ALTERNATIVE BAITS TO IMPROVE THE SUSTAINABILITY OF THE SNOW CRAB (*CHIONOECETES OPILIO*) AND AMERICAN LOBSTER (*HOMARUS AMERICANUS*) FISHERY

By

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Abstract

This study investigated the effectiveness of two attractants containing either betaine or lecithin, and LED light devices as alternative bait options for crustaceans. Sea trials were conducted during the 2018 and 2019 snow crab fishing seasons (CFA 23 and 24). Traps baited with fish bait, betaine attractant and LED light improved capture ($P < 0.001$) in 2018. Because of the limited variation in catch, a cost-benefit analysis was conducted for the 2019 season, which found that using additional betaine attractant and/or LED light could reduce the number of fishing trips needed to meet quota. In-laboratory preference trials were run on American lobsters, testing betaine and lecithin attractants, white LED light, fish bait and an empty control. Lobsters rested for a longer period ($P = 0.001$) by the lecithin attractant and chose it more often ($P = 0.003$) than the LED light to rest.

List of Abbreviations Used

ANOVA	Analysis of variance
B_1	Betaine attractant (large, weighed 108.9 ± 4.60 g)
B_2	Betaine attractant (small, weighed 22.4 ± 1.73 g)
L_2	Lecithin attractant (small, 22.74±0.75 g)
CFA	Crab fishing area
CPUE	Catch per unit effort
CV	Coefficient of variation
DFO	Fisheries and Oceans Canada
DM	Dry matter
FA	Fatty acid
LFA	Lobster fishing area
N-ENS	North-Eastern Nova Scotia
RBD	Randomized block design
S-ENS	South-Eastern Nova Scotia
TAC	Total allowable catch

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Chapter 1: Introduction

Snow crab (*Chionoecetes opillio*) and American lobster (*Homarus americanus*) are the most valuable commercial species of crustacea in Atlantic Canada with a commercial landing value of over $175 and $880 million CAD in Nova Scotia in 2019, based on the landing of 50,854 and 14,271 metric tonnes, respectively (DFO 2021a). The majority of the snow crabs Nova Scotia (S-ENS), the snow crab fishing season lasts from April to September. The season is not continuous in North-Eastern Nova Scotia (N-ENS), and is divided into two seasons: spring fishery (early April to mid to late May) and summer season (mid July to mid August) (DFO 2016a; Figure 1.1.1). The lobster fishery, by comparison, has rotating fishing zones, providing a year-round lobster supply, where the fishing seasons vary around Atlantic Canada. As concerns have emerged regarding the economic and ecological issues associated with fishing, such as threats to the North Atlantic right whales (*Eubalaena glacialis*), improved crustacean fishing practices and ecological protective measures are desired, while maintaining profits for the fishers.

The traditional use of forage fish as bait, such as Atlantic herring (*Clupea harengus*) and Atlantic menhaden (*Brevoortia tyrannus*), presents a challenge to the conservation and availability of fish protein, due to the large-scale consumption of bait in crustacean fisheries. At estimated 32 tons of bait was used to land 10.7 tons of lobster in 2006 in Maine, US (Driscoll et al. 2015). A case study in St. Margaret's Bay and Mahone Bay, Nova Scotia reported a 1.9 bait-to-catch weight ratio in the lobster trap fishery (Harnish and Willison 2009). The use of this fish protein resource to harvest crustaceans rather than for direct human consumption is inefficient (Ayer et al. 2009; Tacon and Metian 2009; Patanasatienkul et al. 2020).

Additional environmental impacts due to crustacean fishing have emerged, influencing the timeline of the fishing season in some regions. Lethal entanglements in fishing gear and ship strikes, for instance, have become a serious threat to the North Atlantic right whale. The breeding season of the North Atlantic right whale is from April to May, overlapping with the fishing season occurring in the same zones, resulting in an increased risk of whales being unintentionally harmed (Baumgartner et al. 2017). In 2018, the snow crab fishery lost its sustainability certification after 12 right whales were found dead in the Gulf of St. Lawrence (DFO 2019a; Ibrahim 2019).

Given this concern, an earlier snow crab fishing season has been implemented in some zones of Atlantic Canada by the federal regulator, Fisheries and Oceans Canada (DFO), shortening the commercial fishing season. In 2018, in Northeastern Nova Scotia, the snow crab fishing season was from April 14^{th} to May 13^{th} in the spring and from July 21^{st} to August 18^{th} in the summer in areas 10 to 22 (DFO 2018a). The snow crab season in the Gulf of St. Lawrence started as soon as the ice broke up in order to avoid the whales, and ended by the end of June in 2018, which ended two weeks earlier compared to previous years (Auld 2018). Effective baits are therefore in demand in order to ensure a sufficient capture during the shortened fishing season. Aside from the shortened season, issues such as the weather conditions are a concern, as it could be dangerous to start the fishing season earlier.

In place of using traditional fish bait, alternative baits and feed attractants with higher efficiency would be beneficial, particularly if they enable fishers to reach their allotted quota in a shorter season, while consuming less fish bait. From the aspect of operating costs, alternative baits with no or few limitations regarding storage are also preferred, as the fish bait must be kept frozen prior to use, which increases costs. Alternative baits and attractants in various forms have been investigated on multiple crustacean species, both in the sea (Dellinger et al. 2016), and the laboratory (Nguyen et al. 2019). Animal by-products sourced from fish or fish extracts, other aquatic organisms, or protein from agricultural waste can be used to partially substitute fish bait (Archdale et al. 2008; Archdale and Kawamura 2011; Middleton et al. 2000). Substituting fish bait with synthetic compounds is another potential solution. Synthetic baits use chemical compounds and sensory stimulants rather than animal products, which simulate attractive compounds derived from marine prey (Masilan and Neethiselvan 2018).

As an alternative to chemical attractants, light is used to catch species that are positively phototaxic. Metal halide, fluorescent and incandescent lamps have been applied on an industrialized scale to catch pelagic species, including herring, sardines, anchovies and squid (Marchesan et al. 2005; Nguyen and Winger 2019a; Solomon and Ahmed 2016). Behavioural response to light is species-specific and depends on multiple factors, such as light source, intensity, colour, wavelength. Although artificial light has been tested for use in ocean shrimp fishing (Hannah et al. 2015), harvesting with light was not considered an approach for use in decapod crustaceans before Nguyen et al. (2017) researched the use of LED light to attract snow crabs.

Behavioural responses are practical indicators of the feed and prey preference of crustaceans. Water-borne chemical stimuli are received via chemoreceptors located in the antennae, leg tips and mouthparts, which initiate changes in locomotion. Malacostracan crustaceans have two pairs of antennae, both of which are sensory appendages. The first antennae (anterior pair) have unique olfactory sensilla (Derby et al. 1997). Foraging behaviour response can be influenced through several mechanisms including locomotion and feeding initiation (Archdale et al. 2011). However, behavioural responses differ among species and may not be apparent by observation if the chemical signals do not exceed the threshold triggering locomotion.

For this thesis, artificial baits were produced containing betaine and lecithin, in which fish or fishery by-products were not used. Betaine is a low molecular weight amino acid found in several prey fish species, and it functions as an attractant by improving the palatability of fish diets (Yesilayer and Kaymak 2020). Lecithin is mainly derived from soybeans and eggs, and has been incorporated into the diets of prawns *Artemesia longinaris* (Haran and Fenucci 2008) and sea bream *Sparus aurata* (Liu et al. 2002, Szuhaj 2016), in which lecithin improved the growth performance. Its application in attracting crustaceans is therefore proposed, which to our knowledge, has not been tested previously. As fishing with light has been practiced in many fisheries, and LED light was previously shown to increase snow crab capture (Nguyen et al. 2017) white LED light was included in this project. The research by Nguyen et al. (2017) concluded that the catch per unit effort (CPUE) of using white and purple LED lights into baited traps was improved by 77% and 47%, respectively, in field conditions, but no difference was confirmed if using LED light solely in traps compared to baited traps. The capture efficiencies of augmenting white LED light and/or betaine attractant were compared, and the attractiveness of lecithin attractants was investigated in this thesis. The goal of the research is to reduce fish bait usage, by increasing the efficiency, and in the case of snow crabs, shortening the duration of the fishing season to reduce the operating cost for fishers, as well as to minimize physical interactions with right whales.

Literature is reviewed in Chapter 2, and sea trials are described in Chapter 3, which were conducted on snow crabs for two seasons (2018 and 2019) in crab fishing area (CFA) 23. The aim was to test if catchability would be improved by augmenting betaine attractant and/or white LED light to traditional fish bait. A cost-benefit analysis followed to evaluate the positives or negatives of using artificial attractants using data from the 2019 snow crab season.

Laboratory trials were conducted on American lobsters at an indoor facility at Dalhousie University, Faculty of Agriculture (see Chapter 4). A preference test was run, where betaine and lecithin attractants, white LED light and traditional fish bait were tested on lobsters. The preference of lobsters for attractants and/or white LED light to mackerel and a blank control was studied, based on their behavioural responses. This was followed by a validation test to determine the response of lobsters to individual attractants or white LED light, as compared with mackerel and a blank control.

The ultimate goal of this project is to improve the efficiency of crab and lobster trapping by improving the effectiveness of artificial attractants containing betaine or lecithin, and white LED light in crustacean fisheries from a capture and economic aspect. The use of alternative options could be potential to partially substitute fish bait, reducing forage fish bait use and enhancing the sustainability of crustacean fisheries in the future.

Figure 1.1.1 Crab fishing areas (CFAs) 20-24 and area 4X in the Maritimes (Figure source: DFO, Integrated Fisheries Management Plans).

Chapter 2: Literature Review

This chapter will cover the history of the snow crab and lobster fisheries in Atlantic Canada and current issues in these industries. Crustaceans and their behavioural responses to chemical signals are reviewed in order to distinguish their feeding preferences and identify options that could be used in developing artificial attractants, with the goal of improving the sustainability of these industries. Feeding preferences and the mechanisms of crustacean prey-seeking behaviours are summarized. Types of traditional fish bait used in crustacean fishing, and the moves that have been made toward developing alternative options for fish bait will also be explored.

2.1 Fishing Crustaceans

2.1.1 Snow Crab

Snow crabs are naturally distributed across the Arctic regions and in the northwest Atlantic Ocean, from northern Labrador to the Gulf of Maine. They are a major commercial epibenthic macro-invertebrate inhabiting the Scotian Shelf. The snow crab stock is distributed dynamically and regionally, but a northward shift has been observed in the past thirty years, due to warming trends (Divine et al. 2017). Snow crab habitats in North-Eastern Nova Scotia (N-ENS), from Cape North to Scatarie Island, consistently range from nearshore, within 8 km, to 24 to 32 km (Figure 1.1.1, 2.1.1). In South-Eastern Nova Scotia (S-ENS), by comparison, from Scatarie to Kempt Point and south, the range is from 24 km up to 193 km (Figure 2.1.1).

The snow crab distribution in Scotian Shelf areas can be significantly altered due to environmental variations, such as currents and temperatures, as it is the most southern habitat in the western Atlantic. Snow crabs of a commercially desired size (carapace width larger than 95 mm) can be found in soft-bottom benthic areas at a depth from 60 to 280 m. The temperature of their habitat ranges from -1 to 6 ºC, preferably below 4 ºC. Unfavorable influences may appear beyond this range, although crabs are occasionally found in areas below the limit of -1 ºC. The salinity of snow crab habitats is optimal > 26 ppt (DFO 2019a).

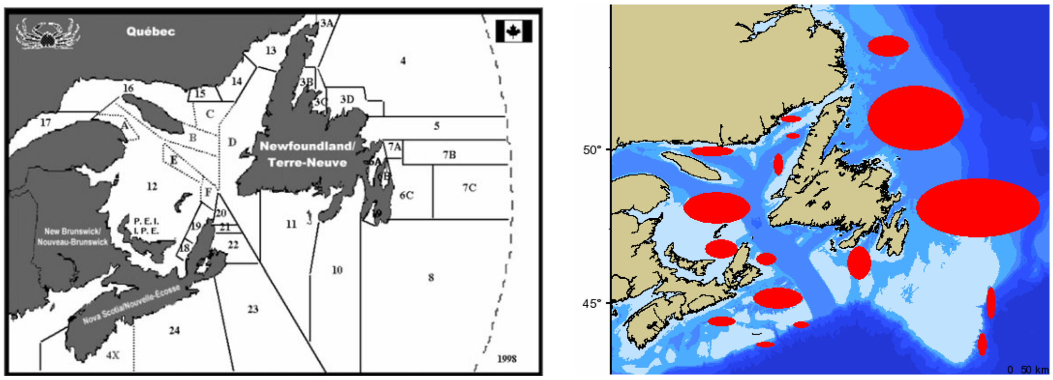


Figure 2.1.1 Fishing areas for the snow crab commercial fishery in the Atlantic Provinces and Quebec (left, Brzeski 2015), and distribution of snow crab stocks harvested in Atlantic Canada indicated in red (right, DFO 2015).

Strict protective methods are enforced to conserve and maintain natural stocks. The commercial landing of hard-shelled males is only permitted for crabs with a carapace width greater than 95 mm, where female crabs and males under the legal size need to be released back to the ocean. Areas will close if more than 10% of the capture are newly molted (soft-shell) crabs (Brzeski 2015). Other management actions include individual boat quotas, total allowable catches (TACs), dockside monitoring, mandatory logbooks and at-sea monitoring (DFO 2018b).

The reproductive period for snow crabs is between February and April, where mating occurs between winter and spring (from March to May), followed by a hatching period for three months in late spring or early summer (DFO 2016b). The pelagic period (zoea stage 1 and 2, the intermediate megalopae stage) lasts for 12 to 15 weeks, after which hatched juveniles settle to the bottom and grow rapidly, experiencing two moults in a year with an approximate 250% increase in weight over each moult (DFO 2016b).

Females grow until their abdomen is wide enough to carry eggs, where the eggs are brooded for one to two years. Primiparous females mature at an average carapace width of 60 mm (DFO 2016b). Males mature at a carapace width of 95 mm, and grow until they acquire large claws on the first pair of legs, which can happen when the carapace width between 40 to 150 mm (DFO 2016b; DFO 2018a; DFO 2019b). After terminal moulting, snow crabs can live up to 6 more years, and the maximum life span is of 12 – 13 years (DFO 2016a, b; DFO 2019b).

The snow crab fishery started in the late 1970's in Nova Scotia, then expanded in the 1980's. Over 60% of the snow crabs consumed globally are from Canada, and 70% of the Canadian harvest is exported. More than 50 crab fishing areas (CFAs) are managed under DFO regulations, and large stocks are in northern Newfoundland and northern Quebec, as well as in areas 20-23 (Figure 2.1.1; Brzeski 2015). In the Maritimes, there are five areas, all in ENS, assigned as CFAs 20 to 24, which are known as N-ENS (north-eastern Nova Scotia, CFAs 20-22) and S-ENS (south-eastern Nova Scotia, DFAs 23-24) (DFO 2016a; Figure 1.1.1). Over 93% of the total landed value was caught from S-ENS in 2010 and was worth more than 50 million Canadian dollars (DFO 2016a). More snow crab fishery licences were issued for S-ENS than N-ENS, 116 and 78 licences, respectively, in 2014 (DFO 2018a; DFO 2019a; Brzeski 2015).

The fishing season for snow crab is typically from mid-July to mid-September in N-ENS, and from April to September in S-ENS. Fall and winter fisheries are allowed in area 4X (DFO 2018a). However, the exact opening date differs every year, which can be affected not only by natural conditions and commercial biomass level, but also economic or social concerns (DFO 2019b, c). In Newfoundland and Labrador, the opening date was postponed as a result of market conditions and logistical complications in 2020 due to the coronavirus crisis (DFO 2020a; White 2020).

A total allowable catch (TAC) is determined annually by DFO, and quotas are monitored to limit capture in different areas, which are usually set at a higher number for S-ENS than N-ENS. In 2018, the quota limit of N-ENS (zones 20-22) was 787.78 metric tonnes (t), representing an approximately 5% decrease from the previous year, and the actual catch of 2018 was 89% of the quota (DFO 2018a). In 2019, the quota was reduced to 630.63 t. In comparison, the limit for S-ENS (zones 23-24) was 6057 t in 2018, and 6663 t in 2019 (DFO 2018c; DFO 2019d).

The bycatch rate was low to negligible across all fishing areas addressed in 2016, with bycatch rates in ENS and CFA 4X less than 0.2% in snow crab fisheries (DFO 2016a). A reduced TAC was recommended in both N-ENS and S-ENS because of declining estimated fishable biomass, based on an increase in the catch of soft-shell and adolescent crab, which indicated recruitment (DFO 2019a).

The landed price of snow crab has fluctuated over the past 25 years and showed a general decline, as a result of influences from global supply and total landings. The landed price reached a peak in 1995 at more than $7/kg and was under $5/kg in 2012. The landed value of snow crab is considerably important to Richmond and Cape Breton Counties in Nova Scotia. As an example of its importance, it comprised 46% ($12 million) and 31% ($13.6 million) of the total fishery landed value, respectively, for these regions in 2009 (DFO 2016a).

2.1.2 Influence of The Right Whale on The Snow Crab Fishing Season

The North Atlantic right whale, which is one of three right whale species (*Eubalaena glacialis, Eubalaena japonica, and Eubalaena australis*), inhabits the eastern coast of North America. In 2018, the Marine Stewardship Council (MSC) suspended its "sustainable" fishing certification for New Brunswick crab products after 12 right whales were found dead in the Gulf of St. Lawrence in 2017, among which several were entangled in fishing gear (DFO 2016b; Ibrahim 2019). Crustacean fisheries have been affected by these governmental actions which aim to protect the whales, as measures implemented include area closures and fishing season shifts. The current right whale population is in a serious and urgent situation, with an estimated 366 individuals and less than 100 breeding females in 2021, and is predicted to be functionally extinct in 20 years if the downward population trajectory is maintained (Baumgartner et al. 2017; Chisholm 2021; DFO 2020b; DFO 2021b; Pennisi 2017).

Twelve out of a total of 450 right whales were found deceased in Canadian waters in 2017 and their deaths were inferred to be related to entanglements or ship strikes when foraging, based on necropsy results. In 2018, the number of dead right whales was reduced to three in the US and zero in Canada due to multiple effective protection measures, and no deaths were recorded in Canadian waters in 2020 (Chisholm 2021; Pettis and Hamilton 2018). Research investigating the entanglement rates of North Atlantic right whales documented 82.9% of the whales, which were monitored from Florida, US to Nova Scotia, were entangled at least once from 1980 to 2009 (Knowlton et al. 2012). The lifespan of right whales approximately reaches a minimum of 70 years, however, female and male North Atlantic right whales currently are living to about 45 and 65 years, respectively (NOAA. a). Female right whales that were pregnant or accompanied by calves were reported to have a higher risk of meeting ship strikes, as they spend more time at the surface during the summer (Baumgartner et al. 2017). The entanglements also stressed the females, which led to a longer interval between calving, and subsequently, decreased birth rate (Stokstad 2017).

Government actions to reduce the risk of entanglements and ship strikes include restricting speed limits, rerouting vessels in designated areas and using weaker ropes for traps to allow whales to escape. However, a rebounding population has not yet been achieved as expected as there is no evidence of recruitment (DFO 2019e).

Crab fisheries in whale habitat were closed, delayed or restricted by Canadian regulators in the summer of 2017 in Atlantic Canada following several deaths of whales (DFO 2016b; Quon 2017). In 2018, the closures were conducted temporarily in six fishing areas in the eastern waters of New Brunswick and Quebec, which affected snow crab, toad crab (*Hyas coarctatus*), rock crab (*Cancer irroratus*) and lobster fishing (DFO 2019f; Sturgeon 2018). In the Gulf of St. Lawrence, the crab fishing season started at the end of March and continued until June, shifting the season to begin earlier, in hopes of avoiding the right whales. Crab fishing areas would temporarily close if whales were spotted, and reopen after confirming they were no longer in the area (DFO 2019a; MacKinnon 2018). This would reduce the threat from fishing gear until the whales migrated south, as right whales are primarily caught by the ropes on crab and lobster traps. A Canadian Coast Guard ice breaker was requisitioned in 2018 in the southern Gulf of St. Lawrence to break up the ice in some fishing areas to ensure the fishing season could start as anticipated. Protective measures continued in 2019, where vessel speed was restricted for not only large vessels, but more vessels longer than 13 m in length, and aerial surveillance was conducted more frequently. In 2020, the season-long closure imposed in 2018 was substituted with a dynamic closure system (Chisholm 2021; DFO 2021c; MacDonald 2020). Based on these limitations, alternative fishing options may be required, such as the use of bait with a higher capture efficiency than traditional fish bait, allowing fishers to meet their quota within the shortened fishing season.

2.1.3 American Lobster

As a critical part of fisheries in both the US and Canada, American lobster is a highly profitable species. As an example, in 2006, it contributed 34% of the total value among all fishery landings in Canada (DFO 2008; Driscoll et al. 2015). The geographic distribution of the American lobster spans from the northern coast of Carolina, US, to Newfoundland and Labrador, CA. Its distribution is temporally stable and spatially specific in the Maritimes, where American lobsters live at a depth of 50 meters or less. Areas around Nova Scotia and the southern Gulf of St. Lawrence are populated habitats (DFO 2018b). American lobsters are long-lived, reaching ages as high as 35 years and potentially live longer, experiencing multiple molting periods in their life (Koopman et al. 2015). Among different lobster fishing areas (LFA), conservation measures, minimum legal sizes, and trap limits vary. The minimum commercial-size lobster carapace length (CL) in Nova Scotia is 82.5 mm, which is typically reached at an age of 8 - 10 years (DFO 2020c). Given the records from the Fishermen and Scientists Research Society, 40% of the lobsters captured in the Scotia Fundy region were returned to the sea because they were undersized or berried (females bearing larvae on the pleopods) (Harnish and Willison 2009).

Lobsters are primarily nocturnal and sedentary animals with shelter-related foraging behaviours. They spend most of the day in shelter and are less active during the light phase, although their activities could be impacted by prey abundance. However, acclimated individuals are more active during the day under laboratory conditions, and prolonged holding periods intensify foraging behaviours (Jury et al. 2001; Lawton 1987). This may bring possible bias in behavioural observations when conducting laboratory experiments.

Lobsters have chemosensory sensilla in the major appendages, which detect different chemical compounds and are used to locate food, as well as allow lobsters sense predators. Increased odorant capture can be achieved by exposing the aesthetasc sensilla via flicking of antenna. Chemoreceptor cells on their walking legs can be excited by specific amino acids, nucleotides, quaternary ammonium compounds, and ammonium ions (Lee and Meyers 1996; Zimmer-Faust et al. 1996). The optimal flicking frequency for American lobster was around 4 – 5 Hz to detect concentration changes in odour (Harzsch and Krieger 2018). Male American lobsters can be attracted by urine-related pheromones released by newly molted females, which is a typical mating signal, based on behavioural observations. Both male and female lobsters respond to water including urine from molted lobsters, where males exhibit aggression and feeding behaviour, including raised claws and caution posture; while females notice the signal (increasing antennular rate), but are not attracted.

However, water from intermolt lobsters does not raise this same response (Atema 1986; McLeese 1973).

LFA 34 is one of the zones with most abundant lobster landing, comprising 32% of all lobster landings in 2016 (Figure 2.1.2). The most frequently used bait in LFA 34 is sourced from Atlantic herring (*Clupea harengus*), Atlantic menhaden (*Brevoortia tyrannus*) and red fish (*Sebastes marinus*), as well as less frequently used species such as haddock (*Melanogrammus aeglefinus*), flounder (several possible species) and Atlantic cod (*Gadus morhua*) (Driscoll et al. 2015).

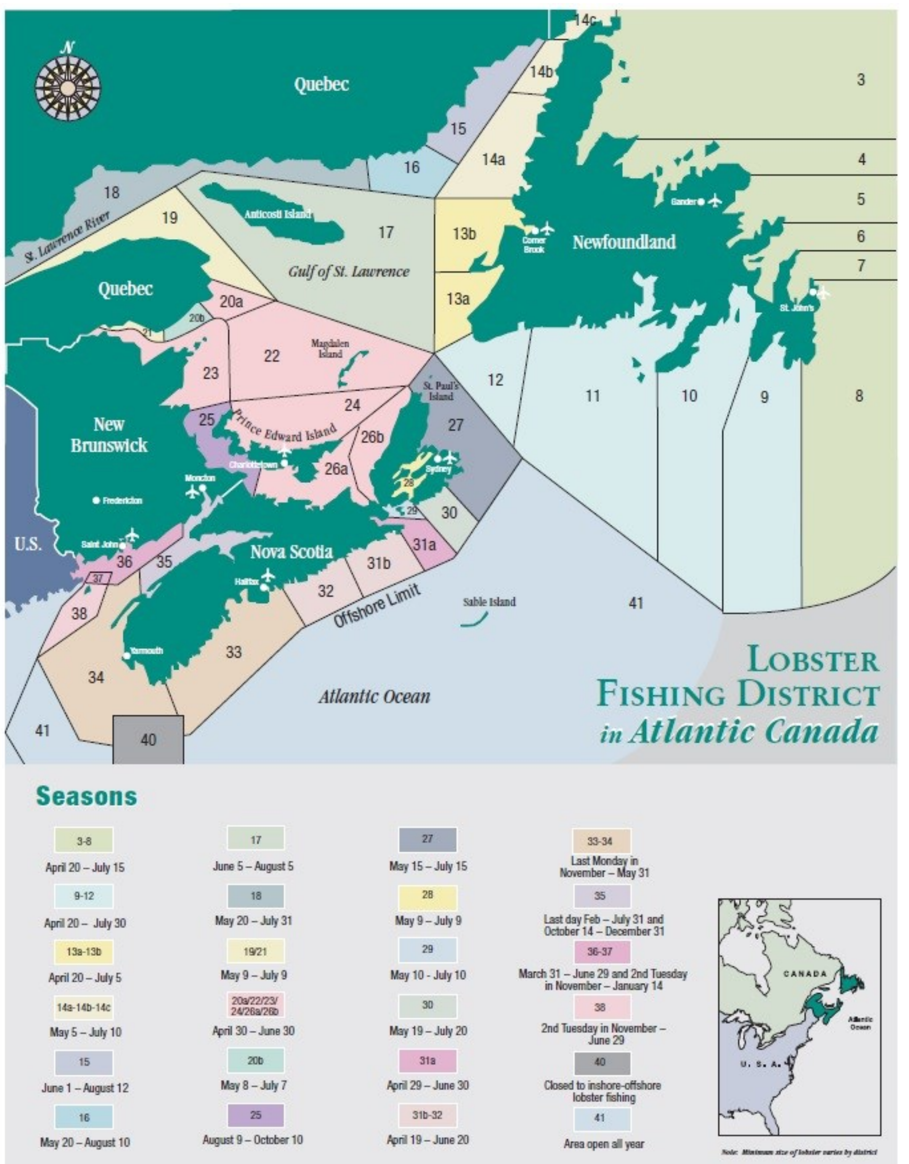


Figure 2.1.2 Lobster fishing districts in Atlantic Canada (source: Laboratory of Innovation in Science and Industry, Université Sainte-Anne)

2.1.4 Current Status of the Sustainability of Forage Fish Species

Based on Fisheries and Oceans Canada (DFO), the stock of a sustainable fishery should be harvested and farmed in a way that "meets our present needs without compromising the ability to meet our future needs" (DFO 2019g). The Marine Stewardship Council (MSC) state that: "fishing must be at a level that ensures it can continue indefinitely and the fish population can remain productive and healthy." Therefore, an important indicator of sustainable fisheries is a biomass stock that can reserve the species continuously.

The stock assessments of snow crab and lobster are overseen by DFO, which are used to establish the total allowable catch (TAC). The increase or reduction in the TAC is influenced by the annually estimated stock. With a peak in stock in the late 1990s, the snow crab supply has generally remained stable, with some periodical fluctuations. The state of snow crab stocks is classified on three levels: healthy, cautious and critical (DFO 2019d). In 2018, the snow crab fishery in N-ENS was classified in the cautious zone, and was moved to the healthy zone in 2019, due to the estimation of a rebounded fishable biomass. The stock in S-ENS, meanwhile, remained in the healthy zone, although no recruitment was found (DFO 2020d).

The total allowable catch of commercial Atlantic mackerel was reduced to 4,000 tonnes in 2021, compared to 8,000 tonnes TAC in 2020. The stock of mackerel has remained in the critical zone in spite of an increasing spawning biomass since 2016. However, the most recent assessment results indicate that mackerel has been overfished, and the spawning biomass has dropped to its lowest observation, leading to the decision to reduce the TAC in 2021 (DFO 2020d, 2021d).

Similar concerns for Atlantic herring stock have been raised, for which a rebuilding plan has been employed since 2013, with several conservation measures (DFO 2014). An assessment of the status of herring stock in 2020 indicated a longer-term increase in herring stock in the 4VWX fishery zone. The total allowable catch of the 4WX herring fishery was 35,000 tonnes off southwest Nova Scotia in 2019, which reduced to 17,500 tonnes in the same region in the following year (DFO 2019h, 2020e). The spawning stock biomass (SSB), however, was still lower than the limit reference point (LRP) in the critical zone.

Meanwhile, the SSB in coastal Nova Scotia and offshore Scotian Shelf increased (DFO 2014).

2.2 Feeding Behaviour and Response to Chemical Stimuli

2.2.1 Natural Diet of Snow Crab and American Lobster

The diet composition of crustaceans is diverse. Although the natural dietary composition differs among habitats, polychaeta, decapod crustaceans, echinoderms (ophiuroids, starfish, and sea urchins), and mollusks (gastropods and sea snails) are frequently counted in the diet of snow crab in the north Pacific (Divine et al. 2017). Other major sources of prey for snow crabs include invertebrate taxa (dominated by shrimp), amphipod crustaceans, worms, large zooplankton, infauna clams and fish (primarily capelin *Mallotus villosus*, lumpfish, Atlantic spiny lumpsuckers *Eumicrotremus spinosus*, redfish *Sebastes spp.*, sea anemones, and other crabs) (Anderson 2014; Squires and Dawe 2003).

The diet preference of snow crabs differs, based on sex and ontogenetic phase. As snow crabs grow larger, they are able to prey on larger and hard-shelled animals. Shrimp and fish contribute the majority of the food mass consumed by snow crabs based on stomach content samples on the northeast Newfoundland Shelf (Divine et al. 2017; Squires and Dawe 2003). Males have a preference for infauna, while females prey more on shrimp and epibenthic prey (DFO 2018a; DFO 2019a). The abundance and availability of infauna and epifauna have a major influence on consumption. Predation on other crabs and cannibalism have been observed in snow crabs. Mature male snow crabs practice predation on other species more frequently than females. In some regions, cannibalism of smaller individuals occurs, which is more frequently observed among mature female snow crabs (Divine et al. 2017; Squires and Dawe 2003).

The natural diet of American lobsters varies based on different regions, as lobsters are opportunistic feeders and source their prey based on availability and abundance. Lobsters are carnivorous during their larvae and postlarvae stages, and are omnivorous when mature, having a diet range including crabs, mollusks, whelks, sea stars and macroalgae (NOAA. b). The American lobsters inhabiting the soft substrates of Northumberland Strait, CA, were mainly carnivorous, as plant material comprised less than 2% of their prey biomass.

Decapod crustaceans were the primary prey, and a strong preference for Atlantic rock crab (*Cancer irroratus*) was found for lobsters of all stages, comprising 46% to 68% of the total prey biomass. Less than 7.5% of the prey biomass of lobsters were molluscs, polychaetes and fish. However, lobsters inhabiting boulders and reefs had a different diet composition, which dominated by detritus, molluscs, rock crab, as well as sea urchins (*Strongylocentrotus droebachiensis*) (Hanson 2009, 2018; Ojeda and Dearborn 1991).

2.2.2 Influence of Chemical Stimuli on the Feeding Behaviour of Crustaceans

Many decapod crustaceans have light/dark rhythms that are impacted by their environment. Due to water turbidity and low illumination in benthic environments, water-borne chemical signals are the principal substance for aquatic animals to orient and locate prey (Anderson 2014; Jury et al. 2005). Chemical information transmits more effectively through water than through air and disperses over a wider area for a longer-lasting period, as it diffuses five orders of magnitude slower in water than in air (Westerberg and Westerberg 2011).

Chemical stimuli are the primary signal in terms of prey seeking, mating, and other behaviours, and chemoreception is the primary sense for most crustaceans. Chemosensory systems, which are comprised of gustation, olfaction, and chemoreceptor cells, determine the acceptance or rejection of feed (Lee and Meyers 1996; Løkkeborg et al. 2014). Crustaceans receive olfaction and taste information through different mechanisms. Olfactory chemoreceptors located on the first pair of antennae motivate forward movement, while the tips of the legs and mouthparts contain the taste receptors (Jackson et al. 2007). Because of the clear association between chemical stimuli and foraging behaviour response, crustaceans are recognized as a fitting experimental animal for analyzing the relationship between chemoreception and foraging behaviours (Dellinger et al. 2016; Middleton et al. 2000; Zimmer-Faust 1989).

Omnivorous crustaceans respond to a wide range of amino acids and chemicals (Bauer et al. 1981; Coman et al. 1996). Molecular mass has a significant effect on crustacean response to dietary attractants. Low-mass molecules, including amino acids, nucleotides and sugars are the primary substances identified to induce crustacean foraging behaviour, even in a much-diluted solution. Olfactory receptor neurons can detect molecules smaller than 8.5 kDa via the aesthetasc. The aesthetasc sensilla are located on two paired antennae, where cuticular extensions house chemosensory hairs. (Archdale et al. 2011; Dellinger et al. 2016; Mackie et al. 1980; Derby et al. 1997). Under laboratory conditions, the European lobster (*Homarus gammarus*) responds to low-molecular-mass fractions (< 1,000 daltons) (Zimmer-Faust 1989).

Responses to attractants can vary, as the threshold of response is not fixed. Under low-risk conditions, such as low predation threat and access to shelter, the threshold could possibly be decreased and crustaceans would respond to low attractant concentrations (spiny lobster *P. interruptus*, Zimmer-Faust 1989). Molt cycles are one of the essential processes in the life of a crustacean and have been demonstrated to lead to changes in chemosensitivity. Levels of response towards chemoattractants may be dissimilar throughout different crustacean molting stages, as has been observed in freshwater prawns (*Macrobrachium rosenbergii*) and giant tiger prawns (*Penaeus monodon*) (Coman et al. 1996).

Behavioural responses raised by chemical attractants can also vary due to different water conditions, which may affect how attractants function. Foraging behaviours of blue crabs (*Callinectes sapidus*) weaken when the homogeneity of chemical plumes is elevated by an increased turbulence level and substrate roughness, while contrasting results have been found in crayfish (Jackson et al. 2007).

Synergistic interactions may be achieved by combining attractive substances, where the attractiveness of a mixture with multiple compounds is greater than the sum of the attractiveness of the individual compounds. As an example, a mixture of amino acids and betaine can attract freshwater prawns effectively and elicit search response at a lower concentration than the individual chemicals (Coman et al. 1996). Traps baited with a mixture of fish bait and sugarcane doubled the catch rate of swimming crabs as compared with traps baited with fish bait only (Archdale et al. 2011).

2.2.3 Behavioural Response

Crustaceans detect signal structures and navigate toward odorant sources by chemical stimulus intensity and distribution, followed by foraging behaviours after entering the odorant plume, which could be asymmetrical and instantaneous. Different thresholds exist in crustaceans for attractive substances such as amino acids or saccharide solutions, where the chemical signal is detected, triggering behavioural responses (Archdale and Anraku

2005). Five phases are typically used to classify the response of crustaceans toward chemical signals: detection, orientation, locomotion, feeding initiation, and continuation/termination of feeding. Four types of behaviours can be observed as responses to stimuli, which are antennule flicking, probing movement, locomotion, and mouthpart movement (Archdale et al. 2011; Carr and Derby 1986; Lee and Meyers 1996).

Amongst the series of behaviours exhibited in response to chemo-stimulants, the most generally observed and clear demonstration in crustaceans is antennular flicking. Besides chemical stimuli, visual and mechanical signals can also trigger antennular flicking. The majority of antennal flicking is elicited in response to chemical cues rather than mechanical or visual (Harpaz and Steiner 1990). However, this type of behavioural response may not always clear to determine or raise a spatial difference.

2.3 Use of Bait and Feed Attractants

2.3.1 Bait Used in Crustacean Fisheries

Forage fish are used predominantly as bait in crustacean fisheries, which includes anchovies, herring, mackerel, and menhaden. Typical finfish baits for American lobster incorporate Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*) (Archdale and Kawamura 2011; Ryan et al. 2014). Herring and mackerel are frequently used in conical traps in snow crab fisheries in the Gulf of St. Lawrence. Herring and squid are both used as bait in Newfoundland and Labrador, where squid is more costly in snow crab fishing. Independent of bait type, catchability is typically variable due to a range of factors including water current, velocity, temperature, levels of satiation, and the abundance of crustaceans in specific areas (Grant and Hiscock 2009).

Since commercial bait is commonly kept frozen until use, refrigeration and low-temperature storage are required before the fish are thawed and cut; thus refrigeration capability is taken into consideration for bait as well as storage space (Mackie et al. 1980; Archdale et al. 2011; Grant and Hiscock 2009). Freezing and storage account for the primary electricity expenses associated with fishing, and the expenditure per haul increases accordingly when bait is supplied and sourced distantly (Ryan et al. 2014). Current fisheries rely on fossil fuel to support fish harvest and refrigeration on board. On average, 1026 L of fuel per tonne of lobster landed was used by fishing vessels in fishing area 34 in Nova Scotia in 2005 (Driscoll et al. 2015).

Forage fish provide nutrition to other predatory animals in the food chain, where almost half of the forage fish are consumed by pelagic fish and seabirds, connecting plankton and predators biologically (Masilan and Neethiselvan 2018). As forage fish could potentially be a nutritious human food source for the rising human demands for protein, alternative baits will be required to replace forage fish in crustacean fisheries (Archdale and Kawamura 2011; Løkkeborg et al. 2014; Masilan and Neethiselvan 2018).

2.3.2 Feed Attractants

The attraction to some natural molecules and substances has been established in several crustacean species. Positive responses of freshwater prawn to certain biogenic amines, sexual pheromones, and natural attractants have been observed (Mendoza et al. 1997). Biogenic amines, such as putrescine and cadaverine, and metabolites with low molecular weight are typical chemo-attractants. However, it is not economically practical to use cadaverine on a commercial scale, considering its high cost of production (Lee and Meyers 1996; Ryan et al. 2014).

Crabs, such as the blue swimming crab (*Portunus pelagicus*), have strong behavioural responses to alanine, betaine, serine, galactose, and glucose (Archdale and Anraku 2005). Among scavenging and predatory crustaceans, betaine and taurine are more attractive and are beneficial to osmotic regulation. Taurine, in particular, is one of the principal amino acids released from invertebrates in the ocean, and is attractive to scavenging and predatory crustaceans (Coman et al. 1996). The attractiveness of glutamine, which functions in ammonia detoxification, has also been verified in various crustacean species. Glycine was also a favourable attractant for decapods in the ocean, however, this speculation was not valid in freshly molted giant tiger prawn as no response was observed, and glutamine raises responses at a lower effective dose than glycine (Dellinger et al. 2016).

Squid and its extracts have a complex chemical composition that are naturally attractive to crustaceans, and were more efficient than other natural baits in snow crab fishing. Despite the fact that some amino acids and molecular compounds of squid bait have been identified, the true chemoattractant(s) in squid bait have not determined (Araya-Schmidt et al. 2019; Mackie et al. 1980; Lee and Meyers 1996; Zimmer-Faust et al. 1996).

Some amino acids, including taurine, glycine, alanine, and arginine, comprise a high proportion of the amino acid composition of squid, which all elicit more stimulating effects in *Portunus armatus* (Archdale et al. 2011).

Crustaceans can recognize water-borne chemical signals despite the complexity of chemical mixtures, and a combination of amino acids and saccharides are attractive to crabs. The preference for saccharides, such as glucose and galactose, was verified in several crab species including crucifix crab (*Charybdis feriata*), porcelain crab (*Petrolisthes cinctipes*), and sand fiddler crab (*Uca pugilator*). Preference was determined given the increased capture when sugarcane or sugar were added to fish bait. Blue swimming crabs were more reactive to galactose and glucose solutions compared to serine, alanine, and betaine at the same concentration within the range of $2*10^{-7}$ to $2*10^{-4}$ M (Archdale et al. 2011; Masilan and Neethiselvan 2018). Capture of swimming crab was doubled using fish bait mixed with sugarcane compared to using fish bait alone (Kawamura et al. 1995). However, this result was not repeated in two subsequent studies. Catchability of blue swimming crabs was independent of additional sugar in fish bait (Archdale et al. 2008; Masilan and Neethiselvan 2018).

Bait attraction for many crustaceans is associated with odours, particularly with a preference for decayed fish. For instance, aged abalone, rather than fresh flesh, was a more desirable option for lobster, but not for crabs (Archdale et al. 2008, 2011; Dellinger et al. 2016). Fish proteins decay into peptides, amino acids, amines, and volatile ammonia, while amines are derived from decarboxylated amino acids. For instance, lysine and arginine convert to cadaverine and putrescine, both major attractants for freshwater prawn. Putrescine is the undeviating catabolic product of amino acids, which indicates that it could evoke a considerable response in American lobster (Ricque-Marie et al. 1998; Mendoza et al. 1997; Dellinger et al. 2016). Biogenic amines act as feeding stimulants in blue shrimp, where the effects of dietary cadaverine on growth was investigated, and it indicated that the concentration of dietary cadaverine (0 – 4600 mg/kg) did not have an influence on growth parameters, including feed consumption and weight gain (Tapia-Salazar et al. 2004a). An accumulated concentration of cadaverine, however, is found in tissues, particularly in the hepatopancreas, suggesting the confined metabolism for cadaverine (Tapia-Salazar et al. 2004b).

2.4 Alternative Bait to Forage Fish

Forage fish used as bait is becoming more expensive, along with the costs associated with bait storage and refrigeration. Its increasing demand influences forage fish depletion, and the sustainability is also imperiled by the current usage of trapped bait, which poses a considerable ecological risk. Fresh bait used in the crayfish commercial industry, for example, is a sizeable expense, comprising up to half of the operating budget (Archdale et al. 2008). Therefore, it is essential to turn to cost-effective alternative bait types with a stable year-round supply on a commercial scale (Dellinger et al. 2016; Masilan and Neethiselvan 2018; Middleton et al. 2000).

While fish bait is the primarily used bait in fisheries, attempts at alternative options have emerged. An alternative bait was developed by Bait Masters Inc. in PEI, for use in the lobster fishing industry. The manufacturer claims the alternative bait reduces the use of pelagic fish, although their product contains other fish flesh, including processed fish and dehydrated fish, comprising 85% of the bait product. Capture resulting from their alternative bait was not significantly different from capture resulting from traditional fish bait, based on the capture mean and the catch per unit effort (CPUE, counted on the number of animals caught) (Patanasatienkul et al. 2020). In comparison with their alternative bait, the attractants developed for the research presented in this thesis will not contain fish products in any form.

The development of artificial baits has been also attempted using by-products from fish and livestock industries sourced from the head and viscera, as well as other agricultural waste (Archdale et al. 2008; Archdale and Kawamura 2011; Mackie et al. 1980; Masilan and Neethiselvan 2018; Middleton et al. 2000). The Yasui Co. Ltd. (Japan), successfully produced a polymer tablet combining fish waste with wheat starch, reducing fish use and recycling some waste from the fish processing industry. However, when tested, the bait was less attractive to sand crabs than fish bait or fish waste bait (Archdale and Kawamura 2011). Oyster hemolymph and shrimp cephalothorax, waste products from oyster shucking houses and shrimp processing were previously tested as alternative baits for blue crab (*Callinectes sapidus*), and the bait created with shrimp cephalothorax had a high selection rate, indicating its potential for being an attractant in an alternative bait (Anderson 2014).

Attractiveness preferences differ among crustaceans, as several decapod species were attracted selectively by distinct bait types (Zimmer-Faust and Case 1982). Lobsters could be selectively attracted by abalone and mackerel tissue. Deterring behaviour was observed in edible crabs by mixing conspecifics into bait, which might inspire exclusive catching methods (Archdale et al. 2008). Green crab bait was compared with traditional baits in laboratory conditions as a potential bait for American lobster, but no significant difference was found among the two baits, indicating green crab bait was similarly effective in attracting lobsters (Ryan et al. 2014). An artificial bait consisting of grain by-products, plant protein, and roughage was produced for crayfish under aquaculture conditions and was commercially applicable (Archdale and Kawamura 2011; Beecher and Romaire 2010).

Poultry by-products have also been assessed as an alternative bait with more attractiveness for blue crab compared to beef stock, pig blood, duck weed, and chicken, while shrimp carapace has also been tested in blue crab traps as a potential ingredient for formulated bait (Anderson 2014). Baits made from extract of sprats and chemical attractant mixtures, which comprised several amino acids and chemical compounds (including taurine, glycine, arginine, hypoxanthine, and glycine betaine hydrochloride), were tested on European lobster compared to fish bait. Fresh fish was the best bait and an acceptable efficiency was obtained using artificial bait (Mackie et al. 1980).

The physical appearance and stability of bait could be altered by binders, which may vary the diffusion rate of compounds and subsequently impact bait attractiveness. Mincing bait could heighten its attractiveness (Archdale et al. 2008). In general, both natural and artificial baits have a high initial releasing rate in the first 1.5 hours of use, followed by a declining releasing rate (Løkkeborg 1990). The attractive compounds in artificial baits, such as amino acids, would ideally be released into the environment gradually over 48 hours of immersion. Gypsum, agar, and gelatin were involved in previous research, where gypsum and gelatin were regarded as more suitable inert supports of artificial bait than agar and were able to release the containing compounds constantly (Mackie et al. 1980; Masilan and Neethiselvan 2018). Low-solubility bait functioned effectively in crayfish, but did not perform well in swimming crabs.

Other types of non-traditional attractants have also been investigated through multiple studies. Synthesized baits consisting only of chemical compounds need a similar composition and concentration ratio to imitate natural baits (Middleton et al. 2000). Bait developed by Dellinger et al. (2016) was designed to mimic derived compounds found in forage fish and demonstrated an equal or better attractiveness for several crustacean species (spiny lobster, blue crab, stone crab) compared to traditional forage fish bait.

Artificial light was accidentally found to be a novel attractant in the crustacean fishery. LED (light emitting diode) lights contribute a significant improvement in the capture of snow crabs, which are more likely to be lured by blue and white light, rather than red or green light. The results, however, vary among crabs with different body sizes (Nguyen et al. 2017).

Among the various compounds and approaches used in fisheries to attract animals, betaine, lecithin and white LED light are the attractants of interests for this study, and will be covered in section 2.4, 2.5 and 2.6 in this chapter.

2.5 LED Light

Fishing with light is a common fishing technique used to increase catch rates in many fisheries, including squid, cod, herring and other pelagic species (Nguyen and Winger 2019b). Spectral sensitivity varies for different marine species, and is influenced by the habitats they occupy and the different stages of their development (Cronin and Jinks 2001). Compared to deep water and pelagic species, coastal species have more sensitive photoreceptive pigments to longer wavelength (Johnson et al. 2002). Studies testing LED light for fishing also investigated the use of different coloured lights. Decreasing by-catch rates, due to the stimulation of escape behaviour, were observed when artificial lights were used. Fishing using green light attached to bottom trawl and gillnet gear reduced bycatch in ocean shrimp (Hannah et al. 2015), flounder (*Paralichtys* spp.), ray (*Batoidea spp.*), guitarfish (*Rhinobatos planiceps*, Ortiz et al. 2016), cod and haddock fishing (Grimaldo et al. 2018). Snow crabs are more likely to be lured by blue and white light, rather than red or green light. The results, however, vary among crabs with different body sizes (Nguyen et al. 2017). Inspired by their research, white LED light was tested in the field trials during two snow crab fishing seasons in thesis.

2.6 Betaine

Betaine ($C_5H_{11}NO_2$), a quaternary ammonium compound (tertiary amine), is a metabolite with a low molecular weight (117.15 g/mol) and is a primary stimulant of feeding behaviour (Carr and Derby 1986; Masilan and Neethiselvan 2018; Wang et al. 2012) that exists extensively in plants, animals and marine prey. As a metabolic intermediate of transmethylation, betaine is the oxidized product of choline, which is found in extracts from fish, crab, and shrimp. Betaine also relates to osmotic regulation for marine invertebrates, as it is one of the nitrogenous osmolytes present in the cells (Bowlus and Somero 1979; Coman et al. 1996).

Betaine is an effective chemo-stimulant in triggering feeding behaviour among several decapod crustaceans (Coman et al. 1996; Harpaz and Steiner 1990). As betaine is found abundantly in marine prey extracts, in the filtrate from injured tissues, it efficaciously indicates available prey. Betaine induces antennular flicking and food searching behaviours in freshwater prawn (Harpaz and Steiner 1990).

Given its feature of triggering feeding behaviour, betaine attractant was developed in this study. The attractiveness of betaine was tested on snow crab and lobster, in sea and laboratory trials, respectively. In the sea trial, betaine attractants were augmented to fish bait to capture snow crab, where synergistic interactions might occur.

2.7 Lecithin

Lecithin is a phospholipid with a molecular weight of 311.225 g/mol (Clarke 2007). Phospholipids are polar lipids that can be synthesized by crustaceans. They play an important role in cell membrane constitution, crustacean growth, metabolism, and survival. It is necessary for some crustacean species to acquire dietary phospholipids for favorable growth and survival, including American lobster, red swamp crayfish, whiteleg shrimp *Litopenaeus vannamei* (Thompson et al. 2003). Phospholipids are also essential to emulsify lipid in digestion and absorption and are the primary transportable lipid in crustacean haemolymph and critical in mobilizing cholesterol and triglycerides from the hepatopancreas to the hemolymph. Glycerophospholipid is one kind of phospholipid, and the primary component of cell membranes and lipoproteins, and lecithin is a mixture of glycerophospholipids (Kumar et al. 2018). Lecithin is a mixture of glycerophospholipids and is widely found in various tissues among different organisms. Lecithin is generally derived from eggs and soy, which mostly contains phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Phosphatidylcholine is the primary active compound in purified soy lecithin diets, which is beneficial for growth enhancement (Haran and Fenucci 2008; Thompson et al. 2003).

Improved growth performance was demonstrated in red swamp crayfish, spiny lobster, American lobster, and whiteleg shrimp due to the addition of soybean lecithin to their diets; while in contrast, supportive conclusions were not achieved in juvenile red claw crayfish and freshwater prawn with a lecithin composition up to 20% in diets (Kumar et al. 2018; Thompson et al. 2003). The crucial role of lecithin in the diet has been verified on *Homarus* species, as a deficiency led to limited cholesterol transport from the hepatopancreas to the hemolymph. A lack of dietary lecithin in American lobsters resulted in a diminished survival rate and molt death syndrome, namely a failure of extrication when molting, as they might have a restricted ability to synthesize phosphatidylcholine (Haran and Fenucci 2008; Thompson et al. 2003).

Artificial lecithin attractant was developed in this study, which had a same concentration of effective ingredient as that of betaine attractant. The lecithin attractant was tested on American lobster in a laboratory trial, along with betaine attractant, and fish bait.

2.8 Objectives and Hypotheses

This thesis had three objectives:

i) To compare the capture rate of snow crabs in sea trials using traps baited with traditional fish bait with traps baited with fish bait augmented with betaine attractant, and/or white LED light.
ii) To evaluate the financial cost/benefits of using alternate bait and attractant combinations in the snow crab fishery.
iii) To compare the attractiveness of betaine attractant, lecithin attractant, white LED light, and traditional fish bait, based on the duration and frequency of response of American lobster in laboratory-based preference trials.

Null hypotheses tested:

i) Traps using only traditional fish bait will have an equal capture as the other treatments (traps augmented with betaine attractant and/or white LED light).

ii) Augmenting fish bait with betaine attractant and/or white LED light will have an equal operating costs as using fish bait only in the snow crab fishery.

iii) Artificial attractant and/or white LED light will be equally attractive to lobster as fish bait that lobsters will respond to the artificial attractant and/or white LED light equally, and lobsters will rest by all attractants, fish bait and LED light for an equal period.

Hypotheses tested:

i) Traps using only traditional fish bait will have a lower capture than the other treatments (traps augmented with betaine attractant and/or white LED light).

ii) Augmenting fish bait with betaine attractant and/or white LED light can reduce operating costs in the snow crab fishery.

iii) Artificial attractant and/or white LED light will be more attractive to lobster than fish bait that lobsters will respond to the artificial attractant and/or white LED light more frequently, and will rest by the artificial attractant or LED light more for longer time.

Chapter 3: Use of Light and Feed Attractant to Enhance Traditional Bait and Catchability of Snow Crab (*Chionoecetes opilio*)

3.1 Abstract

An artificial, fish-free attractant (B_1) was developed and tested along with white light-emitting diode (LED) lights to determine their influence on snow crab (*Chionoecetes opilio*) catchability as compared with traditional fish bait. Sea trials were conducted during the 2018 (10 harvesting days) and 2019 (13 harvesting days) snow crab fishing seasons in southeastern Nova Scotia crab fishing areas 23 and 24. Four treatments were tested: (1) fish bait (control treatment); (2) fish bait and attractant B_1; (3) fish bait and white LED light; (4) fish bait, attractant B_1, and white LED light. Capture was recorded as harvested tote numbers. This experiment was arranged as a randomized block design. Analysis of variance (ANOVA) was conducted on the capture mean, where the harvesting day was blocked. The catch was significantly improved ($P < 0.01$) when additional attractant (B_1) was used with white LED light in the 2018 fishery season but not 2019 ($P = 0.122$). The block among harvesting days was worthwhile in both fishery seasons, indicating there was variation due to harvesting days. No other treatments were significantly different from the control. A diffusion trial was conducted on attractant (B_1) in fixed and flow-through systems, which ascertained its diffusion rate over different time periods under laboratory conditions and confirmed that B_1 would diffuse for at least 72 h at a fishing site. A cost-benefit analysis was conducted, using the 2019 fishery season as a model, to determine the potential economic aspects of using artificial attractants in snow crab fishing. Assumptions of costs and profits were established given available information for current fisheries in the Maritimes. Less fishing trips were found to be required when attractant B_1 and/or white LED light augmented fish bait. The results from an economic aspect could therefore be used as a theoretical support and an encouragement for snow crab fishers to switch to artificial baits in the long run. Future studies could assess the same treatments without fish bait added.

3.2 Introduction

Snow crab is one of Atlantic Canada's most profitable commercial crustacean species, with an international market and a promising potential for further growth. In 2017, the value of snow crab comprised one fifth of the total value of marine fisheries in eastern Canada, following lobster (45% of total value) (DFO 2019a). In 2019 in NS, commercial lobster and snow crab fisheries contributed over 880 and 175 million dollars, respectively (DFO 2021a).

The opening dates of snow crab fishing seasons vary and are assigned on an annual basis, where N-ENS typically opens from April to May and from mid-July to mid-August, while S-ENS opens throughout the summer from April to September. Restrictions and regulations are administered to maintain the sustainability of snow crab stock, as well as environment and ecosystem protection. The fishery is controlled by total allowable catch (TAC), surveillance and evaluations from scientific and industrial perspectives by Advisory Committees, where the exploitation rates are adjustable. Monitoring actions include total allowable catch (TAC), which is based on individual transferable quotas (ITQs), and the limited total number of traps (DFO 2016a). Landing of snow crab in ENS was 7524 metric tonnes in 2017 and was over 6805 tonnes in 2018 (DFO 2018b).

A commonly adopted practice in snow crab commercial fishing is to use fish bait, which tends to increase bait prices and demand. Forage fish are chiefly selected as bait in crustacean fisheries, comprised primarily of anchovies, herring, and mackerel. The sustainability of the use of fish bait is doubted. As an example, about 40% of forage fish catch went into fisheries globally in 2014, which urges a search for alternative options to replace or reduce fish bait use (Masilan and Neethiselvan 2018). From an economic aspect, because of increasing fluctuations in price and demand of these fish species, alternative baits at a cheaper cost are desired.

Additionally, animal protein production has been identified to be responsible for many ecological impacts, including climbing greenhouse gas (GHG) emissions. Fishing vessels driven by fuel are major contributors to energy consumption and GHS emissions (Tyedmers 2004). Highly profitable crustacean fisheries, particularly ones using bottom trawls or pots and traps, are ranked as the most energy and carbon-intensive protein production, excluding ruminant livestock production. It is therefore the least energy-efficient type of fishery. Small pelagic fishing, such as Atlantic mackerel, is the most effective form of fishery. However, protein produced from small pelagic fishing is used less often for human consumption in developed countries (Parker et al. 2018). Marine fishing industries now have multiple concerns including decreasing profitability and growing operation costs. The industry requires technical support to improve its efficiency to overcome these challenges and fluctuating prices.

Previous research has been conducted to develop alternative bait for crustaceans, aiming to reduce or replace the protein-rich fish bait used in marine fishing (Archdale et al. 2008 (swimming crab); Dellinger et al. 2016, Ryan et al. 2014 (American lobster); Middleton et al. 2000 (blue crabs)). The chemical components in natural bait are responsible for attracting crustaceans (Couturier 1984; Sutterlin and Couturier 1983). Betaine is a metabolic intermediate of transmethylation and an amino acid which is oxidized from choline, which exists both in plants and animals. It is found in extractions of injured prey tissues and effectively elicits feeding behaviour (Coman et al. 1996; Harpaz and Steiner 1990). Betaine is commonly supplemented in feeds to enhance intake in aquaculture for species, such as juvenile grouper (*Epinephelus fuscoguttatus*) and rainbow trout (*Oncorhynchus mykiss*) (Lim et al. 2016; Yesilayer and Kaymak 2020).

In addition to the use of chemical compounds, light is an effective fishing method, which now has been equipped on more fishing gear and has been applied at greater depth. Fishing with light has been used more commonly in fish species than crustacean species, and some attempts in using LED light in snow crab have been conducted over the past few years. Previous research on the use of light attractants in snow crabs indicate that blue and white lights significantly increased snow crab catch (highest with white), and purple light has a negative effect (Nguyen et al. 2017).

A cost-benefit analysis can be used in decision-making and to predict future situations, whereby benefits can be evaluated from a variety of perspectives. One of the disputes of a cost-benefit analysis is the diversity of factors that must be taken into consideration. In addition to monetary expenditures and revenues, cost-benefit analysis also monetizes social benefits and costs. A cost-benefit analysis can explore switching from traditional fish bait to alternative baits from an economic aspect. Because of the diversified influences, the focus of this project is placed on the comparisons of cost and benefit predominantly from the perspective of fishers using different baits and attractants under actual and assumed situations. A fishery season includes multiple expenditures, for instance, gas fees for vessels, labour costs, vessel maintenance fees, bait, and equipment expenses. In typical modern industrial fisheries, 75 to 90% of the total energy inputs go to direct fuel costs, which is mainly used for vessel propulsion and for other onboard activities (Tyedmers 2004). In the snow crab fishery, because of quota restrictions for each fisher, fishing stops once quota is met. If the quota is met earlier in the season than scheduled, the duration spent on the sea fishing will be shorter and gas fees and energy inputs will be lower. However, if the efficiency of the fishing process is significantly improved in a single location, there is a potential concern regarding overfishing of that area during a short time period.

In this study, sea trials were conducted to determine the impact of using artificial attractant B_1 and white LED light in addition to traditional fish bait on snow crab catchability. To validate that B_1 would function for a soaking period of up to 1-3 days during the fishing season from April to September in Atlantic Canada, with the temperatures from -1 to 11 °C on the Scotian Shelf (DFO 2016b), where snow crabs are caught. the diffusion rate of B_1 over 72 h was determined at 4 °C, which is within this range, as well as 23.5 °C, which would have a more rapid diffusion, in fresh water and seawater. A cost-benefit case study was conducted based on the 2019 fishery season, which analyzed and compared the costs and profits in two different situations: 1) if only traditional fish bait was used in the 2019 fishery season; 2) by meeting the same capture as that of 2019 fishery season, the differences in profit per haul that would be made using the following bait combinations in all traps: (i) all traditional fish bait, (ii) all fish bait plus attractant B_1, (iii) all fish bait plus white LED light, (iv) all fish bait plus both attractant B_1 and white LED light.

3.3 Objectives and Hypotheses

The objectives of this study were to determine the following:

i) The effects of attractant B_1 and white LED light (4 levels: fish bait; fish bait + B_1; fish bait + light; and fish bait + B_1 + light) on snow crab catchability.

ii) The diffusion process of attractant B_1 over a 72-hour period at two temperature levels in a fixed system and one temperature level in a flow-through system.

iii) The potential costs and benefits of using artificial attractant B_1 and/or white LED light.

Null hypotheses tested:

i) Adding attractant B_1 and/or white LED light will not affect the snow crab capture per haul compared to regular bait in sea trials.

ii) The diffusion process of B_1 will last no more than 72 hours.

iii) Using artificial bait and/or white LED light will have equal operating costs (per haul) as using fish bait only.

Hypotheses tested:

i) Adding attractant B_1 and/or white LED light will increase the snow crab capture per haul compared to regular bait in sea trials.

ii) The diffusion process of B_1 will last for more than 72 hours.

iii) Using artificial bait and/or white LED light will reduce operating costs (per haul) in comparison with using fish bait only.

3.4 Materials and Methods

Standard crab traps used in this study were dropped into the sea, then harvested after a minimum of one day (most frequently after 3 or 4 days). The traps were reused, but different numbers of traps were used every harvesting day (Table 3.1.1). Every trap successfully pulled out of the water was classified as 1 haul, and if *n* traps were hauled in one trip, that was noted as *n* hauls. Snow crab capture was recorded for each haul on the day that they were harvested, thus noted as 1 harvesting day. The interval between every two harvesting days was not fixed, and the traps were generally deployed for 3 to 7 days, but these intervals could be longer and were influenced by weather and other subjective limits. Hard-shelled males larger than 95 mm carapace width were kept as commercial-sized crabs, and other crabs were released back to the sea without being counted. The commercial-sized crabs collected from each haul were separated and counted in totes. Tote number was recorded in whole numbers, and was converted to kilograms in the data analysis, which was at 31.30 kg/tote and 29.48 kg/tote for 2018 and 2019, respectively.

3.4.1 Attractant B_1 and LED Light Preparation

Artificial attractant B_1 and white LED light were tested in both the 2018 and 2019 snow crab fishing seasons (Figure 3.4.1). White light-emitting diode (LED) lights were purchased from Hampidjan Canada Ltd. (NL, Canada), and contained two AA batteries (1.5 V) in each LED light. The intensity of the white LED light was 45 lux. Attractant (B_1)

was of a muffin-shaped appearance, contained anhydrous betaine (minimum 96% feed grade anhydrous betaine powder, manufactured by Finnfeeds Finland Oy, Naantali, Finland) in an "inert matrix" at a ratio of 61:39, and was manufactured in the Chute Animal Nutrition Centre, Dalhousie University (Bible Hill, NS, Canada). A consistent recipe for B_1 was adopted throughout the two fishing seasons, and its formula is proprietary and currently remains unavailable. The mixture was molded using cupcake pans lined with muffin wrappers, each had an average weight of 108.9 ± 4.60 g (SD). All attractants were stored in a -20°C freezer directly after being molded in order to quickly form their shape, which could then be stored at room temperature when handled by the fishers.

Table 3.1.1 Harvested haul numbers of all treatments in the 2018 and 2019 snow crab fishing season (14 and 15 harvesting days, respectively). Trap depth was documented on 9 harvesting days during the 2019 snow crab fishing season.

Fishing season	Date	Average trap depth (m)	Fish bait	Fish bait + B_1	Fish bait + light	Fish bait + B_1 + light	Total hauls per harvesting day
2018	April 19	-	28	15	15	2	60
	April 22	-	36	20	20	4	80
	April 28	-	37	18	20	4	79
	May 02	-	43	11	14	3	71
	May 06	-	48	18	17	3	86
	May 09	-	62	20	21	2	105
	May 17	-	53	8	15	2	78
	May 22*	-	72	0	23	0	95
	May 27*	-	80	0	22	0	102
	June 01*	-	79	0	23	0	102
	June 07	-	77	1	22	1	101
	June 23	-	61	17	22	1	101
	June 24	-	66	14	20	1	101
	June 30*		58	0	18	0	76
Total hauls			800	142	272	23	1237
2019	April 14*	-	60	0	20	0	80
	April 18*	-	42	0	20	0	62
	April 22	-	40	16	16	7	79
	April 26	-	40	17	12	10	79
	April 30	-	39	17	15	9	80
	May 04	-	37	18	17	8	80
	May 07	181.8	39	18	14	7	78
	May 10	183.9	38	16	15	7	76
	May 13	194.7	40	16	15	6	77
	May 17	168.0	39	16	16	6	77
	May 24	182.5	40	16	16	5	77
	May 25	179.5	40	16	16	5	77
	May 29	133.3	46	12	9	10	77
	Jun 07	121.1	41	7	7	7	62
	Jun 13	128.5	35	7	6	5	53
Total hauls			616	192	214	92	1114

* Harvesting days were not included in the statistical analysis due to insufficient data points.

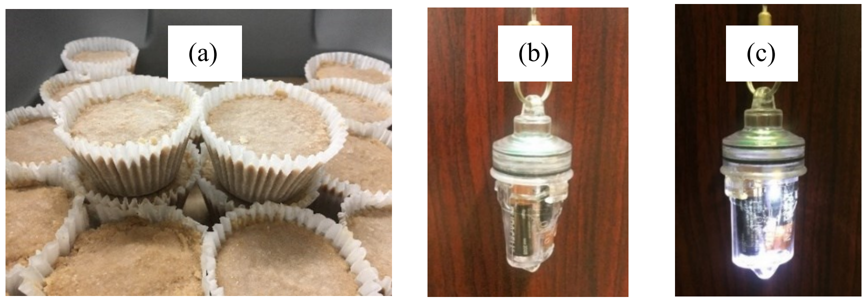


Figure 3.4.1 (a) Attractant B_1, (b) the white LED light (off) (c) the white LED light (on) tested in snow crab sea trials.

3.4.2 Validation of Attractant Diffusion

3.4.2.1 Fixed System Validation of Attractant Diffusion

Fixed systems were designed to test the diffusion rate of B_1 under laboratory conditions. Attractants were tested in a fixed volume of water and the validation trial was conducted in water bath shakers at two temperatures (4.0 and 23.5 °C) and two types of water (seawater (SW) and distilled water (DW)). Four scenarios were simulated: (i) at 23.5°C (room temperature) in DW, (ii) at 4.0°C in DW, (iii) at 4.0°C in SW, (iv) and at 23.5°C in SW.

Given the historic data of surface water temperature monitored from 2016 to 2019 in five different locations along the Eastern Nova Scotian coast (Glace Bay, Halifax, Lawrencetown, Martinique Beach, and Port Hawkesbury), the average surface water (within 200 m depth) temperatures of April, May, and June were at 2.0 ℃, 4.9 ℃, and 8.7 ℃, respectively (Sea Temperature Info 2020). Temperature in the fixed system and flow-through system diffusion validation tests ranged from 4 ℃ up to 23.5 ℃ (4℃ in DW and SW, 23.5 ℃ in DW and SW, 15 ℃ in FW), which encompassed a wider range than the actual water temperature in CFA 23 and 24 in the eastern Nova Scotian ocean.

A small portion of every attractant was manually sampled (14.6 ± 3.32g, SD) and saved for comparison (Fig 3.4.2). The remainder of the attractant (92.84 ± 3.81g, SD) was placed into a beaker filled with 200 ml distilled water or salt water individually (4 replicates/soaking time period/salinity treatment). Attractants were tested and sequentially picked up after being shaken during soaking time periods of 24, 36, 48 and 72 h. Sixteen beakers (one attractant sample in each) were contained in each scenario and held in two water bath shakers separately (8 beakers in each bath shaker). The effective ingredient diffused while the attractants were soaked while shaken. A VWR unstirred water bath and a Julabo SW22 shaking water bath were employed at 60 r. p. m. as confirmed by a stopwatch (8 beakers/shaker/scenario).

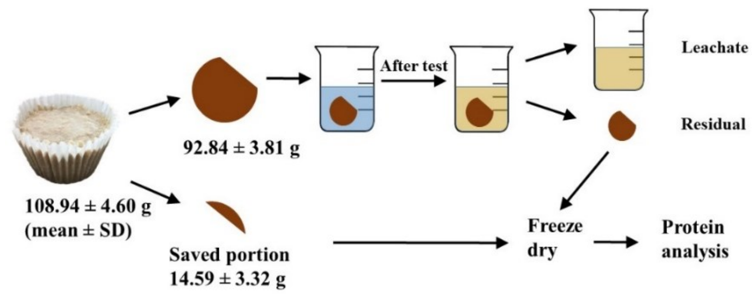


Figure 3.4.2. Illustration of attractants divided into saved and tested portions for protein content determination.

3.4.2.2 Flow-Through System Validation of Attractant Diffusion

A flow-through system was designed to simulate attractant diffusion under running water. This system was conducted with FW, sourced from Atlantic Poultry Research Centre, Dalhousie Agricultural Campus (Bible Hill, NS). FW (15 °C) was continuously dispersed from a tap attached by a hose to a pipe with 12 valves (4 mL/s for each valve). The valves were connected to hoses, which were fixed to the bottom of one of twelve 3.6 L plastic containers using tape (Figure 3.4.5). For consistency, attractant (B_1), produced in the same batch as the ones used in the sea trials were tested. Each attractant was submerged in its separated container and remained immersed at all times in the flowing water. After soaking time periods of 0 h (control), 24, 36, 48 and 72 h (three replicates at each time interval), residues were removed from the system.

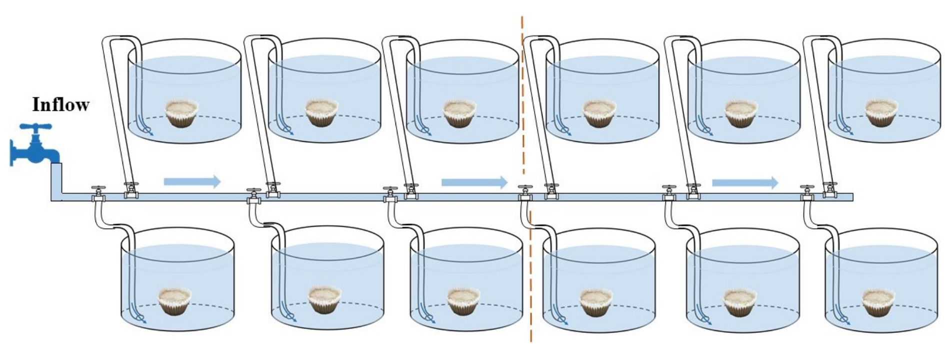


Figure 3.4.3 Illustration of flow-through system design. A consistent inflow of FW at 15 °C was maintained at 4 mL/s and dispensed to 12 containers. A betaine attractant was placed on the bottom of each container, and attractants were collected over one of four soaking periods (24, 36, 48 or 72 h).

3.4.3 Chemical Analysis

All saved portions and residues were individually mashed and freeze-dried for 24 h, then analyzed for protein content on a dry matter (DM) basis. As the effective ingredient, betaine, is the only source contributing nitrogen in the attractant, the analysis on residues was accomplished by a LECO FP-528 protein/nitrogen determinator (analyzed in duplicate). Diffusion efficiency was concluded based on protein content (%), which was reported on a DM basis.

Protein content was examined for all saved portions and residues based on duplicates, and an extra replicate was analyzed if the coefficient of variation (CV) between the duplicates was greater than 3%. For each soaking time period, the mean protein content (%) of residue was adopted and calculated given the four replicates. Protein content values (%) were analyzed to three decimal places, however, two decimals were reported to avoid overestimating accuracy.

Following removal from the flow-through system, the residues were freeze-dried for 24 h, then underwent a nitrogen/protein analysis. The mean protein content (%) of the residue was averaged from three replicates of each soaking period. The remaining protein content (%) was analyzed in duplicate. The mean protein content (%) given the three replicates of each soaking time period was calculated. The CV between the duplicates in protein content determination was controlled within 3%.

3.4.4 Sea Trial

The fishing sites for snow crabs were crab fishing areas (CFAs) 23 and 24, located off the eastern Nova Scotia coast. Standard traps (conical trap with a bottom diameter of 2.1 m) were used with mesh bait bags, and all traps had fish bait. A supply of attractants B_1 and white LED lights were adequately provided for the fishing season. B_1 attractants were also included in bait bags (one attractant per trap every time), and a single white LED light was attached in each trap beside the bait bag. A volunteer crew of snow crab fishers carried out the study and helped collect the data using their own fishing vessel. The fishers went out to the sea, placed traps in water, and waited for a minimum one day before harvesting the traps. Traps harvested on the same day were of a same soaking time, namely being placed on the same day. However, the soaking time of traps between dates was limited by weather condition, which given the data, was typically for 3-4 days (Table 3.1.1). The experimental attractants were used to augment traps containing the standard bait used by the snow crab fishers (fish bait). The attractants were stored in the shade or a cool spot out of the sun to avoid being heated and damaging its appearance, and the exterior paper wrapper was removed before adding it to the mesh bait bag of a trap. After a haul, the attractants were discarded and replaced with a fresh B_1 before the traps were re-deployed, and the old attractant and fish bait were replaced after each haul for all traps, regardless of whether any was left in the bait bag.

The white LED lights were attached to the top of the trap with a clip. Each LED light was turned on when the trap went into the water, and had its batteries changed midway through a crab season. Four treatments were tested by the snow crab fishers in 2018 and 2019. White LED lights and B_1 attractants were randomly assigned to traps by the ship's captain. The treatments were: (1) fish bait; (2) fish bait and attractant B_1; (3) fish bait and light; (4) fish bait, attractant B_1, and light. The fishers complied the request to use a minimum of 10 attractant B_1 and 10 white LED lights for each harvesting day for statistical purposes.

The 2018 snow crab fishing season progressed from April 11th to June 30th and capture results from 14 harvesting days were recorded. In 2019, by comparison there were 13 harvesting days between April 14th to June 13th. All traps were numbered and recorded, but were randomized and were not placed in any sequence at the fishing site. The total haul numbers (sum of number of hauls from all harvesting days) were 1237 in 2018 and 1114 hauls in 2019 (Table 3.1.1; Table 3.4.1).

Haul numbers were inconsistent for each treatment during the two fishing seasons, particularly in 2018, and treatments were not completely included on some of the harvesting days, which were therefore not counted in the data analysis (Table 3.1.1). The fish bait treatment generally had more replicates (hauls) than the other treatments. On April 19th, 2018, compared with 28 hauls using fish bait, only two hauls used fish bait plus B_1 and light, while 15 hauls augmented B_1 and 15 hauls augmented white LED light. The harvesting day was not included in statistical analysis if there were less than three hauls used in any of the four treatments.

The snow crabs in each trap were transferred to totes, and the number of totes were originally recorded as capture data of each trap, and the numbers of totes were recorded in whole numbers, which were subsequently converted into kilograms based on the tote numbers. The weight of one tote was slightly different between the two fishing seasons, counted as 31.30 kg/tote and 29.48 kg/tote for 2018 and 2019, respectively, based on the information provided by fishers. For consistency, results are presented both in totes and kilograms. 10 and 13 harvesting days were included for the 2018 and 2019 fishery seasons, respectively. Male snow crabs with a carapace width greater than 95 mm were collected and counted; however, female crabs were released back to the sea without being placed into totes or counted, and were not included in capture results.

Table 3.4.1 Duration and treatment setup for trial in 2018 and 2019 snow crab fishing seasons in crab fishing areas 23 and 24.

	2018	2019
Location	Crab fishing areas 23 and 24	
Treatments	Fish bait, fish bait + B_1, fish bait + light, fish bait + B_1 + light	
Fishing season	April 11th to June 30th	April 14th to June 13th
Harvesting days	14	15
Haul numbers	60 - 105	53 - 80

Trap placement depths were measured in fathoms, where 1 fathom equals 1.8 meters. During the 2018 snow crab fishing season, depth was recorded on April 19th and June 30th, 2018, where the average trap depths were 179.0 m and 132.3 m, respectively. However, the depth data were not sufficient to conduct statistical analysis. Depth was documented from May 7th to June 13th in the 2019 fishing season, and indicated a tendency to fish first in deep water, then in shallower areas, where the trap depth was over 160 meters on average before May 29th, 2019. As the fishers moved to shallow areas later in the season, the average depth became less than 140 meters (Table 3.1.1).

During the 2019 snow crab fishing season, the fishers deployed a total of 1114 hauls in 15 harvesting days (averaging 74.3 hauls per harvesting day), and a total capture amount estimated at 141,292 kg. All 1114 hauls contained regular fish baits, which were randomly assigned to each trap and were prepared by fisher following their previous practice routine. 192 hauls used with B_1 and fish bait, 214 hauls used white LED light and fish bait, and 92 hauls used fish bait plus B_1 and white LED light. The capture was originally recorded in totes, which weighed an average of 29.5 kg. The capture means of the four treatments weighed 123.8 kg/haul to 135.6 kg/haul in this case (Table 3.4.2).

Table 3.4.2 Haul number and capture mean for each treatment during the 2019 snow crab fishing season (April 14th to June 13th) in CFA 23 and 24.

	Fish bait	Fish bait + B_1	Fish bait + light	Fish bait + B_1 + light	Total
Haul number	616	192	214	92	1114
Capture mean (tote/haul)	4.2	4.3	4.6	4.3	
Capture mean (kg/haul)	123.8	126.8	135.6	126.8	
Capture of the whole season (kg)	76,270.7	24,338.7	29,020.1	11,162.3	141,291.7

3.4.5 Statistical Analysis

3.4.5.1 Validation of Attractant Diffusion

As betaine is the only source contributing nitrogen in the attractant, the analysis of protein/nitrogen determination was conducted on residues. Protein content (%) was plotted against soaking time (h) for the four scenarios (at 23.5°C in DW; at 4.0°C in DW; at 4.0°C in SW; at 23.5°C in SW), which generated four regression lines (second-order polynomial model). An incremental parameter analysis was conducted subsequently, given the model to test the hypothesis that there was a difference among the diffusion processes of the four scenarios. Two scenarios were compared each time to compare the regression curves. The two curves with the largest vertically different distances were compared first, followed by the two curves with the second largest vertical difference. Comparisons stopped once the incremental parameters of any two curves were not statistically different. The difference of incremental parameters (ϕ_1 and ϕ_2) was analyzed by comparing two scenarios each time (two of the four regression lines).

The model was constructed as:

$$y = \beta_0 + (\beta_1 + \phi_1 X^I)*X + (\beta_2 + \phi_2 X^I)*X^2 + \varepsilon$$

Where y was the mean protein content (%) of each soaking time period.

β_0, β_1, β_2 were coefficients.

X^I was specified as 0 or 1 for the compared two scenarios, where two of the regression lines were picked and compared every time (0 = one of the picked scenario, 1 = the other picked scenario);

X = soaking time period.

Two of the scenarios were compared every time, and a difference was determined if the *P*-value of either ϕ_1 or ϕ_2 was less than 0.05. An extension to a third-order polynomial model was employed if the assumption of normality was violated, and *P*-values of ϕ_1, ϕ_2 and ϕ_3 were considered:

$$y = \beta_0 + (\beta_1 + \phi_1 X^I)*X + (\beta_2 + \phi_2 X^I)*X^2 + (\beta_3 + \phi_3 X^I)*X^3 + \varepsilon$$

To compare with the four scenarios in fixed system validation, the mean protein content (%) of each soaking period in flow-through system validation were plotted along with the four scenarios in fixed system validation.

3.4.5.2 Sea Trial

Analysis of variance (ANOVA) was conducted on the capture data of all four treatments for each of the 2018 and 2019 fishing seasons. A randomized block design (RBD) was carried out for each season and the harvesting days were used as blocks. The trap numbers of treatments varied among different harvesting days. On each harvest day, and each treatment average of the captures was used as the response value. There are two main benefits of using average values, including a higher chance of achieving normality of error term, the design allows only one value per block per treatment (Montgomery 2014a, b).

In the sea trials, variations objectively existed among the harvesting days, which were considered a nuisance factor that may affect the results. As the nuisance source of variability is known in this case, an RBD was carried out for each season to remove variable due to harvesting day. This design could systematically eliminate its effect on the statistical comparisons among the treatments, and to form a more homogeneous experimental unit which improved the accuracy of the comparisons (Montgomery 2014b). Under this circumstance, the nuisance due to harvesting days (time) was systematically controlled through blocking. To avoid this influence, the randomized block design was conducted separately for each of the two fishing seasons.

The ANOVA for the RBD was done considering the treatment as fixed effect from treatments being investigated. For hypothesis testing, the model errors are assumed random variables with a normal and independent distribution. The error term is assumed to have a constant variance (Montgomery 2014b). Assumptions were checked in the analysis to ensure the validity. When the effect of treatment was significant (a significant difference among the treatments), multiple means comparisons was conducted using Tukey's method at 5% level of significance.

Further analyses were conducted in order to interpret the results of the two fishery seasons via a table of descriptive statistics of capture, including the number of traps used for each treatment on each harvesting day, the capture mean (in tote numbers), standard deviation, and standard error of mean. Captures were collected and recorded in tote numbers using whole numbers. Because the data were between 1 and 10, decimal places are allowed in the data analysis, and the allowance for this situation could be a maximum of two decimal places (allowance of one decimal place if data were collected between 10 and 99). Therefore, the use of decimal place in analyses for the sea trials should be acceptable.

To clarify the source of variability in the analysis, the values of R^2, the values of the square root of mean square error ($\sqrt{MSE}$), and the coefficient of variance (CV) were included as well. In ANOVA analysis, the square root of the mean square error ($\sqrt{MSE}$) would the best estimator of deviation. By further dividing the square root of mean square error ($\sqrt{MSE}$) by the overall mean, the coefficients of variance (CV) were computed. The variability can be considered low if the CV is less than 10%, and would be considered moderate if between 10 and 30 %.

Analysis was run in Minitab 18 to determine if there was a statistically significant improvement on capture using treatments with additional attractant B_1 or light, with a significance level set at $\alpha = 0.05$. An additional ANOVA that pooled the data from both the 2018 and 2019 seasons was conducted. Assumptions were verified, including the assumption of independence, normality of residuals, and equality of variances, where data transformation was applied when the normality was violated. In addition, the average depth and capture mean of each treatment was examined to determine any potential correlation between the two in 2019.

3.4.6 Cost-Benefit Analysis

3.4.6.1 2019 Snow Crab Fishery Season

The cost-and-benefit analysis was compared in two different situations:

1) The 2019 fishery season if only traditional fish bait was used.

2) If to meet the same total capture as that of 2019 fishery season, the differences of profit per haul using the following: (i) all traditional fish bait, (ii) all fish bait plus B_1, (iii) all fish bait plus white LED light, (iv) all fish bait plus both B_1 and white LED light.

3.4.6.2 Natural Baits, Artificial Attractants, and LED Lights

The cost for fish bait was assumed based on market price, where the typical fish bait includes Atlantic herring, mackerel, sardines, and squid. A considerable range was previously observed among the costs of different bait types, such as squid, which was 2.5 times cost of herring in 2009. Distinct demands for bait are required according to different trap types. For instance, large conical traps require larger quantities of fish bait such as herring or mackerel, where approximately 0.9 kg of fish bait is commonly used in one trap if used exclusively (Grant and Hiscock 2009). The market price of mackerel bait was $4.39/kg in the summer of 2019 in Nova Scotia. Some snow crab fishers would capture fish for their own bait instead of purchasing. However, the bait cost was still assumed and included in this analysis as opportunity cost.

The artificial attractant B_1 was assumed to have been purchased by snow crab fishers from our laboratory at cost, and specific storage conditions were not required for their usage. The average cost of each B_1 was at $1.34. Therefore, the cost of bait during the 2019 fishing season of 284 B_1 used was estimated at $380.60. The cost of each white LED light was $28 including two AA batteries (1.5 V) in each LED light, and the average cost of batteries was $0.75 each. Batteries in the LED lights were all changed mid season, even if they were working.

In this case study, an equal total haul number (1114 hauls) was assumed during a fishery season. Therefore, if white LED light was included in every haul, an equal number of lights was used on every harvesting day. If *n* traps were used every harvesting day, and every trap contained a light, then only *n* lights were needed in total, as they are reusable.

3.4.6.3 Landed Price and Landed Value of Snow Crab

The average landed price of snow crab fluctuated according to global supply, which could be significantly different from one year to another and has maintained an increasing trend over the past ten years, reached an average \$10.76/kg in Quebec and \$9.68/kg in Newfoundland region (DFO 2019a, c). The average quota shares of licence holders were computed at 1.612% and 1.867% in CFA 23 and 24, respectively, given the available information. The yearly landed value per licence holder varied significantly from regions as well. Licence holders in S-ENS earned the most at an average landed value of \$347,000 in 2009, in comparison, the gain was at \$34,000 per licence holder in N-ENS (DFO 2016a). The landed price of Canadian snow crab varied monthly in 2019, which was \$11.86/kg, \$10.80/kg, and \$11.18/kg (in CAD) for April, May, and June, respectively, and the total landed value of this season was computed accordingly (FAO 2019). The assumption established in this case study was that the snow crab fishers sold snow crabs at the average monthly landing price.

3.4.6.4 Labour Cost, Harvesting Cost, and Energy Input

Given the North American Industry Classification System (NAICS), the snow crab fishery is classified as part of the shellfish fishing industry, including commercial catching or taking of shellfish from their natural habitat. The average hourly wage in the shellfish fishing industry was \$25.19 in 2019 in NS, Canada, and the corresponding average weekly wage was \$1219.17 given the survey for both full-time and part-time among both sexes (Statistics Canada 2019). However, this could be different from practical practice, where crew members are often hired seasonally, and payment may include a proportion or a share of the harvest profit. After covering operating expenses, as well as the owner's share and captain's percentage, an unexperienced crew member could potentially receive 1.5% to 5% of the net harvest profit. However, this type of payment leads to variations which exist based on different situations, such as fishing locations and fishery species. In Alaska, a snow crab fisher could gain nothing or tens thousands of dollars in this way, while some other boats may give a fixed daily payment.

Energy input primarily refers to fossil fuels for vessel propulsion, in which direct fuel energy inputs generally account for at least 75% (Tyedmers 2004). Indirect or secondary energy consumption in fisheries includes vessel maintenance, depreciation, refrigeration and freezers onboard, as well as inland facilities (Driscoll et al. 2015; Tyedmers 2004).

3.4.6.5 Comparisons of the Attractants Used in the 2019 Fishery Season

An assumed situation using only fish bait was compared with the actual situation of the 2019 fishery season to determine if there was a gain in benefit. Attractants and white LED lights augmented fish bait in the 2019 fishery season, and the assumed situation was simulated with the same conditions of trap numbers, and fishing season period as the 2019 fishery season, but only involved fish bait. Expense assumptions were primarily made for fish bait, attractant B_1, white LED light, and labour costs (Table 3.5.5 (a), (b)). Other expenses, like gas fees and maintenance costs, were not included in this comparison, which varied subjectively for different vessels and licence holders. As there were 1114 hauls harvested in less than nine weeks (April 14th to June 13th) during the 2019 fishery season, the total haul number was accordingly set at 1114 over 61 days for the assumed situation (using fish bait only).

3.4.6.6 The Situations of Using Fish Bait, Additional Attractants, and LED Lights Respectively to Meet the Same Capture

The total capture amount of the 2019 fishery season was 141,291.7 kg (estimated at $1,582,580.80), which was set as the capture goal (fixed number) in the following assumed situations: using fish bait plus attractant B_1 in all hauls, using fish bait plus white LED light in all hauls, using fish bait plus attractant B_1 and light in all hauls. The comparisons were used to determine if using different bait and attractant combinations could reduce operating costs by improving capture efficiency, and therefore reducing the number of hauls to meet the quota and duration of fishing on the sea (Table 3.5.6 (a)).

3.5 Results and Discussion

3.5.1 Validation of Attractant Diffusion

3.5.1.1 Fixed System Validation of Attractant Diffusion

Increasing temperature in the fixed system resulted in a faster protein release (P-value < 0.05) with a minimal difference between DW and SW (P-value > 0.05). Prior to the diffusion test, B_1 had a consistent protein content (40.27% ± 1.05%, mean ± SD), which indicates a reliable quality control in bait production. The protein content of matrix with no betaine was lower than 1%, thus the protein content attributed by the effective ingredient betaine. A colour difference was visually detectable inside the attractants after diffusion, where the outer layer was faded as only the matrix remained (Figure 3.5.1), and betaine could be seen remaining in the attractant after the diffusion for 72 h.

Decreasing trends were observed in second-order polynomial models for all diffusion treatments ($R^2_{(DW,\ 23.5℃)} = 0.989$, $R^2_{(DW,\ 4.0℃)} = 0.973$, $R^2_{(SW,\ 4.0℃)} = 0.914$, $R^2_{(SW,\ 23.5℃)} = 0.909$). The diffusion processes of two temperature levels were different in both DW and SW (Figure 3.5.2). Both salinity (DW and SW) treatments at 4 ℃ had a higher protein content remaining in the residue compared to the other two scenarios (in DW at 23.5 ℃, and in SW at 23.5 ℃) (*P*-value < 0.05). All residues had a protein content higher than 30% (33.74% in DW, 31.87% in SW) at 4 ℃, while the residue remaining after diffusion at 23.5 ℃ had a protein content less than 25% (9.33% in DW, 14.29% in SW). The diffusion continued from 24 h to 72 h, where the protein content in the residue dropped by approximately 8% in both salinity at 23.5 ℃ (from 17.68% to 9.33% in DW, and from 21.9% to 14.29% in SW).

Figure 3.5.1 Attractants after 24 h (a) and 72 h (b) diffusion testing at 4 °C in sea water. The attractant was initially of a consistent dark brown colour inside out, an outer "shell" appeared after betaine released.

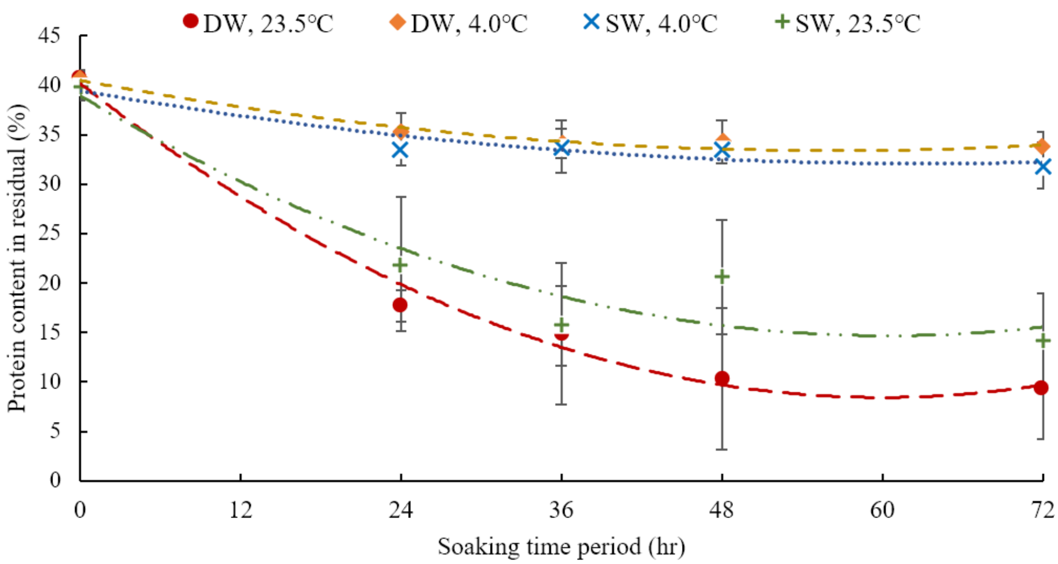


Figure 3.5.2 Mean protein content (%, ±SD) of the residue over 72 h fixed system validation test under four scenarios at two levels of temperature in distilled water and seawater. *n* = 4/temperature level/salinity treatment/soaking time period.

Incremental parameter analysis was conducted based on the second-order polynomial regression of protein content (%) over soaking time. There were differences in incremental parameters (ϕ_1 and ϕ_2) (P-value < 0.001) between the two temperature levels (4 ℃ and 23.5 ℃), but not between DW or SW at the same temperature. Consequently, the rate of diffusion rate in either DW or SW were similar at either temperature, but was faster at the higher temperature.

The solubility of betaine is 55g per 100g methanol, and the solubility is higher when the solvent is water at the same temperature compared to other solvents (O'Neil 2001). There is a positive relationship between the solubility of betaine and temperature (Wang et al. 2012). Given the results of the laboratory diffusion experiment, water temperature influenced the leaching process, where the colder temperature slowed diffusion (4 ℃, *P*-value < 0.001). B_1 maintained its performance and could last during the entire three-day period in all simulated circumstances. In the sea trial, soaking time was not fixed. It ranged from three days up to more than ten days for safety reasons (one 16-day interval in the 2018 season; Table 3.1.1). In a previous study, a longer time period was adopted, but a whole Atlantic menhaden (*Brevoortia tyrannus*) was used and not a piece of bait (Anderson 2014). In the sea trials included in this chapter, the fishers used proprietary combination of forage fish, where whole, frozen fish were cut into chunks used in bait bags, and were replaced for each new haul after each deployment. An uncontrollable source of variation due to the operations during the experiment. As the attractants were manually split into saved and test portions, inconsistent cross sections were left on attractant differently and may have led to varying diffusion results.

3.5.1.2 Flow-Through System Validation of Attractant Diffusion

Betaine diffusion in the flow-through system was compared with the four fixed system validation scenarios (Fig 3.5.3). Based on the results from the fixed system validation, there was no difference between treatments using DW and SW at the same temperature, thus only FW was used in the flow-through system validation, where DW was more sterile than FW. The samples had a consistent initial crude protein content. In comparison to the second-order polynomial model used in the fixed system validation, the loss of betaine in attractants was linear, indicating a consistent decrease of the amount of effective ingredients in the residue over 72 h in the flow-through system ($R^2_{(FW,\ 15.0℃)} = 0.9946$). Residual protein content of the samples in the flow-through system after a 24 h soaking period was 34.6%. This was close to the result of the two fixed system scenarios at 4 ℃ (35.2% in DW, 33.5% in SW). After 72 h, the residual protein content of the samples in the flow-through system was 21.7%, which was more than the final content over the same soaking period at 23.5 ℃ (9.3% in DW, 14.3% in SW) in the previous fixed system validation. During diffusion, the volume containing the effective ingredient decreased due to diffusion, and the surface area ratio was therefore increased. However, the protein content loss remained linear in this flow-through system at 15 ℃. The consistent rate of release trend also implied that the shape, size, or the surface area ratio of B_1 would not affect the diffusion process. However, the linear decrease in protein content indicated that the effective ingredient could be diffused completely if the soaking period continued.

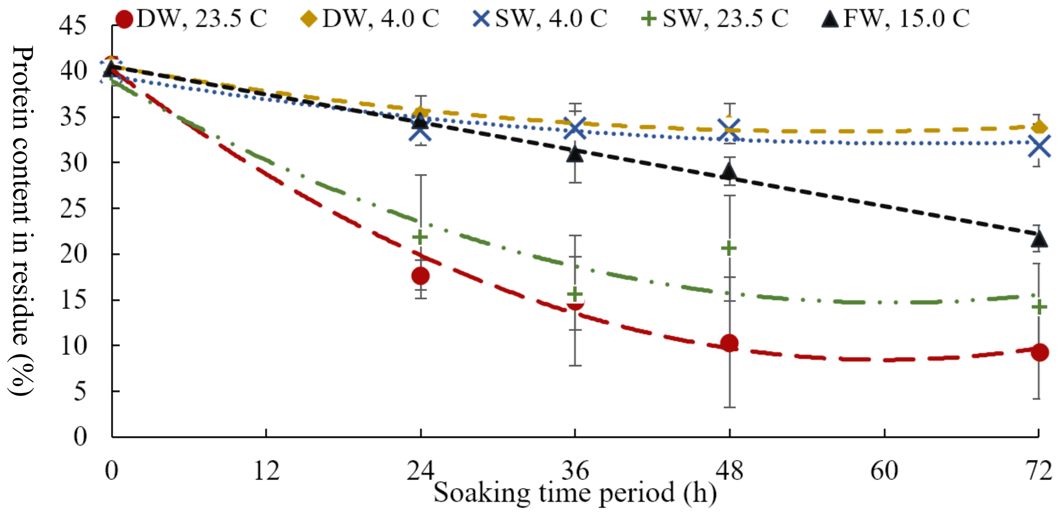


Figure 3.5.3 Mean protein content (%, ± SD) of the residue over 72 h in flow-through system validation test at 15 °C (▲) in comparison with the four scenarios in the fixed system validation at two levels of temperature in distilled water and seawater. In flow-through system validation, *n* = 3/soaking time period.

Given the information provided, fishing site depth ranged from 100.6 m up to 228.6 m, and the interval between harvesting days was typically a minimum of three days (Table 3.1.1). Since the habitats of snow crab on the Scotia Shelf range in temperature from -1 to 11 °C (DFO 2016b), the water temperature range in the validation tests was adequate to include the water temperature at practical sites, but also consider for colder or warmer fishing climates, which would have a faster diffusion rate. Water temperature data could be collected in future trials by adding a temperature logger to the crab trap. Results from the fixed system and flow-through system diffusion validation tests confirmed that B_1 consistently releases attractant for at least 72 h within the experimental water temperature range. Therefore, it is reasonable to propose that attractant B_1 could effectively release the chemical compound for up to 3 days deployment during the snow crab fishing season in Atlantic Canada. The residues in the bait bag from the harvested traps after the soaking period are recommended to be sampled during the fishing season in future research.

Given the feedback of the common fishing practices used by snow crab fishers, the intervals between harvesting days were flexible, largely ranging from 3 to 7 days. As the primary goal of the diffusion rate validation was to determine whether the attractant could be function for longer than 24 hours, the test period was set for three days (72 hours). The analysis consequently focused on the remaining attractant, rather than the concentration of the effective ingredient in the surrounding water. The concentration of ingredients in the water would affect the attractiveness, which may be reflected in the capture.

Synthesized and alternative bait were tested in several previous studies under both field (Dellinger et al. 2016; Archdale et al. 2008; Archdale and Kawamura 2011) and laboratory conditions (Couturier 1984; Middleton et al. 2000; Ryan et al. 2014). Attractant B_1 alternative bait was comparatively of a larger size and a heavier weight compared to tested baits in previous studies. However, the testing water velocity or flow rate was not detailed in most cases, and bait size varied as well. It was not possible to measure the velocity at the many fishing sites during our sea trial. The flow rate in the diffusion test was set at 4 mL/s, which was close to a reference testing velocity, documented up to 4.4 cm/s in a previous leaching rate study on smaller attractants by Couturier (1984), which was conducted in a 60 mL syringe.

3.5.2 Snow Crab Capture Results 2018 and 2019

Capture data from the 10 and 13 harvesting days in the 2018 and 2019 seasons, respectively, for all four treatments were analysed. Harvesting days were blocked in the analyses for both the 2018 and 2019 seasons. The *F*-values were both greater than 2, which indicated the block was worthwhile for both seasons (*P*-value < 0.001 and *F*-value = 17.39 for 2018 season, *P*-value = 0.122 and *F*-value = 42.40 for 2019 season). The assumptions were verified with regards to constancy of variance, normality, and independence.

In the 2018 season, bait type significantly affected catch rates. Using fish bait plus attractant B_1 and white LED light had a capture mean of 5.3 totes/haul, which was significantly higher than using the other three bait and attractant combinations of 3.7 to 4.0 totes/haul (Table 3.5.1). In the 2019 fishery season, in contrast, snow crab capture rate was independent of bait type, ranging from 4.1 to 4.5 totes/haul (*P*-value = 0.122; Table 3.5.1).

As a tote was weighted differently for the two years (31.30 kg/tote and 29.48 kg/tote for 2018 and 2019, respectively), all captures were converted to kilogram in the pooled analysis. The capture means were significantly different among treatments in the 2018 season (*P*-value = 0.001) and the block of harvesting days was worthwhile (*F*-value =

19.46). The fish bait plus attractant B_1 and white LED light treatment had the highest capture mean; the mean captures of the other three treatments were not statistically different, based on Tukey's HSD test.

A table of the descriptive statistics of capture was generated (Table. 3.5.2), where the number of traps used for each treatment on each harvesting day is listed, along with the capture mean (in tote numbers), standard deviation, and standard error of mean.

The ANOVA analysis was conducted based on the entire season over two years, respectively, where the harvesting days acted as blocks and the comparisons were primarily calculated given the treatment means of each block. With the premise of valid assumptions of normality and consistency of variance, it would meet the requirement if there was a minimum of one data point for each treatment on each harvesting day.

The values of R^2, the values of the square root of mean square error ($\sqrt{MSE}$), and the coefficient of variance (CV) were included in addition to the ANOVA results to present the source of variability in the analysis (Table 3.5.3). The R^2 values were 88.67% and 93.47% for the two fishery seasons, respectively. They indicate that 88.67% and 93.47% of the variability could be explained by the two factors that were taken into consideration (bait and attractant combinations, and harvesting days); therefore, 11.33% and 6.53% of the variability would be explained by other factors that were not included in the two fishery seasons, respectively.

The square root of the mean square error ($\sqrt{MSE}$) were 0.55 and 0.47 for the 2018 and 2019 fishery seasons, respectively (Table 3.5.3). The coefficients of variance (CV) were 0.33% and 0.21% for the 2018 and 2019 seasons, respectively, which can offer additional help in interpreting the variability. Since the CV is less than 10%, the variability is consequently considered low in the analysis.

A significant difference was found between treatments in the 2018 fishery season, and the mean comparisons were conducted again for the 2018 fishery season using Fisher's least significant difference (LSD) method, as a significant difference was only found in the 2018 season. By further consultation, the Fisher's least significant difference (LSD) method was suggested, as the Tukey's method would be better for experiments with better control, while the LSD method would be more suitable for situations with less experimental control. The results of means comparison using the LSD method were included in Table

3.5.4. Consistent to the previous results, the traps augmenting attractant B_1 and LED light with fish bait still had a higher capture mean than traps using other combinations.

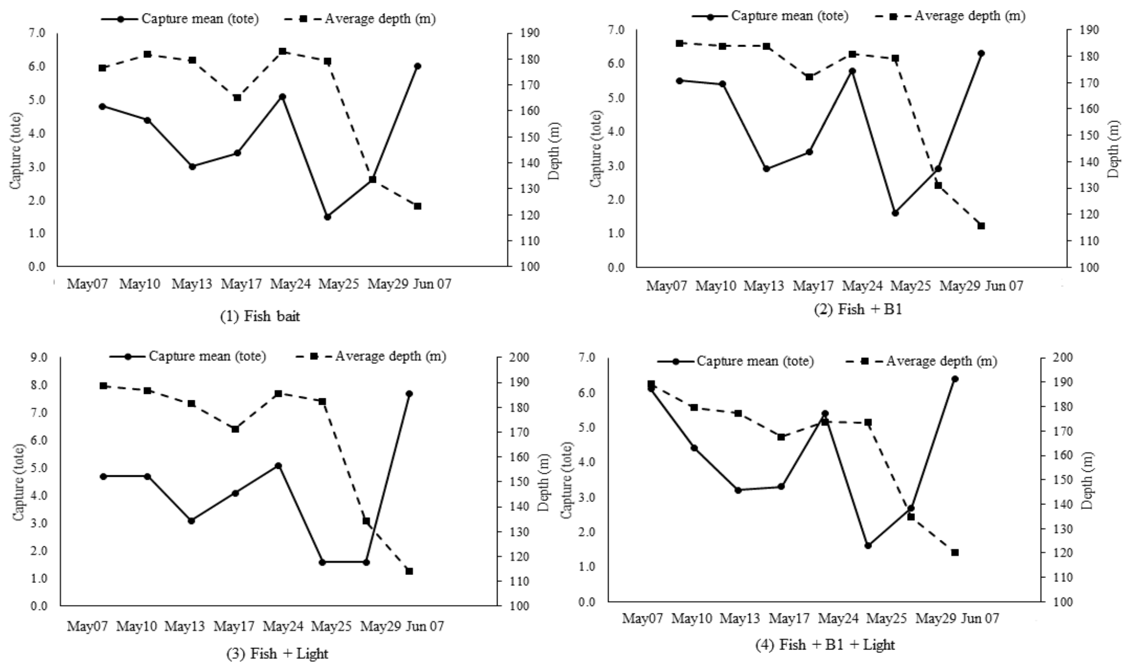


Figure 3.5.4 The mean depth (m) and mean capture (tote) for each treatment in the 2019 snow crab fishing season (May 7th to June 7th) in CFA 23-24.

The depths were similar on a given date and there was no correlation or pattern indicated between capture and depth in any treatment group (Figure 3.5.4). Therefore, the depth was not included as a covariate in the variability and was not accounted for by block (harvesting days).

During the two snow crab fishing seasons, the traditional fish bait subjectively affected the potential margin of improvement in catchability. In previous behavioural observation research, fresh or frozen fish bait was provided via extract, mixture, or mashed form rather than being provided directly, and the quality of the fish bait was not comparable among studies (Anderson 2014). In previous studies where bait attractiveness and behavioural response were investigated, comparisons were conducted with mussel juice (Borroni et al. 1986), squid extracts, commercial attractant (Mendoza et al. 1997), and fish meal made from fresh and stale North Sea herring (*Clupea harengus*) (Opstvedt et al. 2000; Tapia-Salazar et al. 2004a). The fish bait used by the fishers involved in the sea trials reported here was not standardized. However, it was used previously by these fishers with success, and was sufficient to meet its commercial fishing purpose. Potential interactions between the fish bait and the artificial attractant or white LED light could exist, which would be interesting to study in future research.

The tote numbers captured/haul were collected by the snow crab fishers and was documented in whole numbers, which may account for potential bias as no decimal places were recorded if the captures of traps were not of exact totes. An exact capture could be more variable than the whole number to some extent, but was still documented as a whole number during the practice. It had not been possible to count the captured snow crabs individually, therefore I recommend an improved counting method to be considered to collect more precise data in future fishery seasons.

Table 3.5.1 ANOVA on capture (presented as totes and converted to kg, ± SD) and comparison of results of the 2018 and 2019 snow crab fishery seasons* in CFA 23 and 24.

2018	N	Mean	2019	N	Mean
In tote/haul					
Treatment			Treatment		
1) Fish bait	10	3.7±0.9 [B]	1) Fish bait	13	4.1±1.7
2) Fish + B_1	10	3.7±1.0 [B]	2) Fish + B_1	13	4.4±1.5
3) Fish + Light	10	4.0±1.2 [B]	3) Fish + Light	13	4.5±3.7
4) Fish + B_1 + white LED light	10	5.3±1.7 [A]	4) Fish + B_1 + white LED light	13	4.4±2.2
In kg/haul					
Treatment			Treatment		
1) Fish bait	10	116.1±28.7 [B]	1) Fish bait	13	120.7±38.5
2) Fish + B_1	10	116.7±30.8 [B]	2) Fish + B_1	13	130.6±45.1
3) Fish + Light	10	124.3±38.2 [B]	3) Fish + Light	13	133.4±56.7
4) Fish + B_1 + white LED light	10	165.3±53.9 [A]	4) Fish + B_1 + white LED light	13	129.7±44.1
P-value		< 0.001	*P*-value		0.122
Harvesting day			Harvesting day		
F-value		17.39	*F*-value		42.4

* = Means that do not share a letter are significantly different. 10 and 13 harvesting days were included for the 2018 and 2019 fishery seasons, respectively (Table 3.1.1).

Table 3.5.2. Descriptive statistics of the capture (in totes) in 2018 and 2019 snow crab fishery seasons. For every treatment of each harvesting day, the following results are presented in order: the number of traps used (*n*), mean capture ± standard deviation (SD), standard error (SE) of mean.

Fishing season	Date	Fish bait	Fish bait + B_1	Fish bait + light	Fish bait + B_1 + light
2018	April 19	28 5.4 ± 1.9 0.4	15 5.9 ± 2.2 0.6	15 6.2 ± 2.5 0.6	2 8.5 ± 3.5 2.5
	April 22	36 3.7 ± 2.8 0.5	20 3.7 ± 2.5 0.6	20 4.1 ± 2.1 0.5	4 6.0 ± 3.0 1.5
	April 28	37 3.9 ± 1.5 0.2	18 4.3 ± 1.9 0.5	20 4.6 ± 1.5 0.3	4 6.5 ± 1.7 0.9
	May 02	43 2.9 ± 1.0 0.1	11 3.0 ± 0.9 0.3	14 2.6 ± 0.6 0.2	3 3.0 ± 2.0 1.2
	May 06	48 4.3 ± 1.6 0.2	18 3.8 ± 1.5 0.3	17 4.2 ± 1.3 0.3	3 6.33 ± 2.5 1.5
	May 09	62 3.2 ± 1.4 0.2	20 3.5 ± 1.6 0.4	21 4.0 ± 2.1 0.5	2 5.0 ± 0.0 0.0
	May 17	53 4.4 ± 1.1 0.1	8 3.4 ± 1.1 0.4	15 5.1 ± 1.2 0.3	2 4.5 ± 0.7 0.5
	May 22*	73 3.4 ± 1.3 0.2	-	23 4.4 ± 1.2 0.3	-
	May 27*	80 2.9 ± 1.2 0.1	-	22 2.7 ± 1.4 0.3	-
	June 01*	79 2.5 ± 1.2 0.1	-	23 2.6 ± 1.2 0.3	-
	June 07	77 3.7 ± 1.2 0.1	1 4.0 ± n/a n/a	22 3.9 ± 0.9 0.2	1 6.0 ± n/a n/a
	June 23	61 3.6 ± 1.9 0.2	17 3.6 ± 1.8 0.4	22 3.2 ± 1.7 0.4	1 4.0 ± n/a n/a

Table 3.5.2 (continued).

Fishing season	Date	Fish bait	Fish bait + B_1	Fish bait + light	Fish bait + B_1 + light
	June 24	66 2.0 ± 1.1 0.1	14 2.1 ± 1.3 0.4	20 1.9 ± 0.9 0.2	1 3.0 ± n/a n/a
	June 30*	58 3.1 ± 1.2 0.2	-	18 3.4 ± 0.9 0.2	-
2019	April 14*	60 4.2 ± 3.6 0.5	-	20 5.7 ± 4.1 0.9	-
	April 18*	42 6.1 ± 2.3 0.4	-	20 6.5 ± 2.3 0.5	-
	April 22	40 5.1 ± 2.0 0.3	16 4.6 ± 1.9 0.5	16 5.9 ± 2.7 0.7	7 4.6 ± 1.3 0.5
	April 26	40 3.4 ± 1.2 0.2	17 3.6 ± 1.4 0.3	12 3.3 ± 1.1 0.3	10 3.7 ± 1.3 0.4
	April 30	39 3.8 ± 1.5 0.3	17 4.3 ± 1.3 0.3	15 4.1 ± 1.6 0.4	9 4.3 ± 1.7 0.6
	May 04	37 4.3 ± 1.8 0.3	18 4.5 ± 1.7 0.4	17 5.1 ± 2.1 0.5	8 4.9 ± 2.0 0.7
	May 07	39 4.8 ± 2.1 0.4	18 5.5 ± 2.6 0.6	14 4.7 ± 1.6 0.4	7 6.1 ± 3.2 1.2
	May 10	38 4.4 ± 2.0 0.3	16 5.4 ± 2.1 0.5	15 4.7 ± 1.5 0.4	7 4.4 ± 1.4 0.5
	May 13	40 3.0 ± 1.1 0.2	16 2.9 ± 0.9 0.2	15 3.1 ± 1.3 0.3	6 3.2 ± 1.5 0.6
	May 17	39 3.4 ± 2.0 0.3	16 3.4 ± 1.8 0.4	16 4.1 ± 2.4 0.6	6 3.3 ± 1.6 0.7
	May 24	40 5.1 ± 1.9 0.3	16 5.8 ± 1.4 0.4	16 5.1 ± 1.3 0.3	5 5.4 ± 1.5 0.7
	May 25	40 1.5 ± 0.8 0.1	16 1.6 ± 0.8 0.2	16 1.6 ± 0.6 0.2	5 1.6 ± 0.5 0.2

Table 3.5.2 (continued).

Fishing season	Date	Fish bait	Fish bait + B_1	Fish bait + light	Fish bait + B_1 + light
	May 29	46	12	9	10
		2.6 ± 1.4	2.9 ± 2.0	1.6 ± 0.9	2.7 ± 1.2
		0.2	0.6	0.3	0.4
	June 07	41	7	7	7
		6.0 ± 2.5	6.3 ± 3.3	7.7 ± 2.1	6.4 ± 2.4
		0.4	1.3	0.8	0.9
	June 13	35	7	6	5
		5.7 ± 2.1	6.9 ± 3.2	7.8 ± 2.5	6.6 ± 1.5
		0.4	1.2	1.0	0.7

Table 3.5.3. Results of ANOVA analysis and variations over the 2018 and 2019 snow crab fishery seasons

Source of variance	2018 season			2019 season		
	Degrees of freedom	F-Value	P-value	Degrees of freedom	F-Value	P-value
Treatment (Bait and attractant combinations)	3	18.27	< 0.001	3	2.07	0.122
Block (Harvesting day)	9	17.39		12	42.40	
Error	27			36		
Total	39			51		
$\sqrt{MSE}$			0.55			0.47
R^2			88.67%			93.47%
Coefficient of variance			0.33%			0.21%

Table 3.5.4. Result of the comparisons of means for the 2018 snow crab fishery season using Fisher's least significant difference (LSD) method.

Treatment	N	Mean
Fish bait	10	3.71 ^{B}
Fish bait + B_1	10	3.73 ^{B}
Fish bait + LED light	10	3.97 ^{B}
Fish bait + B_1 + LED light	10	5.28 ^{A}

3.5.3 Comparisons of Using Different Bait Combinations

3.5.3.1 Comparison of the Attractants Used in the 2019 Fishery Season

The total capture amount of the 2019 fishery season was 141,291.7 kg and the average capture of using fish bait only was 123.8 kg/haul (Table 3.4.2). The landed value for the 2019 fishery season was computed given different landing prices from April to June ($11.86/kg, $10.80/kg, and $11.18/kg in CAD, respectively), which assumed that all snow crabs were sold after they landed. However, the landed value of the assumed situation (only using fish bait) was referred to as the average landed price from April to June ($11.28/kg in CAD) (Table 3.5.5 (a)).

Fish bait usage was presumed at 0.9 kg/haul, which would cost $3.98/haul. All hauls during the 2019 fishery season were landed using fish bait, and 1114 fish baits were therefore counted. Similarly, 192 B_1 attractants were used in the fish bait plus attractants B_1 treatment, and 92 B_1 attractants were used in the fish bait plus attractants B_1 and white LED light treatment, which led to a total usage of 284 B_1 attractants. Because the lights were reused, the expense of 25 lights for the fishery season was $29.50 each, including batteries (Table 3.5.5 (a)).

For a typical snow crab vessel, the crew size is usually four, but this is subject to change. Fishers typically work in shifts over the season, which was the case in our study, where 14 crew members were hired and worked in shifts. In this case study, labour expenses were assumed based on the weekly wage rate, referred from Statistics Canada, and the duration of the fishery season (61 days, $1,219.2/week/person) (Statistics Canada 2019) (Table 3.5.5 (a)).

3.5.3.2 Comparison of Situations Using Fish Bait, Additional Attractants, and/or LED Lights, Respectively

Based on the 2019 fishery season, the capture means/haul for using only fish bait, fish bait plus B_1, fish bait plus light, and fish bait plus B_1 and light were 123.8, 126.8, 135.6, and 126.8 kg/haul, respectively (Table 3.4.2). As the total capture was fixed for this run of comparisons, the numbers of hauls needed to meet the same capture was computed according to the capture mean/haul.

In the 2019 fishery season, 1,114 hauls were recorded over 14 harvesting days, averaging 74.3 hauls on each harvesting day. The harvesting day of each assumed situation was thus computed given the average of 74.3 hauls/harvesting day, which was rounded up to 75 hauls/harvesting day, as one more haul would be counted to meet the need of 0.3 hauls. Similar to the haul numbers, which had to be whole numbers, the estimated harvesting days were rounded up as well.

As 75 hauls/harvesting day were used in the estimations, 75 white LED lights were thus needed for the assumed situations (fish bait plus white LED light in all hauls, fish bait plus attractant B_1 and light in all hauls) (Table 3.5.6 (a)). The overall expense included the cost for fish bait, B_1 attractants and light, which was averaged based on the total haul numbers calculated previously for each assumed situation.

For each assumed situation, the duration was estimated based on the harvesting days needed. A four-day interval was assumed between harvesting days; therefore, the overall duration would be shorter if fewer harvesting days were needed. Labour expense was computed based on the weekly wage rate; a shorter duration could thus reduce the estimated labour expense (Table 3.5.6 (a), (b)).

3.5.3.3 Considerations to the cost-benefit analysis

The approach of cost-benefit analysis is often considered with limitations due to the uncertainties in the process of evaluation. Catch per unit effort (CPUE) was an index for comparison from non-monetary aspects used in some previous studies, which however, emphasized on the capture per unit (per trap) (Grant and Hiscock 2009; Nguyen and Winger 2019a; Ortiz et al. 2006). Compared to studies focusing on CPUE, there were more factors which were primarily taken into consideration in this case study, including landed value, labour costs, duration of fishing operation, the costs for bait, attractants and light. This economic approach is primarily a framework, which helps with the decision-making in a systematic way and to make comparisons from monetary aspects (Hansjurgens 2004).

The analysis in this chapter is presented more from the scope of sensitivity of the factors which would have influence on the cost and profit, and it indicated the magnitude of effects due to the main factors that were included. However, the complexity of a cost-benefit analysis can be much higher than the included factors, as various expenditures are involved in practical situations, such as fuel costs, maintenance and depreciation, as well as other market factors. Benefits can be evaluated from a variety of perspectives, and the result of an analysis will be notably affected by the range of factors considered, and factors weigh differently in most cases.

Accuracy and certainty of collected data is always of concern in cost-benefit analysis (Hansjurgens 2004). The analysis in this thesis discussed the costs and benefits under the situations during the 2019 fishing season solely. The costs and benefits were analysed in a model under hypothetical circumstances, for example, using the mean values for trap captures, and with limited factors considered for data correction purposes. As the assumed situations in this cost-benefit analysis were based on one fishing season, it might not be ideally representative. However, variation is unavoidable during practice, which might lead to bias eventually due to the accumulation of discrepancy. Systematic corrections on data were not included in this case study, due to the lack of information of the market and actual fishing practices. Additionally, economical information of a fishing season was not always updated soon after the season, which brought difficulties in sourcing the cost and profit data during the same season. Limitations, therefore, existed in this study. In order to provide a better tool for fishers to estimate the situation of using different bait and attractant combinations, the calculating methods are provided in this chapter, which allows fishers to modify the factors taken into account, providing the flexibility to build their own cost-benefit table.

Assigning a default capture and value for all traps, regardless of the bait and attractant combination, which would not be a reasonable practice. The assigned capture would result in an identical result for profits, and only the factors of costs would be considered, which however, would not reflect the purpose of a cost-benefit analysis. The monetary difference is accumulative, and the significance of difference is relevant to the scale of quantity. Meaningful changes would expand particularly when dealing with a larger scale (Bissessur 2008; Dechow et al. 2003).

From the scope of the cost over time, LED light is advantageous as a one-time investment. Based on the average capture of the 2019 fishing season, traps baited with additional LED light would catch 11.8 kg more every haul than a traditional trap using only fish bait (135.6 kg/haul and 123.8 kg/haul, Table 3.4.2). The LED lights in this thesis were confirmed to functioning for at least two years, while the only following investment would be batteries.

As no alike investment for light would be needed every year, the profit of using additional light in baited traps would have a better long-term return on investment, indicating the ratio of net profit and cost of investment. Although the capture was not statistically different during the 2019 season in this thesis, a financial difference would deserve consideration in the long-term. Based on the analysis in 3.5.3.1 of this chapter (Table 3.5.5 (a), (b)), a table was constructed to briefly present the comparisons of return of investment for the two situations, when only considering the revenue of landing, and the cost of fish bait, attractant B_1 and LED light (Table 3.5.7). Another table was constructed in the same way (Table 3.5.8), which was based on the analysis in the section 3.5.3.2 (Table 3.5.6 (a), (b)), where four assumed situations were compared.

Table 3.5.5 (a) The assumed situation of only using regular fish bait compared to the actual 2019 fishery season using additional attractant and white LED light (supplemental calculation).

Categories		The 2019 fishery season*	Assumed 2019 fishery season**
Landed price/kg (in CAD)			
	April	$11.86	
	May	$10.80	$11.228***
	June	$11.18	
Bait, attractant, and LED light			
Usage/haul	Fish bait	$3.98 (0.91 kg)	$3.98 (0.91 kg)
	White LED light (batteries included)	$29.5/light	-
	Attractant B_1	$1.34/attractant	-
Overall usage	Fish bait	1,114 bait items	1,114 bait items
	White LED light (batteries included)	25 lights	-
	Attractant B_1	284 attractants	-
Labour cost			
	Fishing season	Apr 14 - Jun 13	Apr 14 - Jun 13
	Duration (in week)	8.7	8.7
	Crew size	14	14
	Weekly wage rate	$1,219.17	$1,219.17
	Overall labour cost	$148,494.91	$148,494.91

* The 2019 fishery season: all traps were baited, additional B_1 attractant and/or LED light were used in some of the traps.
** Assumed 2019 fishery season: if to use traditional fish bait only in all traps during the 2019 fishery season.
*** = CAD 11.228/kg given the average landed price from April to June 2019.

Table 3.5.5 (b) The assumed situation of only using regular fish bait compared to the actual 2019 fishery season using additional attractant and white LED light (benefits and costs comparison).

Categories		The 2019 fishery season*	Assumed 2019 fishery season**
Benefits:			
Capture information			
	Total hauls	1,114	1,114
	Capture (kg)		
	April	48,251	-
	May	78,571	-
	June	14,468	-
	Total capture (kg)	141,291.7	137,913.2***
Landed value (in CAD)			
	April	$572,261.60	-
	May	$848,562.48	-
	June	$161,756.71	-
	Total landed value	$1,582,580.80	$1,555,660.90
Total benefits		$1,582,580.80	$1,555,660.90
Costs:			
Bait, attractant, and LED light			
	Fish bait	$4,433.72	$4,433.72
	White LED light (batteries included)	$737.50	-
	Attractant B_1	$380.56	-
	Overall expense	$5551.78	$4,433.72
Labour cost		$148,738.74	$148,738.74
Total cost		$154,290.52	$153,172.46
Net Benefit		$1,428,290.28	$1,402,488.44

* The 2019 fishery season: all traps were baited, additional B_1 attractant and/or LED light were used in some of the traps.

** Assumed 2019 fishery season: if to use traditional fish bait only in all traps during the 2019 fishery season.

*** = Based on a mean capture of 123.8 kg/haul from the use of fish bait for all 1114 hauls (Table 3.4.2).

Table 3.5.5 (c) The computing methods involved in Tables 3.5.5 (a) and (b).

Involved categories	Computing method
Total hauls	Accumulative haul numbers summed from the 2019 fishery season.
Total capture (kg)	For the 2019 fishery season: converted from tote numbers given different treatments (Table 3.4.2). For the assumed situation where fish bait was only used: total capture = (total haul number) * (123.8 kg/haul). (the mean capture from the use of fish bait only given the 2019 fishery season, Table 3.4.2)
Total landed value	For the 2019 fishery season: summed landed values from April to June. For the assumed situation where fish bait was only used: total landed value = (total capture (kg)) * ($11.228/kg), where $11.228/kg is the average of landed price from April to June.
Bait, attractant, and white LED light usage per haul	Fish bait = (0.91 kg/haul) * ($4.38/kg) = $3.98/haul. 1 light + 2 batteries = ($28) + ($0.75/count * 2 counts) = $29.50/light.
Bait, attractant, and white LED light overall usage	Because the white LED lights were reused and different numbers of lights were used on different harvesting days during the 2019 fishery season, the maximum light usage was 25 lights/day, therefore the expense of 25 lights was counted. During the 2019 fishery season, 192 hauls in total used fish bait plus B_1, and 92 hauls used fish bait plus B_1 and light, therefore 284 B_1 attractants were accordingly included. The average expense/haul was the average expense of bait, attractant and light based on 1,114 hauls for the season.
Labour cost	Although the 14 employed crew members worked in shifts on the vessel in this case study, the labour expense was calculated based on 61 days for all 14 crew members. The actual payroll could differ from the average rate indicated in Statistics Canada (2019).
Net benefit	(Total landed value) – (total expense of bait, attractant, and light) – (overall labour costs)

Table 3.5.6 (a) The estimated costs in the assumed situations of only using regular fish bait, fish + B_1, fish + LED light, or fish + B_1 + LED light for all hauls to meet the capture of the 2019 fishery season (supplemental calculation).

Categories		Assumed using fish bait only	Assumed using fish + B_1 for all hauls	Assumed using fish + light for all hauls	Assumed using fish + B_1 + light for all hauls
Fixed goal					
	Total capture (kg)	141,291.7			
	Total landed value (CAD)	$1,582,580.80			
	Capture/haul (kg) *	123.8	126.8	135.6	126.8
	Number of hauls needed (rounded up)	1,142	1,115	1,042	1,115
	Harvesting days needed (rounded up)	16	15	14	15
	Duration needed **	64	60	56	60
Bait, attractant, and light					
Usage/haul	Fish bait	$3.98 (0.91 kg)	$3.98 (0.91 kg)	$3.98 (0.91 kg)	$3.98 (0.91 kg)
	Light (batteries included)	-	-	$29.50/light	$29.50/light
	Attractant B_1	-	$1.34/ attractant	-	$1.34/ attractant
Overall usage	Fish bait	1,142 baits	1,115 baits	1,042 baits	1,115 baits
	Light (batteries included)	-	-	75 lights	75 lights
	Attractant B_1	-	1,115 attractants	-	1,115 attractants
Labour cost					
	Duration needed (in week)	9.1	8.6	8.0	8.6
	Crew size	14	14	14	14
	Weekly wage rate	$1,219.17	$1,219.17	$1,219.17	$1,219.17
	Overall labour cost	$155,322.26	$146,788.07	$136,547.04	$146,788.07

* = Based on Table 3.4.2.

Table 3.5.6 (b) The assumed situation of only using regular fish bait, fish + B_1, fish + LED light, or fish +B_1 + LED light in all hauls to meet the capture of the 2019 fishery season (benefits and costs comparison).

Categories		Assumed using fish bait only	Assumed using fish + B_1 for all hauls	Assumed using fish + light for all hauls	Assumed using fish + B_1 + light for all hauls
Benefits:					
Capture information					
	Number of hauls needed	1,142	1,115	1,042	1,115
	Total capture (kg)	141,291.7			
Landed value (in CAD)					
	Total landed value	$1,582,580.80			
Total benefits		$1,582,580.80			
Costs:					
Bait, attractant, and light					
	Fish bait	$4,545.16	$4,437.70	$4,147.16	$4,437.70
	Light (batteries included)	-	-	$2,212.50	$2,212.50
	Attractant B_1	-	$1,494.10	-	$1,494.10
	Total expense	$4,545.16	$5,931.80	$6,359.66	$8,144.30
Labour cost*					
	Duration needed (in week)	9.1	8.6	8.0	8.6
	Crew size	14	14	14	14
	Overall labour cost	$155,322.26	$146,788.07	$136,547.04	$146,788.07
Total cost		$159,867.42	$152,719.87	$142,906.70	$154,932.37
Net Benefit		$1,422,713.38	$1,429,860.93	$1,439,674.10	$1,427,648.43
Net Benefit per haul		$1,245.81	$1,282.39	$1,381.65	$1,280.40
Net Benefit per kg		$10.07	$10.12	$10.19	$10.10

* = Given the calculation from Table 3.5.6 (a).

Table 3.5.6 (c) The computing involved in Table 3.5.6 (a) (b) for the cost-benefit analysis.

Involved categories	Computing method
Total capture (kg)	Fixed at 141,292.7 kg for all assumed situations.
Total landed value ($)	Fixed at $1,582,580.80 for all assumed situations.
Number of hauls needed	(Total capture) / (capture per haul), computed based on the mean capture for each treatment (Table 3.4.2), whole numbers only.
Harvesting days needed	(Number of hauls needed) / 75, because 74.3 hauls (rounded up to 75 hauls) were recorded for each harvesting day during the 2019 fishery season, which therefore was adopted for all situations, whole numbers only.
Bait, attractant, and light usage per haul	Fish bait = (0.91 kg/haul) * ($4.38/kg) = $3.98/haul.
	1 light + 2 batteries = ($28) + ($0.75/count * 2 counts) = $29.5/light.
Bait, attractant, and light overall usage	Fish bait and attractant B_1 were used one time only for each haul.
	Lights were reusable, and 75 lights were computed for the assumed situations using fish bait plus light, and fish bait plus B_1 and light, because 75 hauls were estimated for each harvesting day, which was the maximum usage.
Labour cost	Computed using the same method as in Table 3.5.5 (a) (b).
Net benefit	Computed using the same method as in Table 3.5.5 (a) (b).

Table 3.5.7 Comparison of difference in profit (profit = revenue - cost) between the 2019 fishery season and the assumed fishery season, when only landings and cost for bait, attractant and light are considered.

Basic assumptions			
Landing price (in 2019)		April: $11.86/kg May: $10.80/kg June: $11.18/kg	
Fish bait/haul		$3.98/haul	
Attractant B_1/haul		$1.34/attractant	
LED light		$29.5/light	
	Cost [1]	**Revenue [2]**	**Profit**
The 2019 fishery season*	Fish bait: $4,433.72 25 LED light: $737.50 Attractant B_1: $380.56	April: $572,261.60 May: $848,562.48 June: $161,756.71	$1,577,029.02
	Overall: $5551.78	Overall: $1,582,580.80	
Assumed 2019 fishery season**	Fish bait: $4,433.72	April: $557,941.84 May: $827,627.76 June: $159,169.66	$1,540,305.53
		Overall: $1,544,739.26	

* The 2019 fishery season: all traps were baited, additional B_1 attractant and/or LED light were used in some of the traps.

** Assumed 2019 fishery season: if to use traditional fish bait only in all traps during the 2019 fishery season.

[1]: information based on Table 3.5.5 (a) and (b)

[2]: Landing value was calculated based on the monthly capture.

Table 3.5.8 Comparison of difference in profit (profit = revenue - cost) between the four assumed situations, when only landings and cost for bait, attractant and light are considered.

Basic assumptions			
Landing price	Average*: $ 11.228/kg		
Fish bait/haul	$3.98/haul		
Attractant B_1/haul	$1.34/attractant		
LED light	$29.5/light		

In all traps	**Cost****	**Revenue****	**Profit**
Assumed using fish bait only [1]	Fish bait: $4,545.16	$1,582,580.80	$1,540,305.53
Assumed using fish + attractant B_1 [2]	Fish bait: $4,437.70 Attractant B_1: $1,494.10 Overall: $5931.80	$1,582,580.80	$1,576,649.00
Assumed using fish + LED light [3]	Fish bait: $4,147.16 75 LED light: $2,212.50 Overall: $6359.66	$1,582,580.80	$1,576,221.14
Assumed using fish + attractant B_1 + LED light [4]	Fish bait: $4,437.70 75 LED light: $2,212.50 Attractant B_1: $1,494.10 Overall: $8144.30	$1,582,580.80	$1,574436.50

* = CAD 11.228/kg given the average landed price from April to June 2019.
** = information based on Table 3.5.6 (a) and (b).
[1]: 1,142 hauls needed over 16 harvesting days.
[2]: 1,115 hauls needed over 15 harvesting days.
[3]: 1,042 hauls needed over 14 harvesting days.
[4]: 1,115 hauls needed over 15 harvesting days.

3.6 Conclusion

Using additional white LED light plus B_1 attractant improved the snow crab capture in the 2018 fishing season, but not in 2019. LED light is advantageous to maintain reliable performance under various practical situations regardless of water flow, water depth, temperature and soaking time period. Fishing efficiency was also improved from an economic aspect by using additional B_1 or white LED light. A potential downside to using LED light could be the use of batteries and their disposal, which would raise environmental concerns if to apply the LED light in a massive scale. Other concerns include illumination, which may interfere with other aquatic animals. Previous field and laboratory tests conducted on snow crabs supported that LED lights significantly improved catchability, and preference was found for blue and white light (Nguyen et al. 2017). However, since only male individuals were captured and counted, differences in attractiveness are unknown between sexes among existing studies to date. Artificial attractant B_1 does not introduce environmental hazards into the sea, and further effort could be devoted to developing an alternative packaging method instead of using paper wrap, which may reduce waste.

As this study involved practical applications, the sea trials were restricted by multiple factors, such as inconsistent water depth, temperature, and soaking time period. Results from the validation tests suggests that the B_1 attractant undergoes a stable diffusion process under the typical temperature conditions of the Eastern Nova Scotian water body. Additionally, water depth was not associated with capture. The soaking time period, however, may influence B_1 attractiveness, as the remaining effective compound can diffuse completely if they are left in water beyond 72 h, which may occur if limited by weather conditions during the fishing season. That would also be an issue with fish bait, as the fish bait would be consumed by crabs. In the future, underwater video cameras can be helpful to see how crabs behave, and the residue of returning B_1 can be checked to see if any betaine is left. Focus could be placed on having a better control and treatment setting in sea trials regarding the consistency of haul numbers, depth of fishing site, and intervals between harvesting days. In further studies, it would be best to test a uniform number of hauls for each treatment and more regular intervals between harvesting days. In this case, it was not possible, as fishers need to catch crabs to maintain a living. Fish bait was included in all treatments in this study by reason of potential risk, which therefore provided a comparative result upon harvest improvement. A direct result comparison between fish bait and artificial attractants on actual catchability might be obtainable by discarding traditional fish bait and solely using artificial attractants or lights in additional treatments.

Although captures resulting from the use of the experimental treatments were not statistically improved, they were not negatively impacted either. During the 2019 fishing season, the alternate baits may affect the benefits from the economical and environmental aspects. An economic improvement was found via the cost-benefit analysis comparing the absolute profit between the 2019 fishing season and the assumed situation where traditional fish bait was only used. Comparisons among different assumed situations using different bait combinations concluded a minor improvement in profit by using the alternative B_1 attractant or LED lights. Results from this analysis show a financial evidence that alternative attractants and lights can be potential an option to reduce the use of traditional fish bait with an equal performance of fish bait. Financial gain can be a motivation in all fisheries, including not only an increase in capture, but a reduction in operating cost or time as well, which would also reduce the volume of forage fish used as bait due to reduced trips. Fishers may be encouraged to switch from traditional fish bait to artificial alternatives if using artificial attractants would save costs and bring more profits. Future tests where attractant B_1 and white LED light completely replace fish as bait are recommended.

Chapter 4: Bait Preference of the American Lobster (*Homarus americanus*): Comparison of Fish Bait versus Artificial Betaine and Lecithin Attractants and LED Light

4.1 Abstract

To explore the attraction of the American lobster to a betaine-containing attractant (B_2), a lecithin-containing attractant (L_2), traditional fish bait (mackerel), and white light-emitting diode (LED), a nine-day preference test and a five-day validation test were conducted in a static system using saltwater (28 to 30 ppt; average temperature = -2.3 to 7.0 °C). A randomized block design and a cross-over design were applied to the preference and validation tests, respectively. The preference test included 54 runs over nine days on 18 lobsters in six experimental pools. Twenty-five runs were conducted in the validation test on five lobsters in five experimental pools over five days. All test runs were video-recorded as data references. The attractiveness of the attractants and white LED light were determined via the duration and frequency of each treatment being touched, and the duration and frequency of the lobsters resting by each treatment. Attractants and white LED light were presented together in the preference test, and were presented individually in the validation test. In the validation test, there was no statistical difference in attractiveness among treatments (P = 0.337 for touching duration, P = 0.207 for touching frequency, P = 0.485 for resting duration, P = 0.095 for resting frequency). L_2 performed better than traditional fish bait in the preference test, with regards to duration and frequency (P = 0.001). Given the results and behavioural responses from the two tests, L_2 has potential as an alternative to fish bait, however, uncertainty in practical fishing due to the differences existing among individuals needs to be confirmed in further research.

4.2 Introduction

Multiple alternative bait types have been researched for a number of shellfish and finfish species to substitute forage fish bait. The alternative options included plant-based material, by-products from fish and livestock industries, and other agricultural wastes. Poultry mortalities (for blue crab, *Callinectes sapidus*, Middleton et al. 2000), fish waste (for sand crab, *Ovalipes punctatus*, Archdale and Kawamura 2011), and shrimp head waste (Masilan et al. 2018) were also tested previously. A more sustainable source of bait is desired to reduce fish bait use in crustacean fisheries. Currently, crustacean fisheries account for a large portion of forage fish usage (Archdale et al. 2008; Dellinger et al. 2016; Masilan and Neethiselvan 2018).

Apart from the use of chemical signals, the use of light is a successful fishing method as positive phototaxis occurs for many aquatic species (Breen and Lerner 2013; Solomon and Ahmed 2016). Light can used in both finfish and crustacean fisheries, and it has been used for selective fishing to reduce bycatch (Hannah et al. 2015; Ortiz et al. 2016). Lobsters also prey based on visual signals, and attractiveness may vary due to the colours and shapes of prey. However, knowledge of the eye structure of decapod crustaceans is limited, and structural differences may exist between snow crab and lobsters (Nguyen et al. 2017). LED devices have been widely adopted due to their strong illumination power and lower energy cost (Nguyen and Tran 2015). LED white light and betaine attractant (B_1) were previously tested in snow crabs in Chapter 3. Snow crab capture in CFAs 23 and 24 indicated an improvement during 2018 fishing season by augmenting traditional fish bait with additional white LED light and B_1 attractant. Because of their clear-defined behavioural responses, crustaceans including lobsters are considered model species under laboratory conditions (Zimmer-Faust 1989). The purpose of this experiment was to determine the potential for the use of artificial attractants in commercial crustacean species, such as American lobster, in a controlled laboratory environment.

B_1 attractants were reduced in size for the lobster trial with the same formula, which was subsequently named B_2. Betaine exists in marine prey extracts and damaged tissues, which is an effective ingredient in raising food searching behaviour in many decapod crustaceans (Felix and Sudharsan 2004; Harpaz 1997; Polat and Beklevik 1999). A second artificial attractant was developed using lecithin, which is noted as L_2 in the preference and validation tests. Lecithin is a mixture of glycerophospholipids and widely exists in different organisms (Thompson et al. 2003; Szuhaj 2016). Lecithin improves growth performance in several crustacean species such as red swamp crayfish, western white shrimp, and American lobster (Haran and Fenucci 2008; Thompson et al. 2003). However, the use of lecithin as an attractant has not yet been studied in American lobster.

4.3 Objectives

Experimental data were obtained from video footage based on the observed duration and frequency of the lobsters touching or resting by each treatment. The objectives of the preference and validation tests conducted for this study were to determine the following:

i) The attractant(s) preferred by lobsters as determined by their touching behaviour, considering the frequency and duration of touching.

ii) The attractant(s) preferred by lobsters as determined by their resting behaviour, considering the frequency and duration of resting.

4.4 Hypotheses

The null hypothesis for the experiments conducted in this study was that the artificial attractants or white LED light would equally attract lobsters as the fish bait and blank treatment (control). The hypothesis for the experiments was that the artificial attractants or white LED light would attract lobsters more than the fish bait and blank treatment (control).

4.5 Materials and Methods

4.5.1 Experimental Animals

Eighteen market-size lobsters (minimum carapace length of 82.5 mm) were obtained from a local market (Sobeys, Truro, NS) for the validation and preference tests. All 18 lobsters were tested in the preference test, and five of them were randomly selected to be involved in the validation test. The lobsters had their chelipeds restricted by rubber bands at all times. Upon arrival at the research facility, food was withheld to allow the lobsters to acclimate, where they were left to rest for two days in an insulated holding tank before conducting the tests.

4.5.2 Animal Housing and Experimental Pools

Experimental animals were held in an insulated tank (approximate 900 L, depth = 0.65m). The holding tank was filled with seawater with a water temperature, which was monitored daily and maintained ranging from 2.3 to 2.8 °C. The holding tank was opaque on all sides, and was shielded by an opaque lid to avoid potential visual interference that may impact the diel rhythm of the experimental animals. Continual air was supplied to the holding tank via an air pump (Marina 100 air pump, 150 L/h) and the water was changed every other day to maintain a safe ammonia level. The seawater used in both tests was sourced from the Dalhousie University, Faculty of Agriculture Aquaculture Centre (Bible Hill, NS), with a salinity between 28 to 30 ppt. Lobsters were accommodated in three separate plastic baskets in the insulated holding tank.

All experimental pools were held indoors in an unheated building, and static water was used to minimize influences due to wind and unnecessary agitation, which might enhance the compounds mixed in the experimental pools. The pools were circular with a diameter of 1.5 m and had a repeated uniform pattern on their flat bottoms. The experimental pools contained still, cold seawater at a depth of 15 cm. The water held in each experimental pool was not exchanged with any other pool or the holding tank. The water temperature was monitored daily, ranging from -2.3 to 7.0 °C during the two tests (Figure 4.5.1). Seawater in the experimental pools was changed and oxygenated for a minimum of three hours before an experimental lobster was placed into the pool. No soap was used during a water change but the bottom of the pool was rinsed and wiped with paper towel. Lobsters were placed in their respective pools at 4 p.m. on a daily basis and air stones were removed from the pools before the lobsters were introduced. The entrance to the experimental area of the behavioural tests on the lobsters was closed and was not accessed by individuals during the data collection process. Light and sound conditions were limited to minimize external influences. The experimental pools were in complete dark during the night, and were not interrupted or influenced by human activities. The experimental area was also shaded, thus treatment jars and shelters in the pools would not have been exposed to shadows during the day.

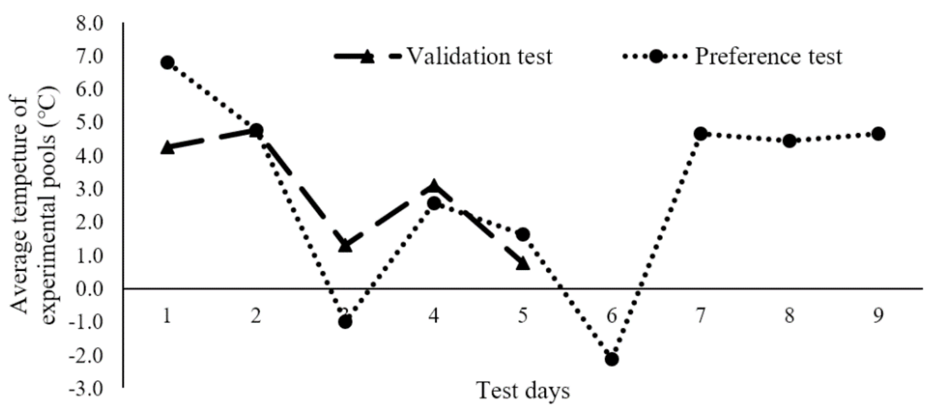


Figure 4.5.1 Average temperatures (°C) of experimental pools during the preference and validation tests conducted on lobsters, which were for nine and five days, respectively, in November 2019. Each test run lasted 16 h (from 4 p.m. to 8 a.m.).

4.5.3 Video Recording and Data Collection

The laboratory trials were both conducted in November 2019. For the validation and preference tests, all runs were conducted overnight for 16 hours. Each test run and video recording started at 4 p.m. and ended at 8 a.m. the following morning. Cameras were hung above each pool (1.8 m in height) and videos were recorded (cameras and 8 channel HD TVI digital video recorders by Speco) for all runs as a reference for behavioural response observation and data collection. Footage was reviewed and data was collected in four categories:

i) The frequency (number of times) a lobster touched each treatment.
ii) The frequency (number of times) a lobster rested by each treatment.
iii) The duration (in seconds) a lobster touched each treatment.
iv) The duration (in seconds) a lobster rested by each treatment.

Consistent criteria were adopted in both tests (Table 4.5.2, Figure 4.5.5). Each test run was counted from the moment when the lobsters were placed in their experiment pools. Timing for touching and resting behaviours were separated. A touching behaviour was counted if the lobster approached and touched a treatment jar, followed by moving away from the treatment jar. A resting behaviour was counted if the lobster approached the treatment jar, and no movement followed.

4.5.4 Bait, Attractants and Light Preparations

Each of the five treatments were placed in glass jars (volume in 475 mL), and the lids were punched with seven evenly spaced holes each 3mm diameter. Treatment jars were completely immersed and filled with salt water. White LED lights were purchased from Hampidjan Canada Ltd (Spaniards Bay, NL, Canada) and two fully charged AA batteries (1.5 V) powered each light, which were turned on and placed in the jars.

Mackerel fish bait was purchased from a local market (Catch of the Bay, Debert, NS, Canada), which was gutted and deboned, and only the flesh was used for testing (22.2 ± 1.41 g used/run). Attractant B_2 was prepared as described for B_1 in Chapter 3, but smaller (average weight = 22.4 ± 1.73 g). The formulas for B_2 and L_2 are proprietary and currently remain closed, but both were produced with the same production and weight ratio of active ingredient to “inert matrix” (60.7:38.3). The betaine used to make B_2 was anhydrous betaine (minimum 96% feed grade anhydrous betaine powder, Finnfeeds Finland Oy, Naantali, Finland). The lecithin used to make L_2 came from soy lecithin granules (58% fat, Bulk Barn, Truro, NS). Attractant L_2 (22.7 ± 0.75 g) and fish bait (mackerel) were prepared to have a similar weight as B_2 and all B_2 and all L_2 used in this study were made from the same individual batches to ensure consistency. Both B_2 and L_2 were lined with paper wrap, which was removed prior to testing.

Figure 4.5.2 Attractant B_2 and L_2 preparations.

4.5.5 Preference Test

Eighteen American lobsters served as subjects in this trial. They were divided into three groups, each with six individuals. A two-day rest interval was scheduled between every two runs for the same lobster: Group 1 was tested on day 1, 4 and 7; Group 2 was tested on day 2, 5 and 8; Group 3 was tested on day 3, 6 and 9. Therefore, all individuals were tested with three replicates over nine days with a two-day rest interval (Table 4.5.1). A 16-hour run was conducted daily in every experimental pool overnight from 4 p.m. to 8 a.m. the following morning. Every day, the six animals within the group being tested that day were arranged randomly into six experimental pools. As lobsters naturally take crevices or burrows as refuge if they feel threatened by predators, a weighted shelter was provided in the middle of each pool to mimic the refuge they might need. Resting under the shelter would indicate a negative response to all treatments. Lobsters were tested singularly for each run and were released into experimental pools so that they were initially resting under the shelter.

Table 4.5.1 Experimental schedule to test preference of lobster for attractants and light (nine-day preference test*).

Test day	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}
Replicate	1	1	1	2	2	2	3	3	3
Lobster #	1 - 6	7 - 12	13 - 18	1 - 6	7 - 12	13 - 18	1 - 6	7 - 12	13 - 18

* = 54 runs (six runs/day) of preference test were conducted on 18 lobsters with three replicates in six experimental pools over nine days. A two-day rest interval was arranged between every two runs.

Five treatments (blank, fish bait, B_2, L_2, and white LED light as the control treatment) were tested simultaneously in all pools. Treatment jars were placed in a random order along the inner edge of experimental pool with an equal distance between jars (Figure 4.5.3).

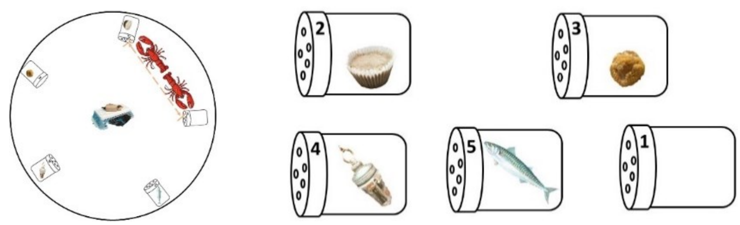


Figure 4.5.3 Illustration of the experimental pool layout and treatment jars for the preference test. A shelter was provided in the middle of experimental pool. Five treatments, including one empty (control) jar, were numbered and equally spaced around the inner edge of pool with an equal distance marginally more than two body lengths of a lobster.

4.5.6 Validation Test

A validation test was conducted over five consecutive days, in order to determine if there was any difference in behavioural response when only one treatment was presented to the lobsters. Five lobsters were randomly selected for the validation test. Daily, the five selected lobsters were assigned randomly to one of five experimental pools, but no lobster was assigned to the same pool twice over the five days. Each treatment was tested every day (1 treatment jar/pool), randomized among pools, and no treatment was tested in the same pool twice. The treatment jar was placed on the inner edge of an experimental pool for each run. All treatments and treatments jars were from the same batch as those involved in the preference test to ensure consistency and avoid potential bias. Lobsters were released into the pools at the end farthest from the treatment jar (Figure 4.5.4). To prevent distraction, no shelter was provided in the validation test. A 16-hour overnight run (from 4 p.m. to 8 a.m. in the following morning) was conducted daily in every experimental pool. All experimental animals were released back to the holding tank for eight hours (from 8 a.m. to 4 p.m.) after each run before the following run started at 4 p.m. the next day.

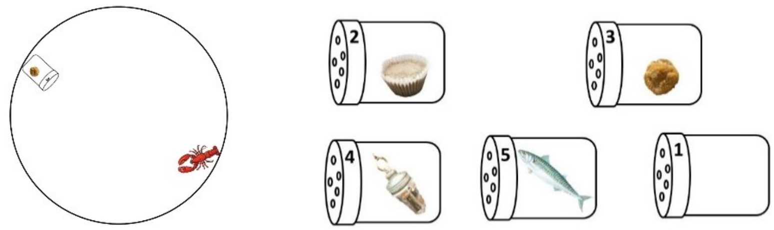


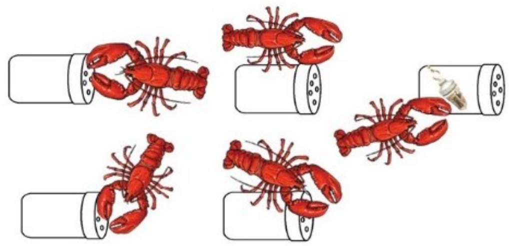
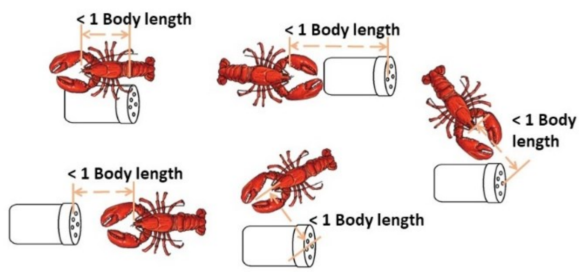
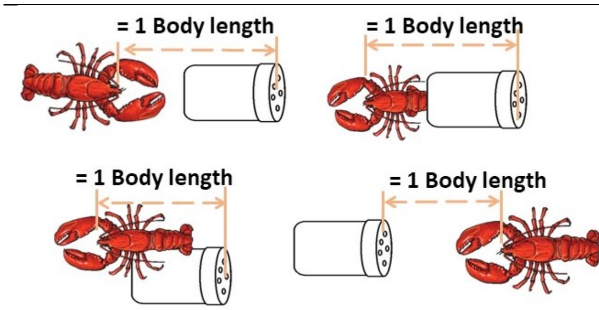
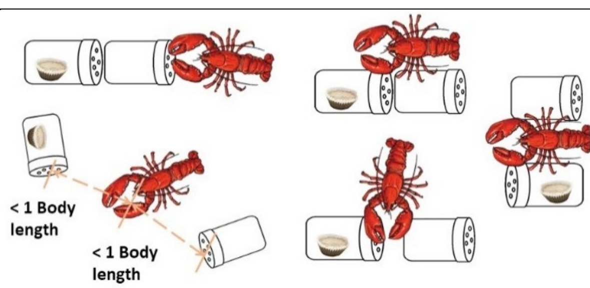
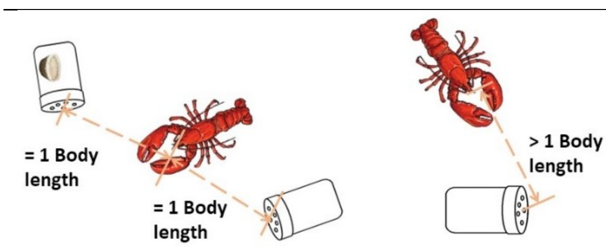
Figure 4.5.4 Illustration of the experimental pool layout and treatment jars for the validation test. One treatment was placed at the inner edge of an experimental pool per run and a lobster was placed at the farthest opposite end. Five treatments were tested in each pool over five days.

Table 4.5.2 Criteria used in determining the locations of touching and resting behaviours.

	Treatment jars (Blank, B_2, L_2, Fish bait, white LED Light)	Undetermined*	Rest without preference*
Touching	Lobster touched one treatment jar with moving behaviour, such as flicking, walking leg movements, climbing on, pushing, or pulling the jar.		
Resting	Lobster approached one treatment jar and stayed within a small range of the jars, as described in Figure 4.5.5.	Lobster stayed between two treatment jars with a distance less than 1 body length to both jars**.	Lobster rested at a location at a distance more than 1 body length to any jar**.

* Records of "Undetermined" and "Rest without preference" were not counted as data points in touching analysis, only in resting analysis.
** Illustrations attached below (Figure 4.5.5). Distance was measured manually given the video footage by repeated measurements from different locations.

Figure 4.5.5 Illustrations of criterions used in determining the locations of touching and resting behaviours.

Locations	Descriptions
	Resting (by a jar): Chelipeds either touched, or grabbed the lid, or touched the jar at a location where the eyes and antennule were directly by the lid.
	Resting (within 1 body length of a jar): Chelipeds were attached to the other end of the treatment jars. Part of the body was attached to the jar, but the eyes and antennule were not directly by the lid. The lobster did not physically touch the jar and the distance from the eyes and antennule to the lid was within 1 body length.
	Resting (at 1 body length): Tail was curled and attached to the other end of the treatment jars and the distance from the eyes and antennule to the lid of treatment jar was at 1 body length; part of the body was attached to the jar and the distance from the eyes and antennule to the lid was at 1 body length; the lobster did not physically touch the jar and the distance from the eyes and antennule to the lid was at 1 body length.
	Undetermined: Treatments jars pushed away from intial location, and distance between two jars was too close that the lobster was within 1 body length to either of the jars. Time period was recorded but not counted as a data point for any treatment.
	Rest without preference: The lobster was sitting between two treatments with an equal distance of 1 body length to either of the jars; the lobster was at a distance of more than 1 body length from any treatment. Time period was counted but not counted for any treatment.

4.5.7 Statistical Analysis

4.5.7.1 Preference Test

The means of the four data categories (duration of touching treatment jar, duration of resting by treatment jar, frequency of touching treatment jar, frequency of resting by treatment jar) were calculated for the six experimental pools every day, where the means were adopted in the analysis of variance. A randomized completed block design (RCBD) was employed for data analysis for the means from nine days, blocking by days, as temperature varied daily. The significance level was set at $\alpha = 0.05$, and Tukey's HSD test was followed if the P-value of treatment was lower than 0.05. The block was considered worthwhile if the F-value of the block was greater than 2. Assumptions were checked, including independence of data, normality of residuals, and equality of variances, where data transformation was applied if normality was violated (Montgomery 2014b).

4.5.7.2 Validation Test

A cross-over design was employed to analyze the validation test, which involved five treatments over five days. The treatment order of each pool was randomized, in order to eliminate position effects. Water in the experimental pools was changed daily, chemical interference from the previous run was limited, and eight hours between every two runs was considered to be a sufficient wash-out period. The wash-out period was set between every two test runs to avoid the potential influence on the following run due to the previous run (Montgomery 2014b). The mean duration and frequency of each treatment were obtained from the data from the five days. Differences in duration or frequency between treatments were compared via ANOVA, blocking by day, as temperature varied daily. Data transformation was applied in the analysis when the normality was violated. The significance level was set at $\alpha = 0.05$, and the block was considered worthwhile if the F-value of the block was greater than 2.

4.6 Results and Discussion

4.6.1 Preference Test

Among the four data categories (duration of touching treatment jar, duration of resting by treatment jar, frequency of touching treatment jar, frequency of resting by treatment jar), the *F*-value of the block (days) was exclusively greater than 2 for touching frequency (*F*-value = 6.76), indicating the block was worthwhile in this data category. No significant difference was confirmed in either duration (*P*-value = 0.167) or frequency (*P*-value = 0.254) of touching treatment jars when the animals were actively moving in the experimental pools (Table 4.6.1). The treatment effect was not distinct. The duration and frequency of touching the blank treatment was not statistically different from that of the other treatments, and the white LED light treatment was not advantageous regarding the duration or frequency of jar touching. The standard deviations for all treatments were high regarding touching duration and frequency, which indicated that data distributions were spread over a large range.

A significant treatment effect was found in both the duration (*P*-value = 0.001) and frequency (*P*-value = 0.003) of resting by treatment jars. Data points were distributed within a wide range for both resting duration and frequency. Given the following multiple means comparison, L_2 was a more favorable choice in terms of both resting duration and frequency (Table 4.6.1). The white LED light was statistically less attractive than L_2 in both resting duration and frequency. However, the blank treatment and B_2 attracted the lobsters as effectively as the other treatments, except L_2. For both the resting duration and frequency, the block for this statistical analysis (days) was not worthwhile (*F*-value < 2), which indicated there was no significant variation due to differences among experimental days, implying that variation in daily temperature did not affect treatment effects.

Table 4.6.1 Comparisons based on the mean (±SD) of four data categories (duration of touching treatments, duration of resting by treatments, frequency of touching treatments, and frequency of resting by treatments)*.

		Duration mean (sum of seconds)	Frequency mean (number of visits)
Touched treatment jar	Blank	290.3±188.7	7.0±4.6
	Attractant B_2	338.7±286.2	7.0±3.4
	Attractant L_2	490.5±395.0	9.5±5.7
	Fish bait	349.5±212.6	7.2±2.9
	White LED light	180.8±102.4	6.8±4.0
	P-value	0.167**	0.254
	F-value of block	1.70	6.76
	R^2	39.09%	65.12%
Rested by treatment jar	Blank	6639.7±4493.0 ^{AB}	4.1±2.8 ^{AB}
	Attractant B_2	5449.5±3561.3 ^{AB}	3.9±3.7 ^{AB}
	Attractant L_2	11011.2±6937.2 ^{A}	8.6±5.0 ^{A}
	Fish bait	4062.6±2740.5 ^{B}	4.3±3.4 ^{AB}
	White LED light	1158.6±1554.2 ^{B}	1.3±0.9 ^{B}
	P-value	0.001	0.003
	F-value of block	0.50	0.76
	R^2	46.07%	45.36%

* = Significant difference among treatments confirmed if *P*-value < 0.05.
** = Data transformation applied in analysis as the normality was violated.

4.6.2 Validation Test

F-values of blocks were less than 2 in all four data categories, where the blocks were consequently not considered worthwhile, thus the difference among days did not count for the effects on the results. There were no differences in attractiveness among treatments at $\alpha = 0.05$ in any of the four categories. However, a wide range was shown in both means and standard deviation. The means of resting duration by the white LED light and L_2 were 137.2 s and 14,186.2 s, respectively. Results of the duration and frequency of resting were obtained given the transformed data, which was due to the violated data normality and no statistical difference was confirmed. Distinct from the preference test, the dataset of the validation test contained more 0 values, which were taken into consideration rather than being removed as outliers since it occurred multiple times on multiple animals throughout the test. Additionally, data points were widely spread, and the standard deviation was notably affected. On the fourth day of validation, although the lobsters did approach the treatment jars, none of the five animals rested by any treatment jars, which resulted in zero values for resting duration and frequency on that day. The water temperature on the fourth day was recorded at 3.1 °C, which was not extreme and was not considered to have influenced the behaviours that resulted in a resting time of zero.

Table 4.6.2 Comparisons based on the mean (±SD) of four data categories (duration of touching treatments, duration of resting by treatments, frequency of touching treatments, and frequency of resting by treatments) given the validation test via a cross-over design*.

		Duration (sum of second)	Frequency (number of visits)
Touched treatment jar	Blank	193.6±115.4	6.4±2.9
	Attractant B_2	270.2±216.3	7.2±4.4
	Attractant L_2	503.4±643.3	11.4±11.6
	White LED light	118.8±205.9	4.6±6.0
	Fish bait	132.2±153.9	5.8±6.8
	P-value	0.337	0.207
	F-value of block	1.38	0.78
	R^2	39.51%	37.93%
Rested by treatment jar	Blank	6866.8±15122.7	4.4±7.2
	Attractant B_2	5440.6±6410.6	3.6±3.0
	Attractant L_2	14186.2±20116.1	9.2±12.7
	White LED light	137.2±157.9	1.4±1.3
	Fish bait	2419.8±5248.6	2.0±3.5
	P-value	0.485**	0.095**
	F-value of block	1.75	0.52
	R^2	39.88%	41.98%

* = Significant difference among treatments confirmed if *P*-value < 0.05.
** = Data transformation applied in analysis as the normality was violated.

Previous studies have investigated the behavioural response elicited by different attractants in commercial species. Betaine not only has a positive effect on growth enhancement (Harpaz 1997), but is also used as a feed attractant (Archdale and Anraku

2005; Polat and Beklevik 1999). Betaine can stimulate chemoreceptor neurons in crustaceans (Tolomei et al. 2003), which trigger locomotion behaviours. In the preference and validation test, however, the B_2 did not attract lobsters to make more visits to this treatment than to the other treatments. The duration of lobsters resting by the B_2 was not outstanding either. Although the lobsters rested by the B_2 for a longer duration compared to the fish bait and white LED light, it was not significantly different from the blank treatment.

The white LED light was not advantageous in the laboratory trials, and was the least preferred treatment by the lobsters to rest by. Lobsters have the potential to discriminate different light intensities and may respond accordingly. In a discrimination learning study on American lobster, light cues were used between 20 to 60 lux, and lobsters showed the ability to distinguish different light intensities under dark conditions (Tomina and Takahata 2012). The white LED light used in this study was initially designed for commercial fishing, where it functions in a complex hydrological environment with possible high turbidity. American lobsters naturally inhabit a low-intensity light environment, and defensive responses can be triggered if there are changes in light intensity (Jury et al. 2001; Tomina and Takahata 2012). The illuminance of the white LED light in this research, therefore, could have been too strong for the lobsters in the experimental pools, which reduced their foraging behavioural response.

Previous studies on lecithin, mainly focused on its positive influence on fish growth, rather than attractiveness. Phospholipids are necessary for maintaining cellular function and are important as a mediator of lipid transport in the hemolymph of decapods (Gong et al. 2000). Dietary phospholipid supplementation also has beneficial effects on several crab (mud crab, *Scylla serrata*; swimming crab, *Portunus trituberculatus*) and American lobster in terms of growth, survival, and prevention of skeletal deformities in larvae and juveniles (Coutteau et al. 1997; Holme et al. 2007; Li et al. 2016; Thompson et al. 2003). Supplemental lecithin from soybean can improve cholesterol metabolism in American lobster juveniles by increasing of serum lipoprotein cholesterol levels (Kumar et al. 2018). Enhancements in lipid deposition were also reported among shrimp species provided with dietary lecithin supplementation, including *Penaeus japonicus* (Teshima et al. 1986), whiteleg shrimp (*Litopenaeus vannamei*, Gong et al. 2000) and tiger shrimp (*Penaeus* *monodon,* Vasagam et al. 2005). Based on the results of the preference and validation tests in this study, lecithin may have potential application as an attractant for lobsters. The lobsters rested by the L_2 with a longer accumulated duration than by the fish bait. The mean duration of resting by the L_2 was 1.6 times more than the blank treatment, which had the second largest mean. However, this was not confirmed with a significant difference. This may be beneficial in areas where competing fishers have traps close to one another and would like to lure a lobster to their trap.

In the validation test, in addition to the 5 experimental pools with the treatment jars (one treatment jar in each pool with one animal being tested), a sixth lobster was placed in another empty pool was set up with no treatment jars. Testing in the empty pool was also conducted over 5 days, and the duration (in seconds) and frequency (number of times happened) of the animal's moving behaviour was recorded. The total motion duration in the empty pool was compared with the total motion duration of the other four treatments by a randomized block design via ANOVA analysis, where the days were blocked. Based on the results (Table 4.6.3), there was no significant difference ($P = 0.409$) in the duration of motion in the pools with treatment jars or in the empty pool, therefore, lobsters were equally active in all the experimental pools, whether or not there was a treatment jar placed in them. The R^2 value of the duration of motion was 24.42%, indicating that 24.42% of the variation could be explained by the factors that were taken into consideration, which were the treatments and the days.

By comparing the frequency of lobsters resting, the results for the ANOVA analysis of frequency of resting were also included in Table 4.6.3. The frequencies of resting were not significantly different among the experimental pools either ($P = 0.533$), indicating the numbers of times that animals rested were not different among the pools, either with or without treatment jar. The R^2 value of the duration of motion was 22.29%, indicating that 22.29% of the variation could be explained by the treatments and the days. Because the R^2 values of both the duration of motion and frequency of resting were lower than 30%, it indicated that less than 30% the variation of behavioural response was explained by the pools and days. Therefore, adding treatment jars to a pool did not drastically change the behaviour of the lobsters.

The F-values of blocks (days) were both less than 2 (0.29 and 0.38 for the duration and frequency of resting, respectively), indicating that the difference between days might not be a major source of variance, where one of the factors counted could be the different daily water temperatures.

Table 4.6.3. Results of ANOVA analysis and variations of the validation test

Source of variance	Total motion duration (sec)			Total frequency of resting		
	Degrees of freedom	F-Value	P-Value	Degrees of freedom	F-Value	P-Value
Treatment	5	1.06	0.409	5	0.85	0.533
Day	4	0.29		4	0.38	
Error	20			20		
Total	29			29		
$\sqrt{MSE}$			5019.93			26.28
R^2			24.42%			22.29%
Coefficient of variance (%)			2.22			1.79

4.6.3 Behavioural Response Observations

In addition to approaching and resting by the treatment jars, other behavioural responses were observed in the video footage of the preference and validation tests. These responses included pushing jars, probing, circling in the pool, attempting to climb the wall, sudden movements and crossing through the shelter. Amidst these behaviours, the most distinct and most often observed was pushing treatment jars, which occurred 10 times during the five-day validation test and 192 times during the nine-day preference test. No glue was used to situate the treatment jars firmly onto the pool, which was to avoid any bias due to the smell or flavour of glue. Due to this, the lobsters were able to push the jars off its position. Sometimes lobsters scratched at or attempted to push the treatment jars, but it was not noted as jar pushing if the treatment jars did not move. Lobsters were observed pushing jars more often during the preference test when there were multiple choices in the pool. In accordance with the daily frequency, there was no particular relationship observed between the daily variation (including water temperature) and pushing frequency during the two experiments. During the validation test, pushing behaviour was not observed on three out of five days; however, 7 out of the total 10 occurrences were on the third day among all experimental pools. Less frequent pushing behaviour (less than 20 times/day) was observed from day 7 to day 9 in the preference test, during which the daily frequency ranged from 7 to 33 times (Figure 4.6.1).

Among the total 192 occurrences of jar pushing behaviour during the preference test, the blank jar was chosen most often and pushed 48 times, followed by L_2 and mackerel (both 45 times) (Table 4.6.4). B_2 was pushed less (36 times), and the jar with white LED light was pushed least often (18 times). Among the 10 pushing occurrences observed during the validation test, six were counted for L_2, followed by the blank treatment (3 times) and mackerel (1 time), while B_2 or white LED light was not moved.

In both experiments, white LED light was less associated with jar pushing behaviour than the other treatments. A possible interpretation could be that the luminance of white LED light applied in this study, was relatively strong relative to the size and volume of the experimental pools as the visual system of lobster adapted for low-intensity light environments (Tomina and Takahata 2012). Meanwhile, the luminance of the same LED light would have been higher in the experiments than would be observed in sea applications, as the turbidity of the laboratory salt water was lower than what the sea water turbidity would be at fishing sites. Therefore, the effectiveness and attractiveness under laboratory conditions could be reduced compared to practical situations. In addition, response behaviours were also affected by individual difference, as some lobsters were more sedentary and thus less movements and behaviours were presented.

Table 4.6.4 Total frequency of pushing each treatment jar during the preference and validation tests, respectively.

	Blank	Attractant B_2	Attractant L_2	Fish bait	White LED light	Total
Preference test	48	36	45	45	18	192
Validation test	3	0	6	1	0	10

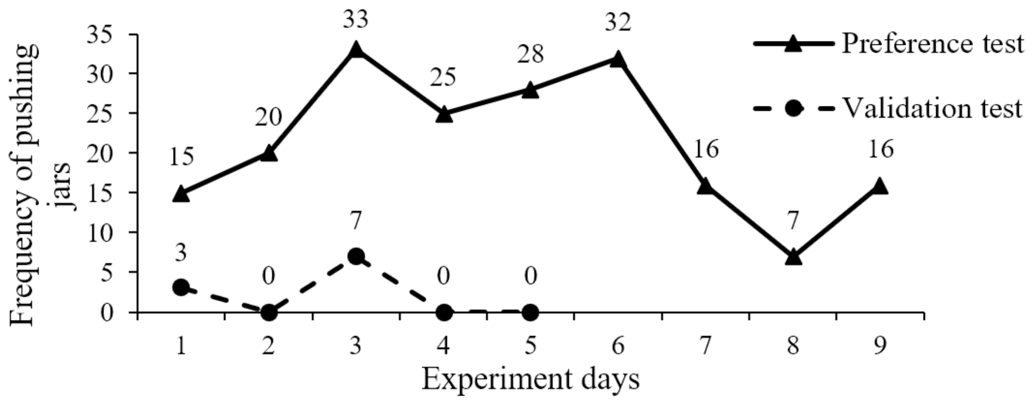


Figure 4.6.1 Daily frequency of pushing treatment jars (blank, attractant B_2, attractant L_2, fish bait, and white LED light) during the preference and validation tests.

In addition to touching and resting by treatments, lobsters also spent a significant amount of time resting in the experimental pools, but did not rest by any treatment jars. This information was not included in the statistical analysis. Particularly, in the validation test, as every experimental pool only contained one treatment jar, more free space was available to the lobsters, and the animals also tended to explore less in the pools during the validation test, compared to the preference test. In this case, the responses to treatments may not be evaluated accurately as non-locomotion responses were not included.

Antennal flicking was considered as the primary response of detection by some species, and other behaviours include probing, locomotion and mouthpart movement. However, these behavioural responses are not always clear or easy to determine. Therefore, the movement of animals toward test compounds were adopted as the criteria in some cases. Behaviours such as antennular flicking, may be helpful to take into consideration as it is a typical response to chemical stimulus (Zimmer-Faust et al. 1996).

Based on the videos of the trials, no influence on behaviour, such as avoidance behaviour, was found in response to the repeated patterns on the flat bottom of the experimental pools. Screenshots from the videos support the fact that lobsters moved freely through the experimental pools, crossing images without hesitation (Figure. 4.6.2).

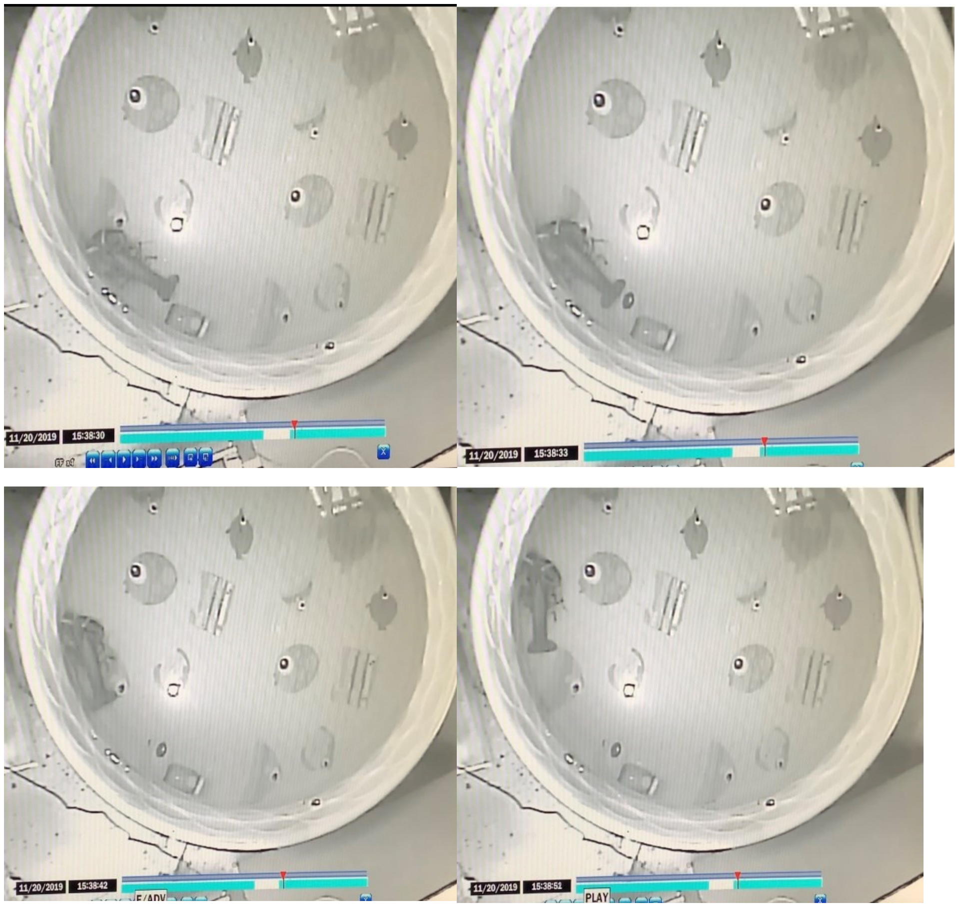


Figure 4.6.2. Lobster walking along the inner edge of an experimental pool, crossing one of the images on the bottom.

In a practical situation, lobsters may present different responses towards baits and attractants from the results based on laboratory observations. Similar to many decapod crustaceans, American lobsters have been observed displaying a circadian rhythm of locomotion and nocturnal increases in locomotion (Jury et al. 2005). Lobsters have also been reported as equally active with respect to locomotion during the day and night. Based on video surveillance, lobsters will enter and leave a trap several times over a soaking period, and lobsters caught in traps may only account for a small portion (6%) of all the attracted lobsters (Jury et al. 2001). This information indicates an uncertainty for the application of attractants in fishing practice. Lobsters could be attracted but escape after, and the attractiveness would not be evaluated correctly. Capture data may not reflect the true attractiveness unless the traps are able to keep all lobsters once they go in using palatable bait. Given this factor, it is essential to have video surveillance in the research of attractants, which can prove exact data regarding attractiveness. The attractiveness based on the capture therefore may not correspond with the results obtained from laboratory trials, making it difficult in determine the actual efficiency of attractants.

Preference tests have been conducted in various types of containers in previous studies, and glass aquaria and video cameras have been adopted previously as well. A divided glass aquarium was used in testing the preference of the giant tiger prawn via the observation of the animals' activities, where video cameras were used (Coman et al. 1996). Behavioural responses of American lobsters to several chemical stimuli have been examined, where the trials were conducted during the dark phase of the cycle, while aquaria and CCTV camera were used (Daniel et al. 2001). American lobsters were also previously tested in flow-through systems (Borroni et al. 1986), in which Plexiglas tanks (glass) were used, and the mixture of chemicals was made in the form of slow-leaching cubes using agar. Partitioned Plexiglas aquaria was used again afterward on research of the foraging behaviours of American lobster using time-lapse video recording (Lawton 1987). Movements of rock lobsters was observed by Tolomei et al. (2003) via cameras in glass aquaria, which was optically isolated using black plastic in each aquarium.

Besides aquaria, tanks, runways and Y-maze are additional the methods previously used in testing crustacean behaviours toward stimuli, and static water was chosen in several studies relating to chemoattraction. Tanks were used in behaviour and sensitivity studies in spiny lobsters and crabs (1.5 m diameter, Zimmer-Faust et al. 1996), and large concrete tanks were used by Mackie et al. (1980) to investigate the preference of European lobsters among several bait options. Behaviour response to attractants and stimulants was tested in Pacific white shrimp using a Y-maze by Nunes et al. (2006), where two options were available during each test run, and static water was used to avoid reotaxia influence. Water flow was also avoided in tanks for attractant research in freshwater prawn (Mendoza et al. 1997). Response under laboratory conditions may not reflect the true behaviours exhibiting in the wild, the trials in this thesis are valid by using similar techniques as previous studies, as well as the demonstrating by the comparisons to the control settings.

Another factor affecting the results might be the water temperature. As water of 15-18°C is preferred by juvenile and adult lobsters, the water used in this laboratory trials were colder, which might elicit less behaviours for lobsters. However, the habitats of lobsters were reported with a temperature range from 0 – 25 °C, and fishing practices not all happen during warmer seasons, which could also happen in winter with a temperature colder than optimal. Among the lobster fishing areas (LFAs, Figure 4.6.2) along the eastern coast of NS, the average bottom water temperatures of LFAs 27 – 32 in the spring and summer (late April to late June) of 2019 were below 14 °C. During the fall and spring seasons from 2018 to 2019 (from December 2018 to May 2019) in LFAs 33 and 34, the lowest average bottom temperatures were -1 and 0 °C, respectively, and the highest records reached 6 and 8 °C (Fishermen and Scientists Research Society 2019). Despite the water temperature in the laboratory trials was below the optimal temperatures for lobsters, it was within the range of bottom temperature for fishing sites in NS. Regardless, our study confirmed that the lobsters will approach an item they find attractive several times (Watson and Jury 2013), in contrast to being attracted and resting for the remainder of the time.

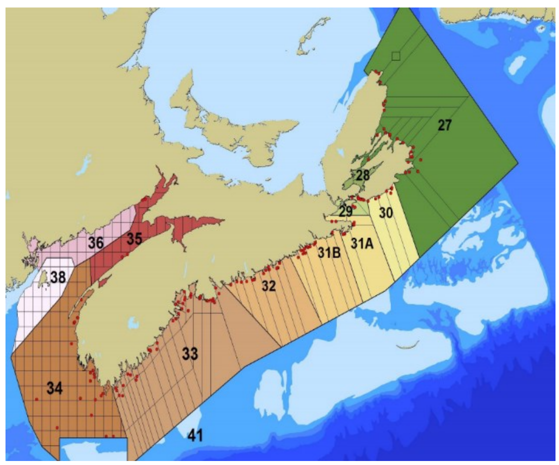


Figure 4.6.3 Lobster fishing areas (LFAs) along of the eastern coast in NS (source: Fishermen and Scientist Research Society 2019).

4.7 Conclusion

Preferences of lobsters to artificial attractants and white LED light were compared with the fish bait and blank treatment via preference and validation tests, and lecithin attractant (L_2) was found to be more advantageous than fish bait and white LED light. When the lobsters had access to all five treatments within an experimental pool, animals rested by the lecithin attractant (L_2) for a significantly longer period than by the mackerel fish bait or white LED light. When the lobsters had access to only one treatment in an experimental pool, no significant difference in preference was observed. A possible explanation is the interaction between different baits and attractants, which could make the lecithin attractant more advantageous when multiple attractiveness sources presented, rather than using the lecithin attractant alone. The thresholds for chemical attractants triggering detection and locomotion can vary, of which a higher threshold is needed to trigger locomotion. The response of locomotion is more important, as artificial attractants aim to attract lobsters to traps in practical fishing. Due to this, the response of locomotion by lobsters was mainly counted in this experiment, as this represents the animals approaching traps, and potentially being captured. Other observations, including antennular flicking and probing, which were recorded, are therefore not chiefly taken into consideration and analysis, due to not having a spatial difference. Further research may include the difference in the behaviours with no spatial difference, which may give a better evaluation of the attractiveness. There was variation in individual preference in both the validation and preference tests, meaning the activity level and locomotion response among individuals can be very different. Although daily temperature varied, results from the block design confirmed that it did not influence lobster preference. Further trials can consider validating the interaction effect, which can be beneficial for fishers to ensure captured lobsters remain in their traps and so their bait is selected rather than the bait in a nearby competitor's trap.

Chapter 5: Discussion

Snow crabs and American lobsters are two crustacean species that are economically important to NS. Since 1990, the landing of snow crabs has quadrupled in Canada, with more than 14,000 tonnes harvested in NS in 2019, and the landing of American lobster in NS was over 50,000 tonnes in the same year (DFO 2021e). From a sustainability aspect, the crab and lobster fisheries are the two major users of fish bait compared with other crustacean fisheries, and this use raises with increased capture, which is a wasteful use of fish protein resources. Increased fish bait use also leads to increased operating costs, due to the refrigeration costs associated with fish bait storage. As fuel costs are a significant expense for fishing vessels, a more efficient artificial bait may also reduce operating time and shorten the fishing season, therefore using less fuel. The sustainability status for crab products was suspended after the death of 12 right whales in NB in 2017, which was related to accidents with crab fishing vessels (Ibrahim 2019). Protective measures for the right whale were taken including shifting fishing seasons to avoid accidents, and the restriction of fishing areas if right whales are spotted.

It may be advantageous to develop alternative baits that function similarly to fish bait, but contain less or no fish products. The sustainability of fisheries is affected by various factors, including the use of fishing gears, fishing vessels and fuel efficiency, and fishable amount. As a part of fishing practice, the sustainability of crustacean and other fishing industries could be improved within a certain extent in by allowing more fish to remain in the oceans or by using the fish protein for human consumption. An ideal alternative bait in the future would be made of sustainable materials, more affordable than fish bait, and could be stored at room temperature.

The objective of this research was to investigate the effectiveness of an artificial attractant containing betaine (B_1) and white LED light on the capture of snow crabs by conducting sea trials in real-life fishing circumstances, and to investigate the effectiveness of artificial attractants (B_2 and L_2, containing betaine or lecithin, respectively) and white LED light in attracting American lobsters via laboratory trials.

In the snow crab sea trials, traps using betaine attractant (B_1) and white LED light to augment fish bait significantly improved snow crab capture in the 2018 season, but not in the 2019 season. No improvement was confirmed when the fish bait was augmented with only betaine attractant or only white LED light in either season. Similar to the results seen in the snow crab trials, the betaine attractant (B_2) did not attract lobsters, as determined by a preference test.

These results were unexpected, as betaine is generally regarded as an effective attractant. Studies suggest that betaine can be detected via gustatory receptors by salmonids, as the synergistic properties of betaine enhance the flavour of amino acids, functioning as a feeding stimulant for species including Salmonidae, Cyprinidae and Cichlidae (Yamashita et al. 2006; Yesilayer and Kaymak 2020). In animal diets, betaine increases feed intake by improving palatability, thus enhancing growth performance (Lim et al. 2016). This improved growth rate has been observed in winter flounder (*Pleuronectes americanus*, Fredette et al. 2000), pike perch (*Sander lucioperca*) fingerlings (Zakipour et al. 2012), and in juvenile rainbow trout (Yesilayer and Kaymak 2020). Betaine has a strong attractiveness for freshwater prawn (*Macrobrachium rosenbergii*) at a low concentration of 10^{-4} M (Felix and Sudharsan 2004).

The concentration of betaine in the B_1 and B_2 used in the snow crab and lobster trials described in this study was considerably higher than 10^{-4} M. The lobsters in the laboratory trials did not exhibit active locomotion in response to the betaine attractant (B_2) and this poor response by the lobsters could have been due to the concentration of betaine in the attractant formula. The concentration of betaine in the attractants may have exceeded the detection threshold, resulting in an avoidance response, as the concentration gap between detection and movement can be distinct.

Based on the results from the diffusion test, the betaine attractant (B_1) would have lasted in the snow crab sea trials when the harvesting interval was no more than three days if the attractants were not consumed by crabs or smashed by external force. Conversely but less likely, in the lobster preference test, the concentration of the released betaine may have achieved the detection threshold, but may not have exceeded the level required to trigger motion responses (Archdale et al. 2011; Lee and Meyers 1996). Further studies investigating betaine as a feed attractant for snow crabs or lobsters should be desired.

With the purpose of capture, the bait and attractants used in fisheries must be able to release chemical signals that exceed the stimulating level and trigger physical movements to lure animals into the traps (Archdale and Anraku 2005). Attractants with a series of betaine levels can be produced to compare the diffusion process and the preference of snow crab and lobsters to determine the optimal level. Additionally, antennular flicking and probing were observed in lobster laboratory trials, but were not included in this analysis, as no spatial difference occurred. As indicators of chemical signal detected at a low concentration can be indicated by antennal flicking or mouthpart movement (Schmidt and Mellon 2010), it may be beneficial to take these behaviours into consideration in future attractiveness evaluations.

In this study, the lobsters rested by the lecithin attractant for the longest period and it was the most frequently touched treatment. The behaviour of these lobsters indicate they preferred the lecithin attractant to the fish bait (mackerel) control. Dietary lipids are essential for crustaceans as an energy source and for the maintenance of bio-membranes. Although lecithin had not previously been tested as an attractant for crustaceans, lecithin offers physiological benefits including improved growth and survival rate, and weight gain (Haran and Fenucci 2008; Thompson et al. 2003). In penaeid shrimp, lecithin supplementation enhances weight gain and survival rate (Teshima et al. 1986; Hien et al. 2005), and survival and metamorphosis rates in the larvae of giant freshwater prawns (*Macrobrachium rosenbergii*, Hien et al. 2005), as well as increased survival rate in mud crab (*Scylla serrate,* Holme et al. 2007). Dietary lecithin is used in finfish diets as well, increasing the body weight of juvenile stellate sturgeon (*Acipenser stelatus*, Jafari et al. 2018). As only one concentration level of lecithin was used in the laboratory trials, it would be beneficial to conduct sea trials using lecithin attractant in the future, and to test different levels in order to determine optimal inclusion levels. A further investigation may also be conducted to determine if another attractant, such as lecithin, would be preferable by snow crab.

Statistical results indicated no difference between betaine (B_2) and lecithin (L_2) attractants regarding the duration and frequency of resting and touching behaviours. In the preference test, lobster chose the lecithin attractant (L_2) over fish bait and white LED light. When tested separately in the validation test, there was no statistically significant difference among treatments. The fish bait may have been chosen over the lecithin attractant (L_2) if it was due to a food-driven behaviour. However, it is unknown how much time had passed since the lobsters had last eaten before they arrived at the laboratory, and they were not fed during acclimation or during the trials. The response to the lecithin attractant (L_2) may have been due to a specific lack of phospholipids. The blank control performed as effectively as all other treatments in the preference test. Possible reasons for this could be due to influences from the mixing of odours from other treatments, the animals using the blank treatment as a shelter to rest by, or the lobsters may have been deterred from the fish bait and the white LED light. Although the weight of mackerel used as bait in the laboratory trial was similar to the weight of the betaine and lecithin attractants, the ratios of the surface area to the cross-section area might not be identical, which could affect the initial concentration of attractant and lead to a different maximum concentration of odour (Westerberg and Westerberg 2011).

Water temperature may be an influencing factor in both our snow crab sea trials and the following preference test on lobster as well, which slowed the diffusion of the effective ingredient betaine in Chapter 3 and resulted in a non-significant attractiveness. The slowed diffusion could be an influencing factor during the snow crab seasons, particularly if the season moves to earlier in the year when the water temperature is low at fishing sites. The betaine attractants (B_2) were not attractive to lobster in the preference test, during which the water was cold as well. The walking rate of the American lobster increases more than twofold when the water temperature rises from 2 ℃ to 10 ℃ (Miller 1990; McLeese and Wilder 1958), and a preference for a warmer temperature (2.8 ± 0.7 ℃ warmer than ambient environment) has been observed in winter (Jury and Watson 2013). The catchability of southern rock lobster also varied seasonally, which is highest in early summer and lowest in winter (Ziegler et al. 2002). However, water temperature was not an influencing factor in our preference tests on lobsters, given results from the block design.

Nguyen et al. (2017) compared the catch per unit effort (CPUE: the number of crabs per trap) and the catchability of snow crabs harvested using LED lights with different colours under field and laboratory conditions. The study indicated a significant increase in capture rate using white LED light, which improved the CPUE by 77%, with no females counted. In following studies, novel luminescent netting pots made with luminescent twine were used in traps, which further improved the CPUE by 21.6% (Nguyen et al. 2019a; Nguyen et al. 2020). Inspired by their results, the snow crab sea trials in this research were conducted, which involved the treatment using fish bait augmented with the same brand of white LED light.

Our sea trials shared several similarities with the field experiments of Nguyen et al. (2017), including the time of fishing season (April 26th to May 23rd, 2016), the depth of fishing sites (ranging from 165 to 173 m), the use of white LED lights to augment fish bait, as well as the considerably different trap numbers of each treatment, where more traps used only fish bait than the other treatments. However, the improvement in capture from white LED light was much higher in the research of Nguyen et al. (2017) than the results we obtained from our snow crab sea trials. Improved capture only occurred in traps with both additional white LED light and betaine attractant in the 2018 fishing season in our study, and the increment was far less than 77% (3.7 totes/trap using fish bait only, 5.3 totes/trap using betaine attractant and white LED light augmenting fish bait, an increment of 43.2%).

One reason for this difference may be that treatments augmented fish bait and did not replace it. The fish bait used in the sea trials is assumed to have been high quality, as it functioned adequately to catch snow crabs and met quota in both seasons; therefore, there may have been less potential for improvement by adding betaine attractants and white LED light. Future sea trials could be conducted using sole artificial attractant or white LED light to replace fish bait, which could directly compare the catchability. Additionally, fish bait usage was different across alternative bait studies. Fish baits used differed in size and weight (750 g squid or herring, Murphy 2014; 453 g mixture of herring and squid, Nguyen et al. 2017; mackerel and minced greenling, Archdale and Kawamura 2011; 0.9 kg squid or herring, Grant and Hiscock 2009; 1 kg squid, Araya-Schmidt et al. 2019), which makes it difficult to compare attractiveness. Additional reasons for differing results in our study may include the differences in fishing site location, soaking time, fishing gear, and fish bait type. Bias could have been introduced in our study during data collection, as only whole numbers were recorded for the harvested tote numbers.

Standard traps and Japanese traps are the two most commonly used trap types in snow crab fishing, where a standard trap can hold twice the capture of a Japanese trap (DFO 2019a). Although Nguyen et al. (2017) did not provide trap size details, based on the capture means, the traps used in our sea trials can be inferred to be larger. In the 2019 snow crab sea trial, our standard traps containing only fish bait captured an average 120.7 kg, which would equal approximately 241 crabs, estimating based on an average crab weight of 0.5 kg (Jamieson 1981). In the experiment by Nguyen et al. (2017), small Japanese-style conical traps with mixed squid and herring were used, and captured an average 12.1 crabs per trap. As larger traps were used in this study, a more significant increment would be required to achieve the same percent improvement (e.g. a 70% improvement based on 241 crabs = 169 crabs, a 70% improvement based on 12.1 crabs = 8.5 crabs). Trap catchability may also be related with the abundance of the snow crabs at a fishing site, where the Japanese trap is smaller and may not be able to catch all crabs available at the fishing site. Attaching underwater cameras to traps would be useful to gather more catchability data in future research.

The abundance of snow crabs may also vary based on different fishing locations. In this study, the depth of the snow crab fishing site ranged from 101 to 229 m (55 to 125 fathoms) in the 2019 fishing season, which did not influence capture for any treatment. Greater depths of snow crab habitats were included in previous studies. Murphy (2014) conducted research at a depth ranging from 340 to 530 m, testing light usage in plaice fishing. The snow crab by-catch was greatly reduced by using green lights in pots, however, the depth of research site was deeper than that of typical practical fishing sites. Nguyen et al. (2017) concluded that the CPUE and depth were negatively associated in the depth range of 80 to 300 m, and a low crab density was inferred in their study. This negative relationship may also be relevant to their fishing location and the trap size, as the trap catchability in our trials was higher using standard traps and there was no evidence that the catch was negatively affected by depth.

The traps used in our sea trials had a minimum soaking time of one day, but often remained in the water for three to four days, depending on weather conditions. The soaking time of Nguyen et al. (2017) was less than 27 h, with the conclusion that the lights functioned better with longer soak times. As the soak times in fishing practice largely depend on weather conditions, artificial baits that would remain effective for several days would be more desirable. The results of the diffusion validation in Chapter 3 confirmed that the betaine attractants can last for at least 72 hours, and that the diffusion process was significantly slower in cold water. Nguyen and Winger (2019a), employed a minimum four-day soaking time, demonstrating a longer soaking time had a positive benefit when using LED lights in crab traps, increasing the CPUE of legal-size crabs increased by 48%, although more sublegal-sized crabs were caught as well. A longer soaking time, could also be disadvantageous in enhancing capture, as decreases in capture result, due to escape (Miller 1990).

As wavelength decreases from red to blue light, red light travels fastest in water; Oppositely, blue light has the best ability to penetrate water, which is followed by green light and yellow light (Chiang et al. 2011). White light contains all colours, however, not all can travel through deep water, as they are not able to penetrate such depth. The depth of 100 meters, for instance, is a depth that difficult for red light to reach. Due to the reflection of blue light, which can penetrate the furthest, blue aquatic animals would be more visible to predators (NOAA 2021). Possible influences on the attractiveness of light may be due to the depth of experimental pools in the lobster laboratory trials, which were shallower than lobster fishing sites and could affect the penetration of light. Based on the measurement of light meter in laboratory, the illuminance of the white LED lights used throughout the field and laboratory trials is 45 lux out of water.

In lobsters, LED light was not advantageous, based on the preference test results, although it was equally as attractive to lobsters as the betaine attractant (B_2) and mackerel. A different colour of light may have been more suitable. The maximum wavelength of visual pigments is approximately 500 nm for coastal species habiting above the mesopelagic zone (200 – 1000 m), and marine crustacea are sensitive to blue-green wavelengths (Johnson et al. 2002). Snow crabs have a preference for blue and white lights (Nguyen et al. 2017). The visual pigment of American lobster is most sensitive to blue-green light of the wavelength around 525 nm, and is least sensitive to red light (Gherardi et al. 2010; Tomina and Takahata 2012). Many marine animals are less sensitive to light with a wavelength greater than 600 nm, and red light therefore becomes better theoretically, which has been verified in spiny lobster (Weiss et al. 2006).

Different mechanisms raise behaviours for snow crab and lobsters. The “sensitivity hypothesis” explains the relationship between spectral sensitivity and habitats, which proposes by the “contrast hypothesis” that visual pigments adapt in response to the light in the animals’ habitat, in order to maximize the contrast between objects and background (Johnson et al. 2002). Snow crabs live at a deeper depth than lobsters, which allows for less natural illumination, although snow crabs do not inhabit deep water environments (more than 200 m), where bioluminescence is important for organisms (Herring 1983). Tomina and Takahata (2012) trained lobsters to discriminate light signals via learning with food rewards. Light stimuli were not considered a method for attracting lobsters and the animals needed to be trained to respond to light. The researchers observed that the lobsters were highly alert and vigilant when they were placed in an experimental aquarium for the first time, which led to restless movements. This corresponds with the research presented in this thesis, where many of the lobsters were observed circling in the experimental pools during their first test run. The light intensity of the white LED light may be too strong for lobsters in experimental pools which may prevent from being attractive to the light.

Lobsters naturally inhabit rocky subtidal areas. They are generally nocturnal and prefer crevices and burrows as shelter. Shelter is important for them to escape predation and they tend to occupy shelters when they are available and adequate (Richards and Cobb 1986; Tomina and Takahata 2012). During the validation and preference tests, lobsters were observed going back to the shelter provided or walking through the shelter. One lobster showed no movement and stayed in the shelter throughout the duration of one entire test run (the third run of that lobster, occurring on Day 8) in the preference test. These behaviours indicate the necessity of providing shelter in laboratory-based lobster preference trials, and also imply that an unknown factor kept the lobsters alert and therefore performed less behaviours.

The cost-benefit analysis conducted in the snow crab case study indicated a potential improvement in fishery sustainability from an implicit aspect. Due to fossil fuel usage, fisheries are considered energy-intensive, where the cost for direct fuel inputs commonly accounts for over 70% of the operating cost (Tyedmers 2004; Driscoll and Tyedmers 2010). In 2011, every kg of fish and invertebrates landed was estimated to have a 2.2 kg CO_2-equivalent, and a greater magnitude of fuel use intensity was calculated for crustacean fisheries, accounting for 6% of the global fisheries landing, but more than one fifth of emissions (Parker et al. 2018). The average fuel use intensity (FUI) for the North American crustacean fishery is estimated at 783 L per ton (Parker and Tyedmers 2015).

Bait and labour costs are ranked after fuel costs for vessel owners. CPUE is commonly used in studies evaluating the efficiency of fishing operations, which however, does not take economic aspects into consideration, and may be defined differently in research (kg per trap in Grant and Hiscock 2009; number of crabs per trap in Chiasson et al. 1993, Nguyen and Winger 2019a, Araya-Schmidt et al. 2019). Limited information is available for fishing operation costs in the Maritimes, which often vary among vessel owners (DFO 2019a). CPUE primarily aims attention at capture, and effects from other aspects are limitedly counted. The brief calculation method to determine the costs and benefits of using different bait and attractant provided in Chapter 3, can be used to build a budget based on bait costs, labour costs, and to estimate a minimum fishing period when using different bait and attractant combinations.

Given the case study for the 2019 snow crab fishing season, the number of hauls required to meet quota would decrease in the assumed situation where betaine attractant (B_1) augmented fish bait, comparing to the situation where traditional fish bait was solely used. Using the betaine attractant or the betaine attractant and white LED light combined to augment fish bait could lead to one fewer trip, and the use of white LED light to augment fish bait would result in two fewer trips. Fuel usage, thus costs could be reduced, and this could also be a way to work with a shorter fishing season than they had been accustomed to prior to 2018.

Other influencing factors may exist and should be taken into consideration. As a feature of selective harvesting, only hard-shell males with a carapace width greater than 95 mm are permitted to be harvested, and soft-shell males and females are illegal capture that must be sorted and released, which requires labour (DFO 2019b). Female and sublegal capture can be significant in commercial fishing where an average of over 64% of red king crab (*Paralithodes camtschaticus*) capture was female or of sublegal size in the Bering Sea (Zhou and Shirley 1997). LED light has been proven to enhance snow crab capture, but potential preferences for light colour may occur between the males and females, although this has not been determined. In future research, it would be beneficial and more efficient to seek the possibility of selectively fishing for capture-sized males. However, only legal-sized males were counted based on the current practice of our sea trials, and the capture data of males and females was not collected.

Bait supplies have decreased over the years, while operating costs have risen for fisheries (Ryan et al. 2014). Harnish and Willison (2009) conducted the first study on bait-to-catch ratio of lobster in NS fisheries, which conservatively estimated the average bait-to-catch ratio was 1.9 in St. Margaret's Bay and Mahone Bay (LFA 33). Based on their report, most lobster fishers buy and freeze fish bait. A cost-benefit analysis for a lobster fishing season could be also conducted in future research with capture data from sea trials.

In addition to being adopted in place of traditional fish bait, alternative attractants should be affordable for fishers, and ideally would not cost more than fish bait, or would provide indirect financial benefits, as acceptable cost may encourage fishers switching to use these more sustainable options.

Chapter 6: Conclusion

Fishing crustaceans, such as snow crabs and lobsters, can be more sustainable if fish bait usage can be reduced, maintaining fish stocks in the ocean and/or using captured fish for human consumption rather than bait. In addition to producing an alternative option that is equally as attractive as traditional fish bait to capture crustaceans, a trade-off needs to be considered from an economical aspect. Alternative bait options have been previously studied in crustacean fisheries, which traditionally use a significant amount of fish bait. With the objective of developing potential alternative baits for crustacean fisheries, this study examined the catch resulting from traps augmenting fish bait with artificial betaine attractants and/or white LED light from an economic aspect and catchability during snow crab fishing seasons, and also tested the preference of lobsters for different alternative bait options under laboratory conditions.

Traps baited with fish bait augmented with additional betaine attractant (B_1) and white LED light resulted in an improved capture in the 2018 snow crab fishing season, but not in the following 2019 season. The reason for the snow crabs being inconsistently attracted to the light in sea trials in the 2018 season and not in the 2019 season or in the lobster trials remains unclear, further investigations are recommended to clarify the reason of the difference. Positive responses may have been motived by non-chemical factors, such as the utilization of bioluminescence in deep-sea environments (Herring 1983). Straightforward comparisons between catchability using fish bait and artificial attractants are ideal. However, in order to reduce risk for the fishers participating in this research, rather than comparing directly, the trials only compared the effect on catch rates by adding artificial attractants and lights to fish bait, rather than directly replacing fish bait. It is assumed that the fish bait was of good quality and functioned efficiently to attract crabs, as the fishers previously applied only fish bait in all traps. As the snow crab fishing occurred in *in vivo* situations, capture results were influenced by weather and fishing sites, as well as limited data collection procedures, which were recognized as sources of error and possibly affected the results.

Although the cost of bait is not the biggest influencing factor in fisheries, bait quality could affect capture and consequently affect the fishing period. The cost-benefit analysis of augmenting fish bait with alterative attractants (B_1 and white LED light) indicated little difference. It did not result in a significant change in operating costs in different assumed practical situations, and cost less than using fish bait alone. These results can be helpful to encourage snow crab fishers to utilize alternative attractants and white LED light for fish bait from an economical aspect, which would then have potential trickle-down benefits for fish conservation and sustainability, or be used as a model for testing additional alternatives to forage fish as bait.

In contrast with the conditionally positive performance of white LED light during the 2018 snow crab fishing season, the white LED light did not attract American lobsters under laboratory conditions. By means of observing the response towards different attractants and white LED light, lobsters did not show a preference for betaine attractant (B_2) or white LED light. However, the lecithin treatment (L_2) was chosen the most when lobsters rested beside it, which is accordingly considered attractive to lobsters and may be potential to reduce or replace fish bait use in the future. Aside from the differences between natural habitat conditions and laboratory conditions, the mechanisms of attraction appear to be unique for each species, resulting in different preferences. Although lobsters have previously been presented as a laboratory model for crustacean studies, the difference due to their locomotion circadian rhythm may result in bias (Jury et al. 2005), and the limited response to compounds in low-molecular-mass (< 1,000 daltons) compounds (Zimmer-Faust 1989).

Future direction may include laboratory preference experiments on snow crabs testing the preference for various alternative options, including attractants containing lecithin.

Rather than augmenting artificial attractants or white LED light with fish bait, traps replacing fish bait with artificial attractants or white LED light may be tested during future snow crab fishing seasons. Different betaine and lecithin concentrations could be considered in further laboratory trials on lobsters, as well as in sea trials during lobster fishing seasons. Although betaine is not certain to be the best available chemical attractant, it may enhance the effectiveness when using in combination. The effectiveness of chemical stimuli might need better evaluation when mixtures are included in attractiveness tests, as the receptors could be activated by various compounds. Due to the escape behaviour of lobsters, the attractants would be ideal if they not only attract lobsters to the traps, but encourage them to remain in the traps as well. A possible solution for keeping lobsters in traps could be to add edible or palatable feed ingredients to the attractants, which may prolong the time the lobsters remain in the trap before escaping. Future studies may consider the length of time the lobsters remain in baited traps as a factor when evaluating attractiveness. Video surveillance may be beneficial to determine if there is an optimal soaking time during which there are most abundant lobsters in the traps. Further sea trials could focus on a more uniform trap setup with an equal number of traps for each treatment and the same interval between harvesting days. Ideally, only one item (fish bait, artificial attractants, or white LED light) would be used in each trap rather than augmenting fish bait with attractants and white LED light.

Despite the need for more research, initial findings on the effectiveness of a combination of betaine attractant, white LED light and fish bait, and the effectiveness of lecithin, have been determined in snow crab and American lobster, respectively. Benefits from an economic aspect have also been considered in practical fishing. A reduction in operating costs and a shorter fishing season could be achieved using artificial attractants, making the use of artificial attractants advantageous, even without statistically increasing capture. Further efforts should be made to investigate better options for bait alternatives, and the reliance on fish bait in crustacean fisheries may be reduced by partially substituting fish bait with alternative attractants.

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