting in an influx of rain and a decrease in temperature. From previous feeding experiments done by Oosterwal (2016), it was found that oyster drills could enter a state of hibernation at temperatures below 10 °C, reducing or inhibiting their feeding activity. The sudden change in temperature during this time period could have also provided a physical shock to the drills, inhibiting their feeding activity. Likewise, the effects of unnatural stressors, specific to the captivity and the experimental setup, should be considered. For example, whenever the sample drills tried to climb or escape the experimental setup, they were manually removed and returned to the substrate level. This added and unnatural stressor could have conditioned their behavior, affecting their actions within the experiment (including their feeding activity). In general, the drills were held in an enclosed space, with limited capacity to wander around and to climb; their feeding behavior and prey choice may have differed if the experiments had taken place in the field.

5.2 LOCATION OF DRILLING AND SHELL THICKNESS

Despite the low total feeding rate recorded in Experiment 1, new information could still be obtained from the data, such as the preferred drilling sites on preyed Pacific oysters. Firstly, the adult oyster drills created boreholes through mainly the middle and tip area of the shell, and on the shell lid for all size classes (Figure A-4, Figure A-5). One plausible reason for these preferred drilling sites is shell thickness, a commonly identified factor for feeding behavior in the literature (Fey, van den Brink, Wijsman, & Bos, 2010). Similar to the theory that oyster drills may target certain size classes based on shell thickness, oyster drills may also probe and select sites for drilling based on whether their radulas can easily drill through that area of the shell (Lützen, et al., 2012; Lord & Whitlatch, 2013). This theory was found plausible from the results of the pre-trial, where drilled adult and juvenile Pacific oyster shells were collected to examine the site of drilling, and their specific shell thicknesses. The results from the pre-trial found that the middle section of the shell was most frequently drilled for both size classes; this preferred drilling site also coincided with one of the thinner sections of the shell (Figure A-1, Figure A-2). For Experiment 1, the thinner sections of the shell (i.e. middle and tip) also coincided with the most targeted locations for drilling (Table B-9). Similarly, the thinner valve of the oyster (i.e. Valve 2, the lid) coincided with the most targeted valve for drilling (Table B-10). The given results suggest that it is, hence, plausible for shell thickness to explain why adult oyster drills would choose to drill through the middle or tip of the shell rather than hinge, or through the lid of the shell rather than the cup. Nonetheless, it is important to note that there was a high standard deviation for all shell

thickness measurements in Experiment 1 (Figure A-6), relating to the varying shell morphologies of oysters within and between all size classes (e.g. slightly flatter valves, thicker shell locations). Likewise, the shell thickness difference between valves may only be significant for Pacific oysters, or only for adult oysters.

The post-hoc tests from Experiment 1 also gave evidence for a significant difference in shell thickness between different size classes of the Pacific oyster; Table B-7 indicated that there was a significant difference in shell thickness between spats and the larger size classes. Based on the theory that drills chose their prey size based on the ease of drilling, it would thus be plausible that adult oyster drills chose spats over larger size classes due to their thinner shells (Lützen, et al., 2012). However, this theory for shell thickness could not be applied to prey preference between juveniles and adults, since there was no significant difference found (Table B-7). Furthermore, the high standard deviation for all shell thickness measurements cannot be ignored (Figure A-6); shell thickness varies widely within and between size classes due to the natural differences in shell morphology for oysters collected from the natural environment. Lastly, due to the low total feeding rate and the lack of significance found in Experiment 1, the results regarding prey size choice and shell thickness are inconclusive. Although spats had the lowest shell thickness between size classes, there was no significant difference found in predation (Table B-2). Another plausible theory for prey size choice was found in a study done on southern oyster drills, where larger drills were found to have a preference for larger prey with thicker shells, despite being harder to handle, due to an increase in their feeding rate and their efficiency in tissue consumption as they grew in shell length (i.e. body size). On the other hand, it was found that smaller drills had a preference for smaller prey, as the growth rate from those feeding on oyster spat was six times greater than those feeding on large oysters (Brown & Richardson, 1988). Essentially, all drills chose their prey and prey size based on their maximum potential for growth (in terms of the dry mass of edible tissue ingested and the handling time, and accounting for the losses from respiration and excretion) in proportion to their body size (Brown & Richardson, 1988).

5.3 SPECIES PREFERENCE

Overall, the total feeding rate improved for Experiment 2 compared to Experiment 1; more than half of all the oyster samples were preyed on. The increased feeding activity can be most attributed to the increased number of drills used in each replicate (i.e. 15 drills per replicate, compared to 10 drills per replicate), thereby increasing the total number of active predators in the experiment in proportion to prey available. It is important to note, however, that there were a few dead native and Pacific oysters found among the undrilled samples. This suggests that there may have been samples that died within the 9 days of the experiment, before consumption. In the study done by Keur (2017), it was found that drills only ate healthy, fresh oysters when in captivity. Unlike the spat samples used in Experiment 1, the spat samples for the species preference experiments were taken from a hatchery in the Eastern Scheldt. Before being transferred to the experimental site, they were stored in a shallow basin and given only the bare minimum (in terms of feed and storage conditions) for survival. These

spat samples were given proper care once they arrived at the experimental site, and only visibly healthy individuals were taken as samples; however, it is still possible that some of these individuals were in a weakened state entering into Experiment 2. It is equally plausible that the condition of these weakened individuals worsened throughout the study, thus explaining why the drills avoided them and chose to prey on healthier samples.

As seen in Figure A-7 and Table B-14, the number of boreholes drilled between the Pacific oyster species and the native European oyster species was not significantly different. This contradicts an experiment done between Pacific oyster and the native Olympia oyster in Washington, which indicated that there was ingestive conditioning observed or an effect of diet history on the species preference (Buhle & Ruesink, 2009). The results from the Buhle & Ruesink study (2009) found that sample drills preferred the Pacific oyster, since they were the more abundant species in the location from where they were collected as samples (i.e. their natural environment). The collected sample drills for Experiment 2 were taken close to a local farm in the Eastern Scheldt, where the Pacific oyster species was frequently found and more abundant; hypothetically, the drills should have thus been more attracted to the Pacific spat samples. Since there was no preferred prey species found, the results for the current study support the alternative theory posed by Lützen, et al. (2012), which states that the Japanese oyster drill is a generalist species that is flexible on prey species choice. If the drills are free to switch between prey species, then prey species choice may have been more influenced by the factors of prey availability and abundance at the moment of feeding. In a study done by Carricker (1981) on Japanese oyster drill behavior, it was found that oyster drills would search for another available prey species if their former target species declined significantly in abundance. During the feeding experiments, however, there was a relatively low but equal abundance of each prey species (i.e. 5 spat samples for the native and Pacific oyster species). Since there were an equal abundance of each prey species, it is likely that the oyster drills simply targeted both species available in their environment, in order to satisfy their hunger.

Examining the shell thickness results from Experiment 2, there was also no significant difference found in shell thickness, regardless of whether the samples were drilled or not, and whether they were native European flat oysters or introduced Pacific oysters (Table B-15. Table B-16). The results, hence, do not support the additional theory that oyster drills choose their prey (in terms of species) based on shell thickness (Lützen, et al., 2012; Lord & Whitlatch, 2013). It is important to note, however, that the spat samples for Experiment 2 originated from a local hatchery. Since hatcheries adhere to certain size and shape standards (compared to spats collected from the natural environment), it is possible that the prior conditioning and cultivation of the spat samples influenced their shell morphology and hence, their shell thickness.

6. CONCLUSION

Although the smaller oysters were altogether more drilled than the larger oysters, the results from the size preference experiments did not find any significant difference in predation between the distinct life stages (i.e. spat, juveniles, adults). Hence, the hypothesis that spats and juveniles would be most susceptible to predation is not fully supported. Nonetheless, the results gave insight on the potential vulnerability of all oyster size classes to the Japanese oyster drill population in the Netherlands (particularly in the Eastern Scheldt). Essentially, the lack of a size preference suggests that no size class can fully avoid predation, thereby rejecting the hypothesis that market-size oysters would be able to avoid drill predation. In the context of commercial oyster farms, the final results are especially relevant for oyster farmers that practice bottom culture techniques, as this type of culture creates greater opportunities for contact with and predation from Japanese oyster drills. Regarding the influence of shell thickness on prey size choice, the results found shell thickness to be an inconclusive factor due to the wide variation in shell morphology, both between and within size classes, as well as across the shell and between shell valves. However, there are still unanswered questions regarding the effects of predator size and predator density, as well as the potential effects of unnatural stressors (specific to captivity).

Similar to prey size choice, the results from the species preference experiments indicated that there was no significant difference in predation between the introduced Pacific oyster and the native European flat oyster. Thirteen spat samples from each species group were preyed on, hence, the hypothesis that the drills would prey more on their natural prey species is rejected. The results found that the sampled oyster drills did not display any effects from diet history, and instead, showcased their ability to be generalist species. With a constant and relatively low total prey abundance, prey availability at the moment of feeding was thus identified as the prominent factor influencing prey choice; in the case of the study, the drills chose to feed on all individuals available (regardless of their species) to satisfy their hunger. Since there was no species preference found, the study suggests that both oyster species are equally impacted by the existence and growth of the Japanese oyster drill population. In the context of the native European flat oyster, farms operating specifically for native oyster cultivation and population recovery would therefore be most effective, when restricted to areas unoccupied and uncontaminated by the Japanese oyster drill. Unfortunately, this is not very realistic in practice, considering the established populations of the Japanese oyster drill in the Eastern Scheldt, and their rapid dispersal rate throughout the Netherlands. Concerns for the native oyster population are especially relevant amidst the numerous “unofficial” sightings for oyster drill dispersals, including near Lake Grevenlingenmeer, where native oysters are currently most abundant.

7. RECOMMENDATIONS

7.1 CONTINUATION OF THE RESEARCH

Due to the low feeding activity observed in Experiment 1, conclusive results could not be obtained for the prey size preference of adult oyster drills. As such, Experiment 1 should be repeated, with certain modifications to the time duration and the total number of oyster drills used. Specifically, the experiment should use 30 oyster drills per replicate, and the study duration should be extended to two weeks. The total feeding rate would increase with more oyster drills acting as potential predators, and the oyster drills would be more likely to feed in captivity after a longer period of adaptation to the experimental setup. To explore the additional factors of predator size and predator density on prey size choice, presented by Brown & Richardson (1988), two follow-up feeding experiments should be conducted. The first experiment should focus on the prey size choice of juvenile oyster drills, to determine whether the juvenile drills would target different or specific class sizes compared to the adult drills. The second experiment should focus on the effects of varying predator densities (e.g. 10 drills per replicate, 30 replicates per replicate, 50 drills per replicate), to determine whether group feeding behavior would be initiated and whether such behavior would affect their prey size choice. An experiment could also be done to explore the interactions between predator size and density, involving varying levels of drill body size and drill density.

Although the total feeding rate improved drastically in Experiment 2, there are still various adjustments that could be made to the protocol, in order to improve the accuracy of results. Specifically, Experiment 2 should be repeated using juvenile or market-size samples instead of spats. Different size classes could provide different results regarding species preference, as well as on shell thickness between species groups. Similarly, the oyster samples used for the experiment should be kept in the optimal condition, even in the days or weeks prior to the experiment (i.e. in storage), to ensure that there are no sample deaths or weakened samples during the feeding experiments. Additionally, the number of oyster samples should be increased to 20 oyster samples per replicate (i.e. 10 oysters for each species group). This would ensure that there is an adequate prey abundance for oyster drills within each replicate, and would improve the ability to distinguish prey preference between the two species groups (i.e. the drills will be more likely to make a distinct choice between species, rather than blindly preying on available individuals).

As a follow-up to all feeding experiments, the option of field experiments should be considered. From the limited research done on the Japanese oyster drill, most studies have been done in a controlled lab environment. In order to examine the true feeding behavior of the drill in Dutch waters, it would be useful to perform feeding experiments in the field and to compare these prey preference results to the lab trials. This would verify whether the previous results were affected by unnatural stressors, specific to captivity and the lab environment. It is important to note, however, that field experiments should be carried out with utmost caution, in order to avoid additional dispersals of adult drills and their eggs in Dutch waters. One recommendation for the field experiments would be to tag the individual drills using

electronic chips; by tracking the movement of sample drills, it would be possible to locate drills that escape and to pinpoint possible areas for laid egg capsules (Heimbigner, 2012). Other factors that must be considered during the planning stage of field feeding experiments include the increased difficulty for setup of equipment (i.e. transporting and unloading containers and nets into the field site), and the limited accessibility for monitoring and measurements (i.e. sites may only be accessible in low tide, suitable weather, or certain seasons).

7.2 ADVICE FOR OYSTER FARMERS

As mentioned previously, there is no feasible method to fully eliminate oyster drills in the Netherlands, and only limited options in terms of management; this is especially true for addressing the established populations in the Eastern Scheldt. Nevertheless, since all oyster class sizes are equally susceptible to predation, oyster farmers should place a high priority on understanding and developing appropriate drill management strategies. The recommended advice for local oyster farmers should be to avoid the use of bottom culture practices, thereby reducing direct contact of commercially-grown oysters to bottom-dwelling oyster drill populations. Off-bottom culture practices, such as the use of off-bottom baskets, trellises, and bags should be invested on and implemented in all operations (Lützen, et al., 2012; BST, 2009). Similarly, authorities for the shellfish industry should implement a mandatory screening of shipments (i.e. before packing or unloading) in the protocol of all oyster farmers, thereby preventing the contamination of oyster stocks and secondary introductions to other economically important shellfish areas (Fey, van den Brink, Wijsman, & Bos, 2010).

Regarding the native European flat oyster population, there is no certain method to predict where Japanese oyster drill populations will spread to in the future. Although it is still viable to increase native oyster populations in the current areas where they are successful, it may be too soon to state whether large-scale operations for native oyster cultivation in these areas will be successful. Since there has been a slight increase in the native European flat oyster population in Netherlands reported in recent years, the management of the oyster drill population should be the top priority for continuous success and progress in stock recovery and cultivation. The main recommendation for oyster farmers would be to prevent secondary introductions of oyster drills to areas where native oysters are abundant. This can be achieved through the use of hatchery seeds for the cultivation of native oysters (instead of imports from potentially-contaminated areas) and the screening of shipments entering these areas (Fey, van den Brink, Wijsman, & Bos, 2010). Since there is still limited knowledge on the feeding behavior of the Japanese oyster drill and their prey preference, it would also be useful for local farmers to personally monitor their stocks for drill predation and drill feeding behavior. Through regular monitoring, local farmers could potentially discover new feeding patterns or characteristics of the Japanese oyster drill, which could then build on current knowledge, and advance future research on drill management.

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10. APPENDICES

APPENDIX A. FIGURES

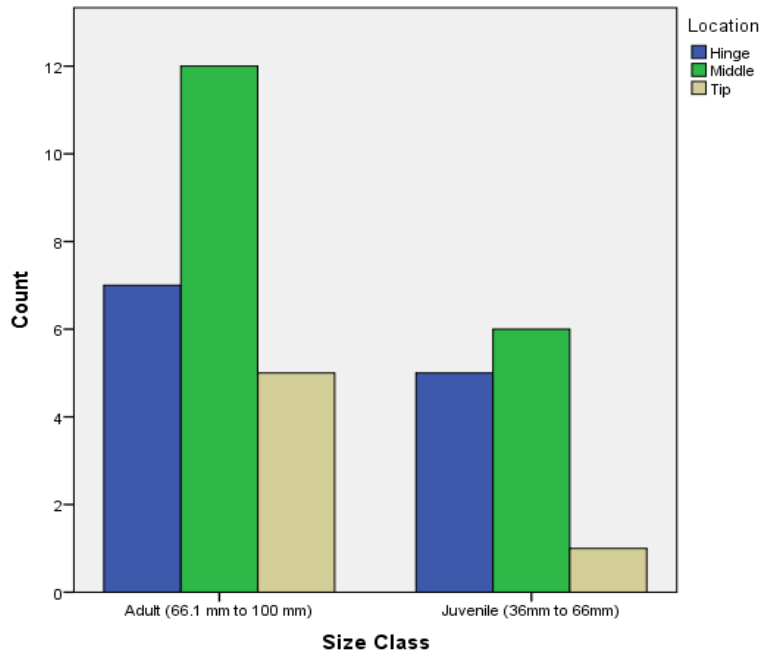


Figure A-1: Comparison of predation (number of boreholes counted) on the different locations for drilling amongst the 31 shell samples, for pre-trial findings

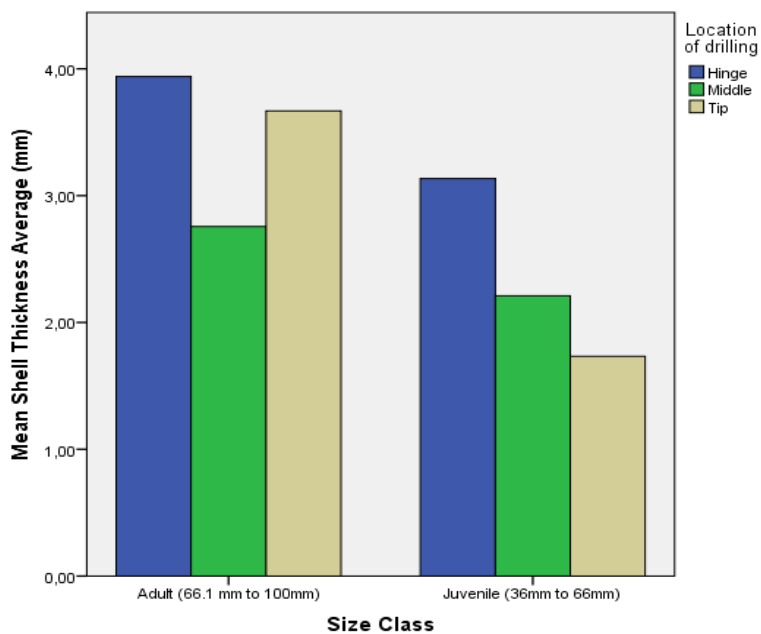


Figure A-2: Comparison of mean shell thickness (mm) at the location of drilling between the drilled shells of adult and juvenile oyster samples, for pre-trial findings

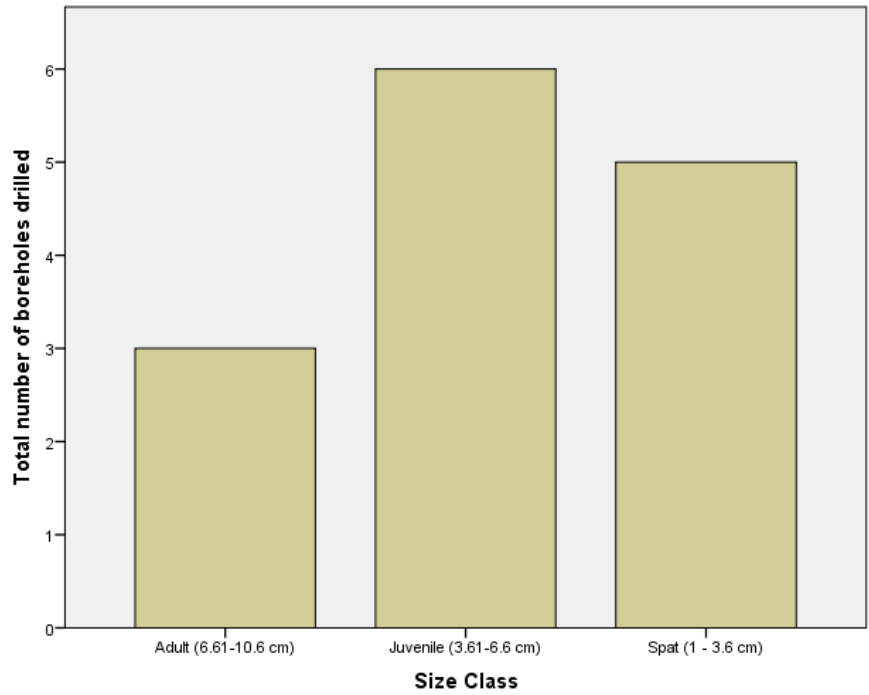


Figure A-3: Total number of boreholes drilled in Pacific oyster samples, within each size class, for size preference experiments

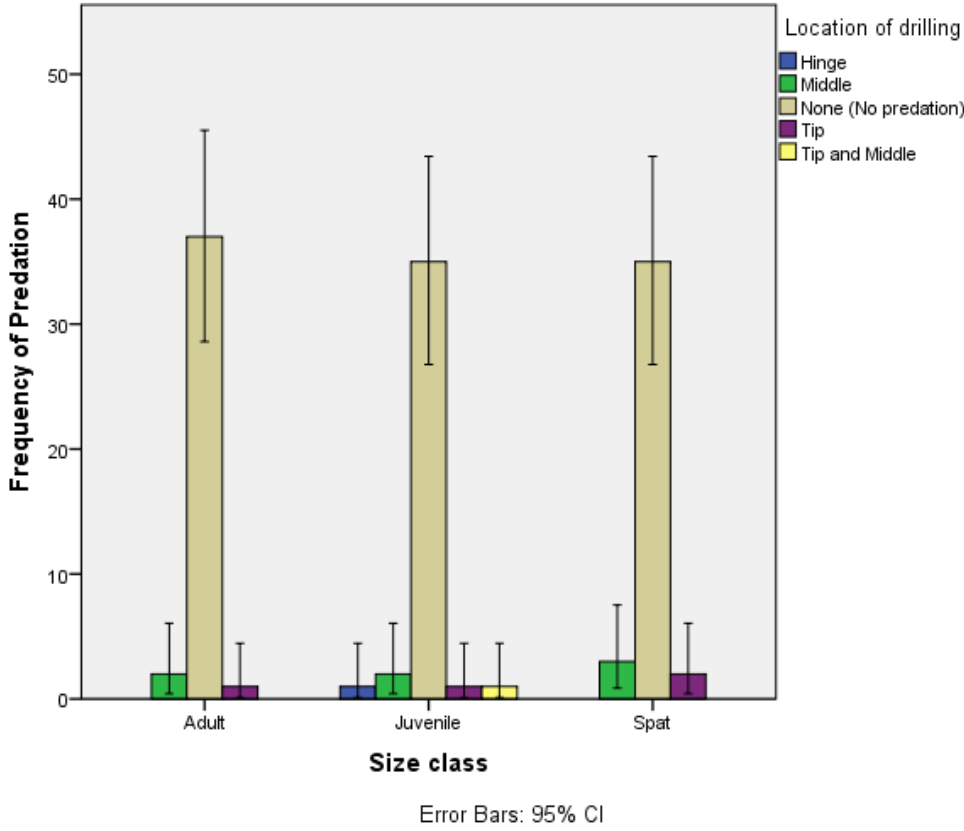


Figure A-4: Location of drilling for Pacific oyster samples of different size classes, for size preference experiments

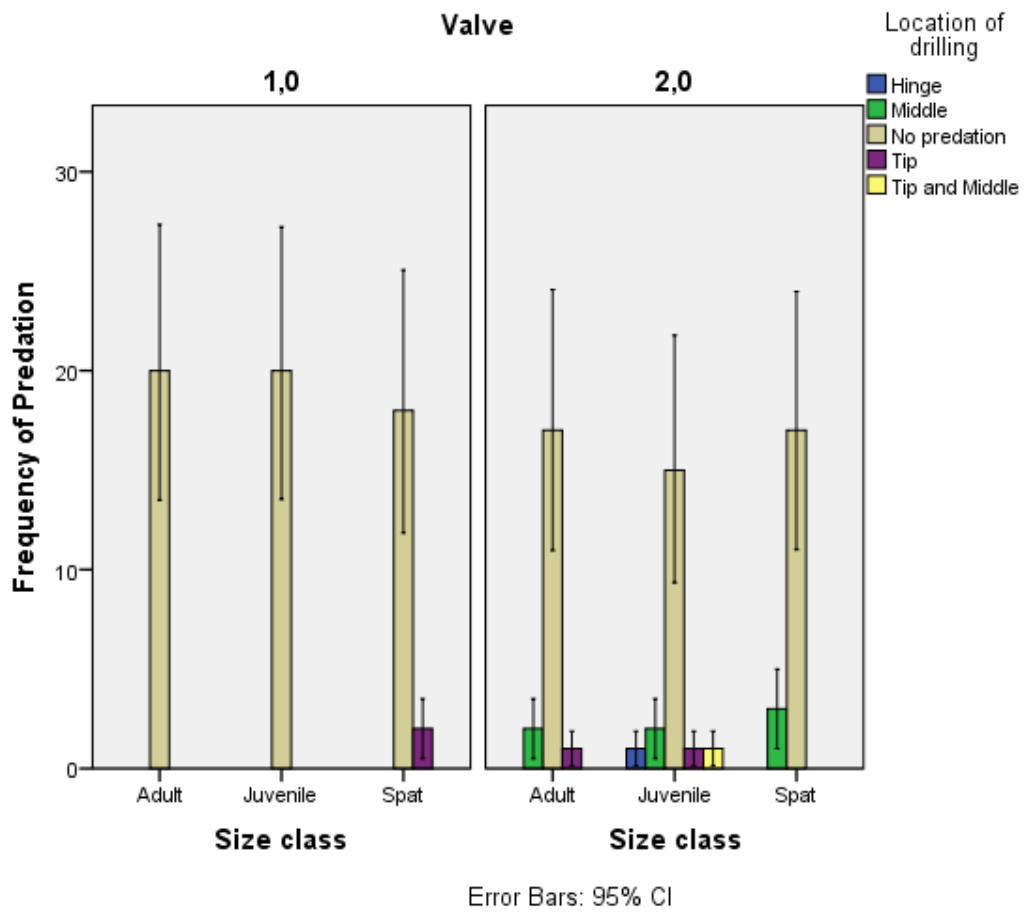


Figure A-5: Location of predation, separated by valves, for all Pacific oyster samples, in different size classes, for size preference experiments

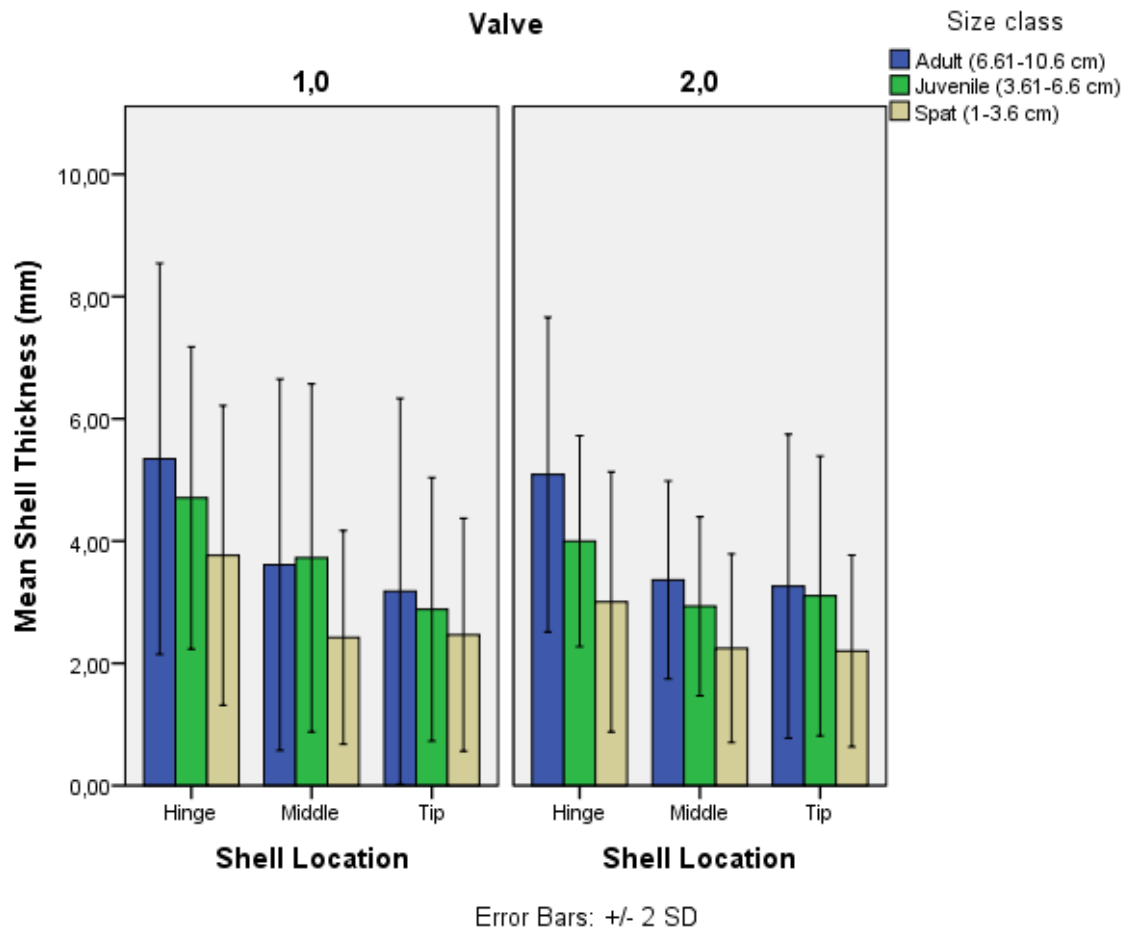


Figure A-6: Mean shell thickness (mm) at specific shell locations on Pacific oyster samples of different size classes, for size preference experiments

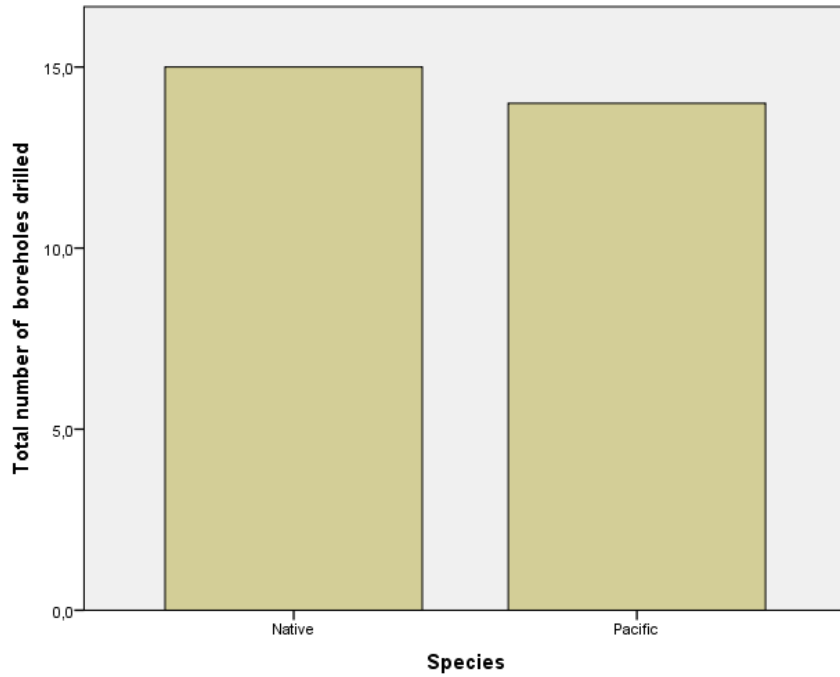


Figure A-7: Total number of boreholes drilled in Pacific and native spat oyster samples, for species preference experiments

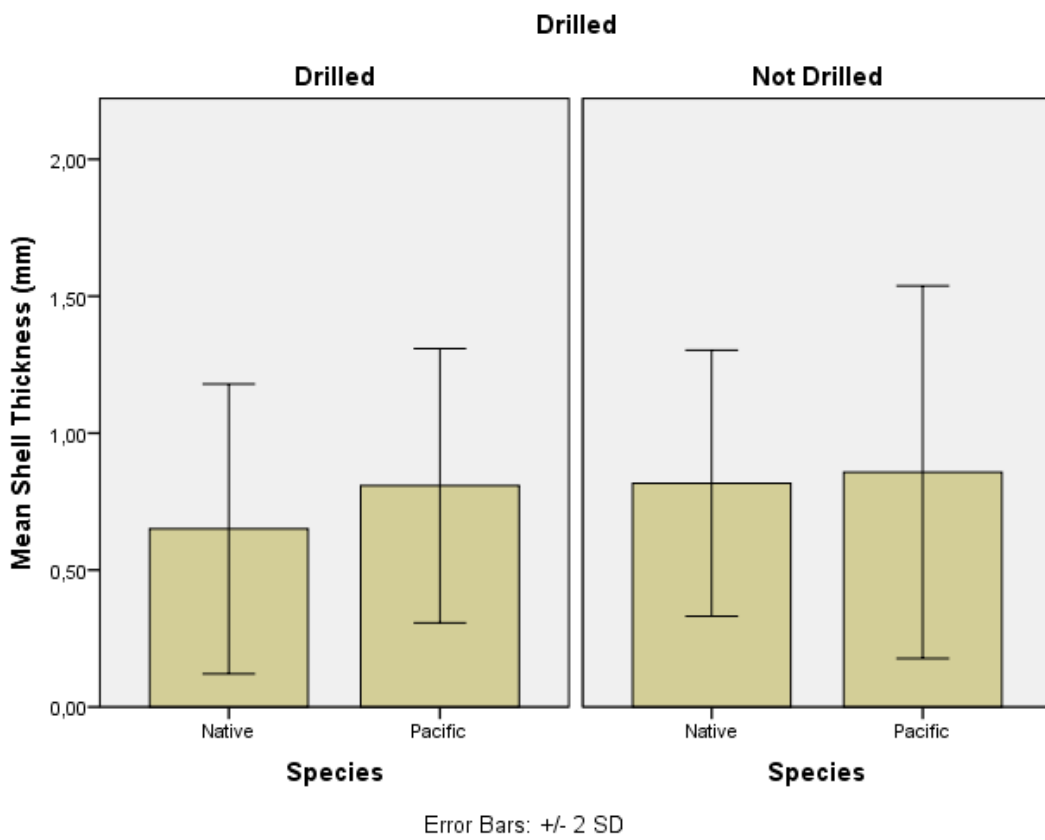


Figure A-8: Mean shell thickness (mm) for drilled and undrilled samples, from both species groups, for species preference experiments

APPENDIX B. TABLES

Table B-1: Summary results of the mean and standard deviation for the number of boreholes drilled and mean shell length for Pacific oyster samples of different size classes, for size preference experiments

Size Class		Number of holes drilled	Shell Length (mm)
Adult	Mean	1,000	87,3267
	Std. Deviation	,0000	13,71432
Juvenile	Mean	1,200	59,8360
	Std. Deviation	,4472	5,23963
Spat	Mean	1,000	33,4280
	Std. Deviation	,0000	1,05208

Table B-2: ANOVA test for the number of boreholes drilled at each size class for Pacific oyster samples, for size preference experiments

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,123	2	,062	,769	,489
Within Groups	,800	10	,080		

Table B-3: Summary results of the mean and standard deviation for the shell thickness at specific shell locations for Pacific oyster samples, for size preference experiments

Shell Location	Mean	Std. Deviation
Hinge	4,3176	1,4551
Middle	3,0500	1,1920
Tip	2,8484	1,1972

Table B-4: Summary results of the mean and standard deviation for the shell thickness for Pacific oyster samples of different size classes, for size preference experiments

Size class	Mean	Std. Deviation
Adult	3,9741	1,6100
Juvenile	3,5574	1,2654
Spat	2,6846	1,0881

Table B-5: Summary results of the mean and standard deviation for the shell thickness of the individual valves for Pacific oyster samples, for size preference experiments

Valve	Mean	Std. Deviation
1,0	3,5670	1,5730
2,0	3,2441	1,2763

Table B-6: Welch's ANOVA test on shell thickness between different size classes of the Pacific oyster, for size preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	31,785	2	232,659	,000

a. Asymptotically F distributed.

Table B-7: Games-Howell post hoc test on shell thickness between different size classes of the Pacific oyster, for size preference experiments

(I) Size class	(J) Size class	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Adult	Juvenile	,4167	,1869	,0685	-,0243	,8577
	Spat	1,2895	,1774	,0000	,8708	1,7082
Juvenile	Adult	-,4167	,1869	,0685	-,8577	,0243
	Spat	,8728	,1524	,0000	,5134	1,2322
Spat	Adult	-1,2895	,1774	,0000	-1,7082	-,8708
	Juvenile	-,8728	,1524	,0000	-1,2322	-,5134

Based on observed means.
The error term is Mean Square(Error) = 1,792.

*. The mean difference is significant at the 0,05 level.

Table B-8: Welch's ANOVA on shell thickness between different shell locations of the Pacific oyster, for size preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	40,407	2	236,244	,000

a. Asymptotically F distributed.

Table B-9: Games-Howell post hoc test on shell thickness between different shell locations of the Pacific oyster, for size preference experiments

(I) Shell Location	(J) Shell Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Hinge	Middle	1,2676	,1717	,0000	,8625	1,6727
	Tip	1,4693	,1720	,0000	1,0635	1,8750
Middle	Hinge	-1,2676	,1717	,0000	-1,6727	-,8625
	Tip	,2016	,1542	,3924	-,1621	,5654
Tip	Hinge	-1,4693	,1720	,0000	-1,8750	-1,0635
	Middle	-,2016	,1542	,3924	-,5654	,1621

Based on observed means.
The error term is Mean Square(Error) = 1,657.

*. The mean difference is significant at the 0,05 level.

Table B-10: Welch's ANOVA on shell thickness between valves of the Pacific oyster, for size preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	4,560	1	343,427	,033

a. Asymptotically F distributed.

Table B-11: Summary results of the mean and standard deviation for the number of boreholes drilled for Pacific and European flat oyster (native) spat samples, for species preference experiments

Species	Mean	Std. Deviation
Native	1,1538	,3755
Pacific	1,0769	,2774

Table B-12: Welch’s ANOVA on species preference between Pacific and native oysters, for species preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	,353	1	22,089	,558

a. Asymptotically F distributed.

Table B-13: Summary results of the mean and standard deviation for the shell thickness of drilled and undrilled samples, for species preference experiments

Status of oyster	Mean	Std. Deviation
Drilled	,7264	,2656
Not Drilled	,8377	,2943

Table B-14: Summary results of the mean and standard deviation for the shell thickness of the Pacific oyster and native oyster spat samples, for species preference experiments

Species	Mean	Std. Deviation
Native	,7546	,2609
Pacific	,8402	,3094

Table B-15: Welch's ANOVA on shell thickness between drilled and undrilled samples, for species preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	2,997	1	63,422	,088

a. Asymptotically F distributed.

Table B-16: Welch's ANOVA on shell thickness between the Pacific oyster and native oyster spat samples, for species preference experiments

	Statistic ^a	df1	df2	Sig.
Welch	1,789	1	75,843	,185

a. Asymptotically F distributed.