DETERMINING THE EFFECTS OF CHEMICAL CUES FROM PREY ON THE PREDATION BEHAVIOUR OF *OCINEBRELLUS INORNATUS*

Final Report

Delta Academy
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FINAL REPORT

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SUMMARY

The Japanese oyster drill, *Ocinebrellus inornatus*, is an invasive marine gastropod that predates upon benthic bivalves, including the cultivated Pacific oyster, *Magallana gigas*. *O. inornatus* was first discovered in the Oosterschelde area of the Netherlands in 2007 (Lützen et al., 2011), and has since established populations in cultivated oyster plots. *O. inornatus* has threatened the oyster farming industry in the area, causing up to 50% economic losses for farmers (Smaal, et al. 2016).

An experiment was designed to study the feeding behaviour of *O. inornatus*, specifically whether they use scent to select certain prey over others. To do this, two different prey species were placed in separate zones where the *O. inornatus* could not see them until they were close. Prey species used in the experiment were selected randomly using prey species available at the university which included: consumption size *M. gigas*, mid-sized *M. gigas* (LFK), blue mussel seed (*Mytilus edulis*), European flat oysters (*Ostrea edulis)* and grooved carpet shells (*Ruditapes decussatus*).

Because there where only 10 *O. inornatus* used during each run of the experiment, to avoid competition under the *O. inornatus* further runs should be conducted to be able to draw accurate conclusions from the data.

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1. INTRODUCTION

1.1. CONTEXT

The Pacific oyster, *Magallana gigas*, is one of the species of oyster that is cultivated around the world, with 4.4 million tonnes of oysters having been produced globally in 2003 ("*Crassostrea gigas* (Thunberg, 1793)", 2005). Oyster cultivation in the Netherlands has recently become threatened by the introduction of the invasive snail, the Japanese oyster drill, *Ocinebrellus inornatus*. *O. inornatus,* which has caused high mortality within on-bottom cultivated oysters plots in the Oosterschelde estuary, in the Dutch province of Zeeland. With little information being available on *O. inornatus*, combatting the snail has been difficult. HZ University has partnered with local oyster farmers to learn more about *O. inornatus* and develop and compare counteractive measures to protect oyster plots. This report focusses on identifying any possible food preference of *O. inornatus*.

1.2. AIM AND MAIN QUESTION

This experiment was designed to identify if *O. inornatus* preferentially travels towards certain prey species over others based solely upon chemical cues from the prey. The experiment was designed to address the main question as follows:

"Does *O. inornatus* preferentially move towards certain species of prey over others based solely upon chemical cues"

2.1. SUB-QUESTIONS

1. How active are the *O. inornatus* when there are nonvisible prey in controlled lab-setting?
2. How does *O. inornatus* react to different chemical cues in a controlled lab-setting?

1.3. BACKGROUND

1.3.1. BIOLOGY AND PHYSICAL DESCRIPTION OF *O. INORNATUS*

O. inornatus, commonly known as the Japanese Oyster Drill (Figure 1), is a predatory species of marine muricid gastropod native to the oceans around North-East Asia (Lützen et al., 2011). Typically, specimens will have 5-6 whorls (complete shell rotations) and approximately 8 axial ribs on the final whorl (van den Brink, 2010).

O. inornatus has been observed to have two breeding periods, spring and autumn (Fey et al., 2010). Because *O. inornatus* is gonochoristic, meaning each specimen has a single sex, mature individuals must therefore congregate to produce fertilized eggs (Fey et al., 2010). *O. inornatus* typically lay between 20 and 40 egg capsules during breeding periods (Lützen et al., 2011). Egg capsules are bright yellow and contain between 10-15 individual embryos (Lützen et al., 2011).

O. inornatus does not have a planktonic larvae phase, and as such the larvae hatch at 2 mm long and will keep the same form through to adulthood (Buhle et al., 2004).

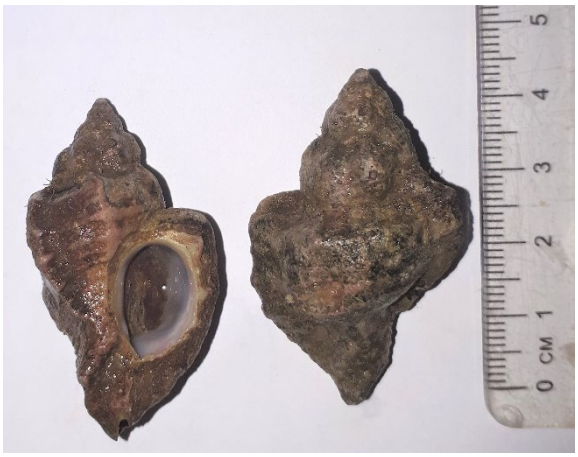

Figure 1: Typical *Ocinebrellus inornatus* shell appearance

While *O. inornatus* prefer warmer water temperatures, they have been observed to withstand temperatures as low as 0 °C (Faasse et al., 2009), lower than what usually occurs in the Netherlands. This ability makes them particularly difficult to manage, as they can survive in a wide variety of habitats.

The diet of *O. inornatus* consists of mainly benthic bivalves such as oysters and mussels (Fey et al., 2010), though in some extreme cases cannibalism has been observed in the related American Oyster Drill, *Urosalpinx cinerea* (Carriker, 1955). To feed, *O. inornatus* follows effluent trails released by their prey and selects a suitable spot on the prey to feed (Carriker, 1981). *O. inornatus* feeds using its boring organ, called the radula, which it scrapes across the prey's shell while secreting sulphuric acid to soften the shell (University of Rhode Island, n.d.). Depending on the shell thickness of the prey, this process can take anywhere from 1-14 days to complete before *O. inornatus* can feed on the prey (Fey et al., 2010).

1.3.2. INVASIVE HISTORY OF *OCINEBRELLUS INORNATUS*

O. inornatus was first discovered outside of its native range in 1924 in Puget Sound, United States (Lützen et al., 2011). *O. inornatus* was later discovered in 1995 to have established populations in Marennes-Oléron Bay, France where it then proceeded to travel north along the coast, to eventually establish a population in the Oosterschelde, Netherlands in 2007 (Lützen et al., 2011). The most likely cause of this spread is due to transportation of *M. gigas* between oyster farms. Any attempts to regulate this transportation have been ineffective due to the large quantity of live oysters being transported between farms every year.

1.4. ECONOMIC AND ECOLOGICAL IMPACT

O. inornatus poses a significant threat to both the economic and ecological stability of affected areas, due to its rapid population growth rate and lack of a natural predator or parasite (Faasse et al., 2007). Predation by the introduced *O. inornatus* has inhibited the rehabilitation of the native European flat oyster (*Ostrea edulis*), whose population has been devastated historically by parasites including *Bonamia ostreae* (Ronza et al., 2018).

The economic losses by oyster farmers and related industries have been observed to be as high as 50% (Smaal, et al. 2016). In one year, the Netherlands produces approximately 5 million euro worth of *M. gigas* (Strietman, 2015) and as such, oyster farming is an important part of the economy. Oyster losses caused by *O. inornatus* could therefore have a significant impact on the lives of many Dutch farmers and businesses who rely on the oysters for income.

1.2. EXPERIMENTAL DESIGN INSPIRATION

The experiment was designed to confirm the previously observed preference of *O. inornatus* to feed on *M.* gigas, within a controlled environment. A controlled environment would provide results which are unaffected by variables, such as sudden water quality changes and scents from wild prey, occurring in the natural environment that could skew the results. The experiment was designed such that the *O. inornatus* were unable to see the prey and would be driven by the cues of the pray that was detectable due to the flow moving from the pray to the starting point of the *O. inornatus*.

3. MATERIAL AND METHOD

3.1. PHYSICAL EXPERIMENTAL SETUP

The experiment (refer to Figure 2) was built inside of a 119 cm by 99 cm plastic basin and consisted of a T-shaped wooden enclosure. The location where the *O. inornatus* were placed, when starting the experiment, was at the base of the T-shape (number 1 in Figure 2). At both ends of the T-shape prey could be placed, protected by removable plastic meshes (adjacent to labels 3A and 3B in Figure 2). To keep track of the movement of the *O. inornatus* over time, the enclosure was divided into 4 zones and marks were drawn along the sides at intervals of 5 cm beginning at the base of the T. A triangle was put in the middle of the T (between labels 3A and 3B in Figure 2) to divert water flow towards the base of the T, and to ensure chemical cues in zones 3A and 3B (Figure 2) would remain homogenous. A GoPro Hero 4 session was mounted on a beam over the setup and was programmed to take a time lapse with a 1-minute interval.

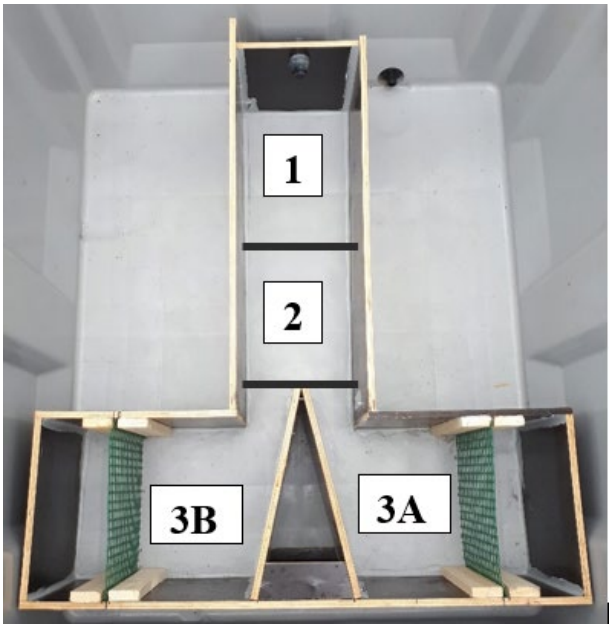

Figure 2: Experimental setup and zone labels

To allow for transport of chemical cues, the basin was placed under an angle of 5% causing the flow move from the both ends of the T towards the Base of the T where the overflow was placed. 4.5 mm hoses were placed in section 3A and 3B, prey areas, and emitted a constant water flow. The flows in each of the prey areas, approximately 6 L/hr, were produced by separate Williamson Cased 200CM water pumps, both of which pumped filtered ground water from a reservoir that was filled every day. Air stones were placed in each prey area to oxygenate the water.

3.2. EXPERIMENTAL PROCEDURE

Each experimental run has a duration of 48 hours in total. Before the start of each run of the experiment, the two water pumps were calibrated such that they would both emit similar flows. The calculation done to calculate all flow rates are shown in equation 1. In this equation, Q represents the flow rate [L/hr] and t is the time it took for the pump to produce 100 ml of water [s].

$$Q\left[\frac{L}{hr}\right] = \frac{100\,[ml]}{t\,[s]} \times \frac{1\,[L]}{1000\,[ml]} \times \frac{3600\,[s]}{1\,[hr]} \quad (1)$$

Flows were calculated 4 times for each pump to ensure a more accurate result. If required, the hoses attached to the pumps were cleaned to reduce the risk of blockages occurring. Then, 10 *O. inornatus,* to avoid competition under the *O. inornatus,* of a similar size class were selected and individually measured. These *O. inornatus* were then each painted with nail polish such that they would be distinguishable from each other. Prey species used in each run were determined randomly using an Excel program. The prey species used were selected based upon availability in the university lab. The prey species available during the time the results were collected were consumption size *M. gigas*, mid-sized *M. gigas* (LFK), blue mussel seed (*Mytilus edulis*), European flat oyster *(Ostrea edulis)*, and grooved carpet shells (*Ruditapes decussatus*).

This program also determined which zone, 3A or 3B, the species would be placed in. All prey species being tested in the experiment were selected on equal mass (around 800 grams) between both prey species. The enclosure (inside of the T) was filled with groundwater and the plastic meshes were inserted into zones 3A and 3B. Then, the prey species were placed in their proper zones and the *O. inornatus* were placed such that the tips of their siphons were aligned with the 10 cm mark on the T. A YSI Professional Plus multimeter was used to determine the percentage of dissolved oxygen and the water temperature in each zone. In all zones the meter was immersed for 10 seconds before being read. Finally, a white tarp was used to cover the setup, reducing the effects of outside weather conditions and disturbance during the experiment.

At 24 hours after setting in the experiment, the positions of all *O. inornatus* were noted down. After 48 hours this was done again and the percentage of dissolved oxygen and water temperature was measured. The *O. inornatus* and prey species were removed from the setup, and, if applicable, the number of dead prey were counted. The flows from the pumps were measured, with the same method, once again using the previously mentioned method prior to their shut down. The setup was drained and cleaned using a pressure washer and scrub brush.

4. PRELIMINARY RESULTS

Due to the small numbers of *O. inornatus* being used in each run of the experiment, the current results are only enough to provide preliminary data. Initially, all the runs involving consumption sized *M. gigas* were compared (Figure 3). From this initial data, *O. inornatus* appear to prefer *M. gigas* over the *O. edulis*. Results when comparing the consumption sized *M. gigas* to the large French *M. gigas* raised in Kattendijke (LFK), showed a different preference each time.

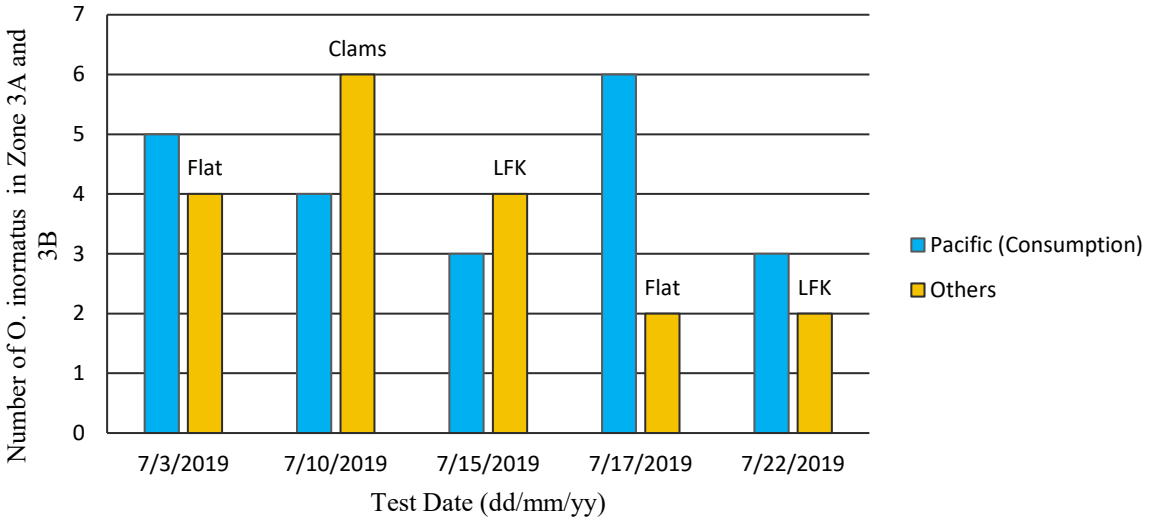

Figure 3: Number of *O. inornatus* observed in zones containing different prey species, all tests involved consumption size *M. gigas* (shown in blue)

All runs comparing *R. decussatus*, labelled as clams in this report, to other prey species were compared (Figure 4). Two of the runs involving *M. gigas*, consumption sized and mid sized (LFK), show that *O. inornatus* appear to prefer the clams over *M. gigas*.

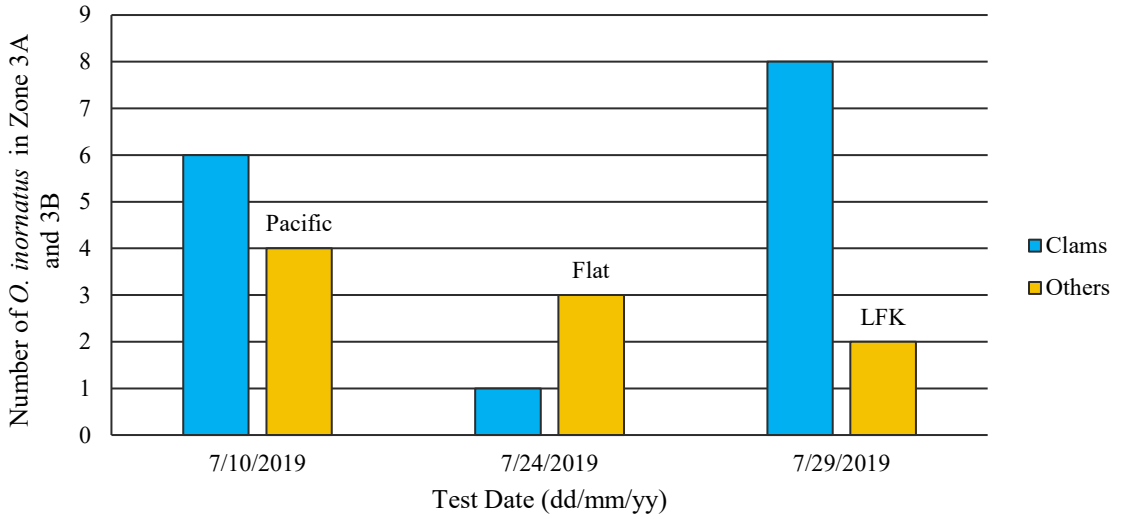

Figure 4: Number of *O. inornatus* observed in zones containing different prey species, all tests involved clams (shown in blue)

No significant observations can be made when comparing all runs involving the LFK *M. gigas* (Figure 5) or when comparing the runs involving *O. edulis* (Figure 6). Although there appears to be a significant preference for *O. edulis* over the *M. edulis*.

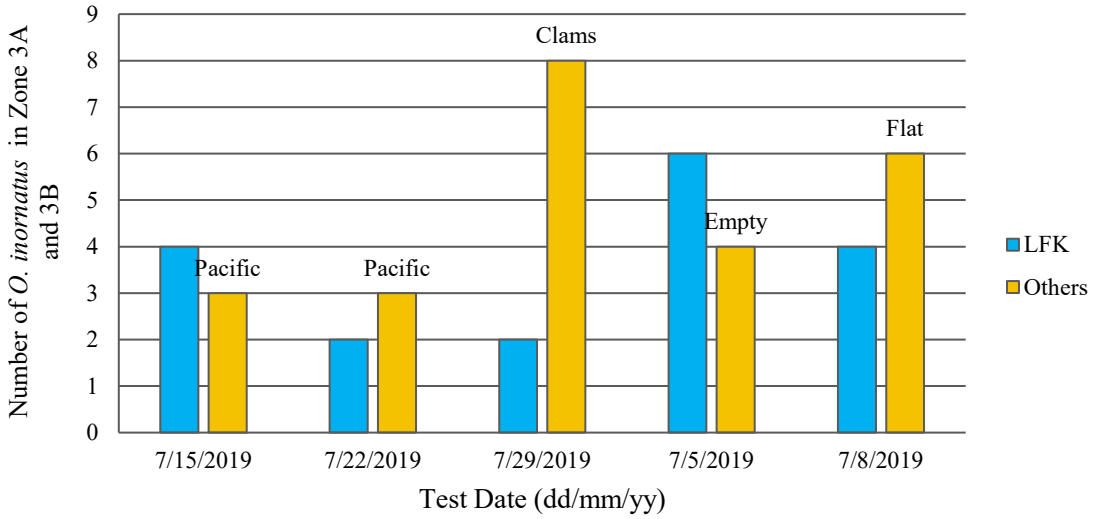

Figure 5: Number of *O. inornatus* observed in zones containing different prey species, all tests involved mid-sized *M. gigas* (LFK, shown in blue)

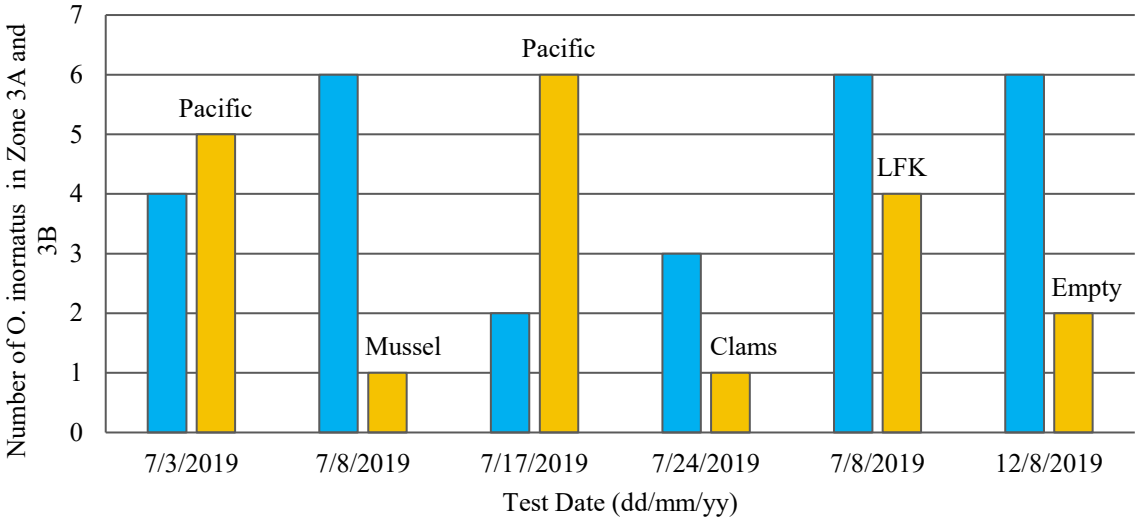

Figure 6: Number of *O. inornatus* observed in zones containing different prey species, all tests involved *O. edulis* (shown in blue)

5. DISCUSSION

From the preliminary data collected, a few points of interest appear. The two runs involving consumption size and mid sized *M. gigas* both showed different preferences by *O. inornatus*. This could support the idea that *O. inornatus* selects smaller *M. gigas* to feed on based upon some factor other than scent. The low numbers of *O. inornatus* present in zones near the mussels could suggest that their scent is less potent than that of other prey species being tested. In tests involving one empty zone, there were still *O. inornatus* present in the empty zone. This may occur as a result of confusion as to where the scent originates from, suggesting that chance encounters with prey may play a role in how *O. inornatus* finds prey.

6. CONCLUSION AND RECOMMENDATIONS

The experiment explained in this report was designed to answer the research question "Does *O. inornatus* preferentially move towards certain species of prey over others based solely upon chemical cues". Although only preliminary results have been collected so far, the results do appear to indicate that *O. inornatus* may prefer to move towards *O. edulis* and *R. decussatus*. Due to the small number of *O. inornatus* being used in each run of the experiment, additional runs of each combination of prey species must be run. Further runs should be done with no prey species in either prey zones. This would help identify whether scent determined the movement direction of the *O. inornatus*, or whether another factor related to the setup affected the results.

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APPENDICES

Appendix A – Water Flow Data

Table A - 1: Calculated flow values [L/hr] **at the start and end of each run**

Date	Prey 3A	Prey 3B	Zone 3A, Start	Zone 3A, End	Zone 3B, Start	Zone 3B, End
03/07/19	Flat	Pacific	5.27	4.50	5.14	6.26
08/07/19	Mussel	Flat	7.06	6.92	7.06	7.25
10/07/19	Clams	Pacific	5.71	6.37	5.67	5.33
15/07/19	Pacific	LFK	5.39	6.70	5.52	6.52
17/07/19	Pacific	Flat	6.70	6.18	6.52	6.73
22/07/19	LFK	Pacific	6.10	7.16	6.13	7.02
24/07/19	Clams	Flat	6.23	8.18	6.05	7.02
29/07/19	Clams	LFK	6.10	7.10	6.00	7.15
05/08/19	LFK	Empty	6.05	6.75	6.00	8.04
7/8/2019	Flat	LFK	6.79	7.25	6.92	7.66
12/8/2019	Empty	Flat	6.00	7.46	6.14	7.96
14/08/19	Empty	Mussel	6.18	6.63	6.34	6.43

Appendix B – Water Quality Data

Table B - 1: Dissolved oxygen levels [%] measured before and after each run

Date	Prey 3A	Prey 3B	Zone 1, Start	Zone 1, End	Zone 2, Start	Zone 2, End	Zone 3A, Start	Zone 3A, End	Zone 3B, Start	Zone 3B, End
03/07/19	Flat	Pacific	85	92.1	71.4	97.4	76.1	93.8	65.5	98.2
08/07/19	Mussel	Flat	68	88.3	57.3	90	62.7	89.3	56	84.9
10/07/19	Clams	Pacific	82.7	92.2	76.1	87.5	82.9	88.4	85.6	88.5
15/07/19	Pacific	LFK	77.3	79.9	68.1	81.6	75.3	83.4	99.6	85.3
17/07/19	Pacific	Flat	58	78.4	58.9	80	67.6	80.9	70	82.9
22/07/19	LFK	Pacific	48.6	71.7	46.3	70.8	51.6	72.5	56.8	75.6
24/07/19	Clams	Flat	58.9	86.3	51.4	90.7	53.6	92.7	54.7	94.2
29/07/19	Clams	LFK	103	95.4	111.5	95.6	114.2	94.2	114.5	94
05/07/19	LFK	Empty	55.6	89.4	51.2	94.6	58	94.5	57.9	96.6
07/08/19	Flat	LFK	60.7	92.8	48.6	90.3	54.5	90.6	60	89.4
12/08/19	Empty	Flat	50	97	41.4	89.5	45	88.4	46	87.5
14/08/19	Empty	Mussel	53.9	90.8	43.5	92.5	46.9	90.3	49.2	88.1

Table B - 2: Water temperature [°C] measured before and after each run

Date	Prey 3A	Prey 3B	Zone 1, Start	Zone 1, End	Zone 2, Start	Zone2, End	Zone 3A, Start	Zone 3A, End	Zone 3B, Start	Zone 3B, End
03/07/19	Flat	Pacific	20.4	26.2	20.4	26.3	20.1	26.2	20.5	26.2
08/07/19	Mussel	Flat	19.5	19	19.5	18.9	19.3	19	19.3	18.9
10/07/19	Clams	Pacific	18.7	22.1	18.7	22.1	18.9	22	18.7	22
15/07/19	Pacific	LFK	18.6	22.8	18.5	22.8	18.4	22.7	18.4	22.8
17/07/19	Pacific	Flat	20.2	22	20.1	22.1	20.4	22.1	19.9	22.1
22/07/19	LFK	Pacific	20.6	26.1	20.6	26.2	20.5	26.2	20.5	26.3
24/07/19	Clams	Flat	23	27.8	23.1	27.8	23.4	27.6	22.9	27.6
29/07/19	Clams	LFK	23.4	20	23.4	19.9	23.3	19.9	23.3	19.9
05/07/19	LFK	Empty	20.5	24.5	20.6	24.5	20.6	24.5	20.5	24.3
07/08/19	Flat	LFK	19.8	21.4	19.8	21.5	19.8	21.4	19.6	21.5
12/08/19	Empty	Flat	17.8	18.6	17.8	18.7	17.9	18.7	17.6	18.6
14/08/19	Empty	Mussel	18.2	20.2	18.4	20.3	18.6	20.3	18.2	20.3