Thesis for the Degree of Master of Fisheries Science

Behavior analysis of sea urchin *Strongylocentrotus nudus* and rock bream *Oplegnathus fasciatus* for identifying chemical cues eliciting attractant/repellant responses

by

Siriya Kankanamage Tilan Chamara Duminda

Department of Fisheries Biology

The Graduate School

Pukyoung National University

August 2020

Behavior analysis of sea urchin *Strongylocentrotus nudus* and rock bream *Oplegnathus fasciatus* for identifying chemical cues eliciting attractant/repellant responses

둥근성게 *Strongylocentrotus nudus* 와 돌돔 *Oplegnathus fasciatus* 의 행동 분석을 이용한 유인 및 기피물질 탐색

Advisor : Prof. Jong-Myoung Kim

by

Siriya Kankanamage Tilan Chamara Duminda

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Fisheries Science

in the Department of Fisheries Biology, The Graduate School
Pukyoung National University

August 2020

Siriya Kankanamage Tilan Chamara Duminda 의

수산학 석사 학위논문을 인준함

2020 년 8 월

주 심	수산학박사	박 정 환	인
위 원	수산학박사	최 윤 희	인
위 원	이 학 박사	김 종 명	인

Behavior analysis of sea urchin *Strongylocentrotus nudus* and rock bream *Oplegnathus fasciatus* for identifying chemical cues eliciting attractant/repellant responses

A dissertation

by

Siriya Kankanamage Tilan Chamara Duminda

Approved by:

(Chairman) Prof. Jeonghwan Park

(Member) Prof. Youn-Hee Choi

(Member) Prof. Jong-Myoung Kim

August 2020

Contents

Behavior analysis of sea urchin *Strongylocentrotus nudus* and rock bream *Oplegnathus fasciatus* for identifying chemical cues eliciting attractant/repellant responses

Siriya Kankanamage Tilan Chamara Duminda

Department of Fisheries Biology, The Graduate School,
Pukyoung National University

Abstract

Monitoring the movement of fish is important to understand various aspects of fish physiology dealt with environmental changes. To explore the applicability of the video tracking system for tracking aquatic animals, movement patterns of sea urchin *Strongylocentrotus nudus* (invertebrate spp.) and rock bream *Oplegnathus fasciatus* (vertebrate sp.) were recorded upon exposure to the materials eliciting the behavioral response. Movement of sea urchins were examined upon exposure to materials including seaweed, coffee waste, and starfish. Behavioral response was first analyzed by direction of movement against the materials in a tank. While most of sea urchin showed a clear preference toward a seaweed, an avoiding behavior from the tested materials were observed for coffee bean waste and dried starfish. The results suggest a potential way to efficiently recycle the industrial waste like coffee waste, and harmful organism for restricting sea urchin distribution in the designated ocean area. To test the applicability of the video tracking-based analysis for identifying a chemical cue attracting the fish, behavioral monitoring was tested with rock bream upon exposure to different chemical cues including commercial feed, and materials used as a bait for fishing. Pellets formulated with cellulose and cornflower powders to contain the homogenized rock worm, bait worm, sea urchin tissue, krill, barley seeds, or powdered fish feed, respectively, were tested as chemical cues. Preference toward cues were calculated by duration and frequency of rock bream detected from a discrete zone around the chemical cues in a tank as analyzed by using automated video tracking software. The results showing a higher attraction toward a sea urchin as compared to that of the rock worm provide a basis for its potential use as baits for rock bream fishing. Behavior tracking tools used for analyzing sea urchin and fish movement behaviors can be also developed for automated monitoring of aquaculture facilities.

Figure legends

Figure 1. Photographs of (A) 2 x 2 m large tank with video camera and (B) Y-shape tanks used for monitoring of sea urchin and rock bream behaviors. Smaller tanks of 1 x 1 m sizes were used. (C) Dimension of Y-shape tank was indicated.

Figure 2. (A)Diagrams shows location of zones (Z1 and Z2) where movements of fishes around the spots surrounding an empty container (Z1) and a container containing tested materials (Z2) were detected in a rectangular tank and Y-shape tanks (B).

Figure 3. Preparation of hardened agars (A) containing test materials including kelp, coffee waste, and starfish powder, from left to right, used for sea urchin behavior analysis. (B) Pellets prepared for rock bream behavior analysis were shown from cellulose pellets, cellulose pellts mixed with rock worm and sea urchin inner tissue from left to right.

Figure 4. (A) Heat map analysis sea urchin (located at the right end) were exposed to kelp and empty shell control as indicated. Heat map showed a movement path of sea urchins. (B) Distribution of sea urchin analyzed upon exposure to an agar containing starfish extract at the center of the tank.

Figure 5. Photograph showing a distribution of sea urchins upon exposure to an agar containing coffee bean waste located at the center of the small size tank (1 m x 1 m). Number of sea urchins found in the center (red square) and the surrounding region (edge zone) were counted.

Figure 6. Number of sea urchins found in the center zone of tank and edge zone of tank of the small tank upon exposure to agars containing seaweed (A), coffee bean waste (B) and starfish crumb (C).

Behavior analysis of sea urchin *Strongylocentrotus nudus* and rock bream *Oplegnathus fasciatus* for identifying chemical cues eliciting attractant/repellant responses

I. Introduction

Animals respond to their surroundings and find the best place for their living. Behavior of living organism is directly reflecting the state of the environment, and physiological and ecological relationship of organism to its environment (Little and brewer, 2001). Behavioral response can be determined by integration of avoidance or attraction (Kane et al. 2005), that may affect their habitat distribution. Intense observation of animals provides a sound tool for understanding the behavioral response against changes of cues of the environment to understand their physiology and ecology (Xia, C. at el, 2018). Comparing to amounts of information regarding chemical cues acting on terrestrial animals, relatively less is known about behavioral response in aquatic animals mainly due to little progress in application of automated monitoring system of aquatic animals (Hay, M. E. 2009).

Marine aquatic environment is a fabulous field of study for monitoring animals' behavioral response through chemical mediated cues and signals. Aquatic animals are living in the water media and water quality parameters are highly affected their homeostasis maintenance and widely use physical and chemical cues to detect various signals from the watery environment for their survival. Behavioral endpoints in aquatic animals can be quantified by movement parameters such as total distance moved, velocity, acceleration, turning angles and frequency, meander, mobility (time spent for swimming), horizontal and vertical distribution of organisms, path changing, space utilization, distance from center point ministering to discern and these figures assist to evaluate the effects of exposure to environmental stressors (Kane et al. 2004, 2005).

Aquatic animals detect and distinguish myriad odor signals and elicit important behaviors such as foraging, courtship, schooling, and assessing threat (Firestein, S. et al. 2001; Kermen, F. et al. 2013) with the help of bio-sensory system. For instance, amino acids induce appetitive swimming behavior in zebrafish. Responses for those cues would be increased number of turns and swimming speed (Lindsay and Vogt, 2004) whereas steroids and prostaglandin F2α hormones produced in the gonads of fish shown to trigger reproductive behaviors in a variety of teleosts (Hurk and Lambert, 1983; Stacey and Kyle, 1983). Compounds released from conspecific injured skin communicate as an stereotyped alarm signal (von Frisch, K. 1941) executing darting followed by slow swimming or freezing behaviors (Speedie and Gerlai, 2008; Doving and Lastein, 2009).

Prey animals in nature under predation situation faced different challenges as competing motivations due to hunger and fear for predation (Julia Mas-Muñoz et al. 2011). Overall activity against such a situation may be determined as an avoiding or attracting behavioral response. These types of complex natural phenomena possible to be explained by behavioral experiments through comparing behavioral/movement response in the presence of different cues at relevant stimulation levels with controls. In this movement behavioural study rock bream (vertebrate) and sea urchin (invertebrate) are used as specimen species due to importance in several aspects as follows.

Sea urchin is a marine invertebrate that is important for marine ecology, in particular for their grazing habits for seaweeds and algal biomass (Lang C, et al. 1976, Lawrence JM, 2007, C. Harrold, et al. 1987). Sea urchins are imperative herbivores on coral reefs, and playing a critical role in maintaining the balance between coral and algae. Therefore, distribution of sea urchin is important to understand the natural disturbance on the ecology of seaweed-dominated area mainly due to sea urchin's grazing behaviour on kelps and other macroalgae (Valentine and Heck, 1999).

Understanding the threat as a harmful organism due to largely foraging impact on the other fauna, and flora species, sea urchin distribution altering seems to be a crucial activity for implementing a sustainable control strategy by means of biological and industrial approaches. Sea urchin *Strongylocentrotus nudus* (Agassiz, 1864) is a dominant species in the northwest Pacific seas and found on intertidal and subtidal sea bottom (Yukio A., 2007) and in shallow waters in Korea. The gonads of this sea urchin are halfmoon shaped with a distinctive aroma and good taste (Silvia et al., 2016, Qin, L. et al. 2011). The sea urchin gonads of yellow-orange color deliver a striking sea food delicacy. Further, this species gonad has long been used as a food nutraceutical (Zhao et al., 2018). In account of above values, it is important to manage sea urchin resources to optimize their economic aspects as well as the environmental sustainability. High density population of sea urchin overgrazing implanted sea weeds is most destructive for sea weed developing grounds, and for fish breeding grounds. Therefore, it is important to prevent the accumulation of sea urchin population to protect the reinforced marine forest.

In order to reinforce the biological contribution of seaweeds to the marine production, and the biodiversity, seaweed being implanted by marine authorities of the South Korea. Controlling of sea urchin number invading implanted seaweeds is one of the main challenges faced to protect the alga environment. Peoples are trying to remove physically these sea urchins in order to control their higher population, and their harmful impact on implanted seaweed, and these operations are bound with high risk, time consuming and costly operations. Therefore, it would be an innovative solution if it is possible to introduce a method to repel these invading sea urchins from that implanted areas by using chemical cues meanwhile introducing a novel option to increase sea urchin consumption on industrial basis by using sea urchin for bait production to catch rock beam. For this, a video tracking system was applied for monitoring the movements of sea urchin together with rock bream, one of the main aquaculture fish species to find chemical cues attracting or repelling the target organisms.

II. Materials and methods

1. Experimental animals

Sea urchin ware obtained from East coastal area of Korea with the help of Korea Fisheries Resources Agency (FIRA). Sea urchins were dissected and freeze dried or kept live in tanks with sea water (33ppt) at 16 ±2 ^oC. Sea urchins were fed with sea weeds and allowed several days to starve before the experiments. Rock breams of 10-50 g in weights were obtained from National Fisheries Research and Development Institute (Geoje, Korea). Fish were reared basically in 2 x 2 x 1 m tank with 33 ±2 ppt recirculating sea water at 20 ±2^{o}C and fed with 3% commercial fish feed. While large size rock bream up to 50 g in weights were used for tracking in rectangular tank, smaller size of 5-10 g in weights juvenile rock bream were used for tracking in Y-shape tank. Rock worms (*Marphysa victori*) were received from Goseong Fishery Technology Center, PKNU, Korea, and live bait worm and dried krill (baits grade) were purchased from a bait selling market in Busan. Experimental handling and treatment of experimental animals were conducted in accordance with the government regulations and relevant standards.

2. Experimental tanks

Water tanks of different shapes and sizes (rectangular, square: 1 x 1 x 1 m, 2 x 2 x 1 m, Y-shape) were used for this study. Sizes of an arm of the large Y tank was 48 cm x 33 cm x 28 cm. Video camera were fixed on top of the illuminated experimental tank and connected with the computer in which the tracking software is installed (Figure 1).

(A) (B) (C)

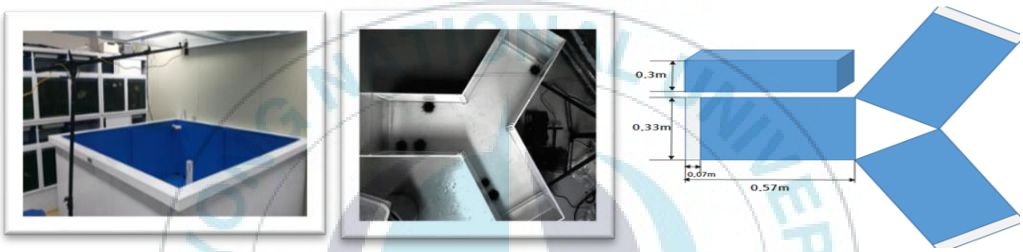

Figure 1. Photographs of (A) 2 x 2 m large tank with video camera and (B) Y-shape tanks used for monitoring of sea urchin and rock bream behaviors. Smaller tanks of 1 x 1 m sizes were used. (C) Dimension of Y-shape tank was indicated.

Rock bream movements mainly tracked in 105 cm x 60 cm x 55 cm rectangular tank. Further monitoring of the movements of rock breams were repeated in Y shape tank (size of a single arm- 48 cm x 33 cm x 28 cm) as a Y maize choice test to compare the attraction of rock bream for different cues (Figure 2).

3. Preparation of testing materials

To prepare materials for behavioral analysis of sea urchin, dried seaweed powder, coffee bean waste powder, and dried starfish crumbs were resuspended in water containing 1.5% agar. Mixtures were autoclaved for 15 min, and hardened on top of the trays (Figure 3, tray sizes, 27.5 x 27.5 cm and 30 x 40 cm). Materials used for rock bream behavioral analysis were prepared as a cellulose-based pellet. Cellulose pellet (10% corn flour in 90% cellulose) was prepared from 25.2 g of cellulose with 2.8 g of corn flour resuspended in 65 g of water, passed through 10 ml disposable syringe, cut into 1 cm in length and then air-dried. Sea urchin internal tissues, rock worms, commercial bait worms and krill were homogenized followed by freeze-drying. Dried materials (0.2 g) resuspended in 1 ml of water and adsorbed onto 1 g of cellulose pellet as prepared above followed by air drying. Known amounts of pellets containing the tested materials were inserted into an empty tea bag for testing. A plastic container (11 cm x 8 cm x 4 cm) with penetrating slits over the top surface of the lid were used for hiding materials enclosed to empty tea bags during chemical cue tests to prevent from visual affect and feeding by fish (Figure 3).

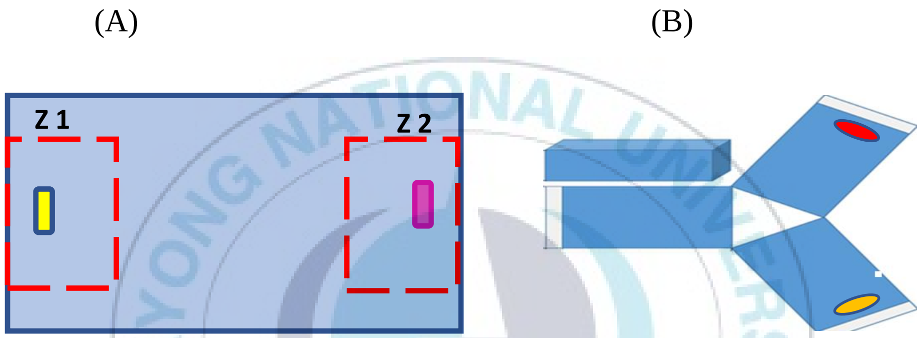

Figure 2. (A) Diagrams shows location of zones (Z1 and Z2) where movements of fishes around the spots surrounding an empty container (Z1) and a container containing tested materials (Z2) were detected in a rectangular tank and Y-shape tanks (B).

(A) (B)

Figure 3. (A) Preparation of hardened agars containing test materials including kelp, coffee waste, and starfish powder, from left to right, used for sea urchin behavior analysis. (B) Pellets prepared for rock bream behavior analysis were shown from cellulose pellets, cellulose pellts mixed with rock worm and sea urchin inner tissue from left to right.

4. Behavioural analysis of sea urchin and rock bream

4.1. Monitoring system for sea urchin movement

The experiment trials were carried out at least in two different tanks with a camera fixed on the top of the tank. Small tank experiment was carried out with 10 sea urchins in 1 X 1 m tank filled with 15 cm height of sea water (33 ±2 ppt and 16 ± 2 ^{o}C) aerated with air stones. Sea urchins acclimated for one day were exposed to an agar plate containing tested cues placed in the middle of tank. Sea urchin movements were recorded for one hour and movement tracking were analyzed by heat map generated by EthoVision XT 10 software (Noldus). Large scale experiment was carried out with 40 sea urchins in a tank (2 m X 2 m) with an agar plate containing tested cues. Materials such as seaweed, coffee bean waste and starfish crumbs hardened on a tray was used. Distribution of sea urchins were recorded 1 day after the exposure.

4.2. Rock bream movement analysis

Behavioural response of rock bream was analysed in rectangular tanks of 105 cm x 60 cm x 55 cm size and Y-shape tank with arm size of 48 cm x 33 cm x 28 cm for comparison of movement parameters. Fish were randomly divided into two groups and transferred to identical size aquaria and fed with commercial feed (2% of body weight). Fish feeding were reduced from one day before the experiment to increase their appetite to keep their normal food up taking process during the experiment. Tank was aerated about 10 min time period in between control trial and treatment trial to provide similar oxygen condition but air stones were removed during video recording. Experiment in a rectangular tank was carried out with randomly selected 3 fishes acclimated in a tracking tank for 15 minutes. Movement of fish was recorded for 15 minutes with control and treatment trials. Rock bream movements were also tested in Y shape tank with smaller size juveniles. Fish (5 fishes per group) were firstly held in acclimation zone separating from other 2 arms harboring a cage containing testing materials, respectively. Upon removing the blocking shield, movements were recorded for 15 minutes.

5. Vision-based automated tracking software

Behavioral responses of sea urchin and rock bream recorded by a camera fixed on top of the tank were analyzed by EthoVision video tracking software. For this, analog video signal captured by the camera was digitized by a frame grabber and fed into the computer for automated analysis. Movement patterns were analyzed by using heat maps, movement parameters including the distance moved, duration and frequency detected in the designated zones around the materials.

6. Statistical analysis

The experimental data were noted as mean ±SD and statistical analysis was performed using PASW statistical program ver.18 (IBM Co, USA), and the statistical significance between experimental groups ($P<0.05$) was examined using one-way ANOVA, Paired t-test, and percentage values.

III. Results

1. Video-based monitoring of sea urchin movements

To develop a repellant-based strategy for reducing sea urchin population in marine forest, it is critical to establish a behavioral monitoring system to identify materials that can repel or attract sea urchin. Vision-based monitoring system was tested for its applicability for monitoring the movement of marine animals using sea urchin and rock bream as a model system. To examine the behavioral response of sea urchins, experimental tanks of square and Y-shape tanks of different sizes were used. Two sizes of square shape tanks of 1 m x 1m and 2 m x 2m were employed for finding time duration (one hour to overnight) and number of sea urchins (10 to 40). The larger tank was employed to confirm a sound diffusion effect on the distribution over the tank as per the attractive/ repulsion behaviors. Y-shape tank also was used to compare the difference in attraction/repelling efficacies between materials (Figure 1 and Figure 4).

To examine whether the condition employed in this study is good enough to distinguish chemicals with different attraction efficacy, preliminary trials were done with chemical cues such as sea weeds (Sea Mustard/ Kelp). Testing materials were also incorporated into agar with the aim of a constant gradient dispersion for a longer period allowing sea urchin to respond. Control experiments showed a clear direction of attracting response toward the kelp (Figure 4).

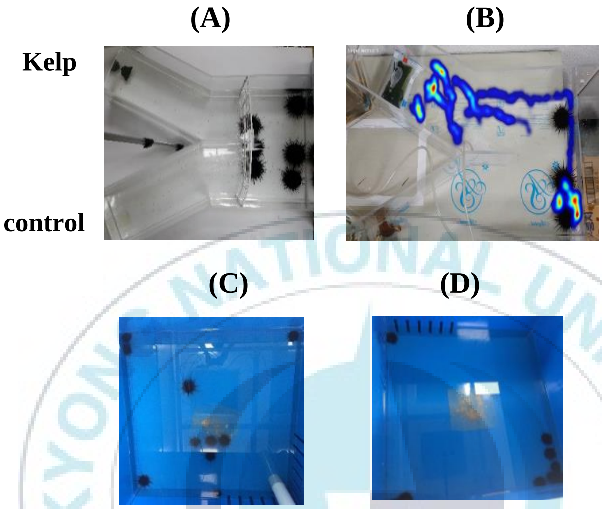

Figure 4. (A) Heat map analysis sea urchin (located at the right end) were exposed to kelp and empty shell control as indicated. Heat map showed a movement path of sea urchins. (B) Distribution of sea urchin analyzed upon exposure to an agar containing starfish extract at the center of the tank.

This together with an evading behavior of sea urchin from coffee waste and starfish indicated that our method could be applicable for examining an attraction/repellant activity of the chemicals. The results from indicate that 1 hr is enough to distinguish repellant/attracting activities indicated in a small tank.

2. Identification of materials avoided by sea urchins

To find chemicals with a repelling efficacy toward sea urchin, various materials that are easily obtained from natural resources with a lower cost and less harmful were tested. These include coffee been waste that is almost freely available and can be recycled. Starfish is another candidate destructing marine forest regarded as a target for removal. There has been concerned about a strategy for recycling many starfish collected from their relief project. A possible use of starfish was tested for its repelling activity toward sea urchins were tested in this study.

Behavioral response of sea urchin against diverse materials were carried out consecutively with small and large size tanks and Y-shape tanks. For quantitative comparison, distribution of sea urchins upon exposure to respective cues in small were analyzed for counting number of sea urchins distributing in 2 zones including a center (inner area) zone around the chemical cues and distant the surrounding zone (edge area) as shown in Figure 5. Small tank experiments were carried out with 10 sea urchins and repeated at least five times. Out of 5 replicate trials with 10 sea urchin for each experiment, while almost no sea urchins (only average value 0.02 out of 50 sea urchins) were found in the center regions upon exposure to agars containing coffee

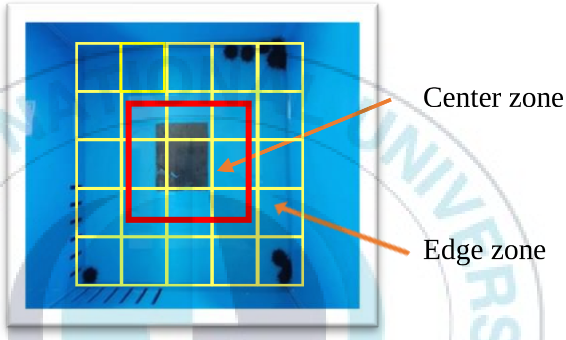

Figure 5: Photograph showing a distribution of sea urchins upon exposure to an agar containing coffee bean waste located at the center of the small size tank (1 m x 1 m). Number of sea urchins found in the center (red square) and the surrounding region (edge zone) were counted.

been waste and starfish crumb, respectively, but most of them were found at the edges which are distant from the testing materials (Figure 6). In contrast, a higher proportion of sea urchins were found from the center around an agar containing seaweed. The results showing an attraction toward a sea weed but repelling from coffee been waste and starfish supports a hypothesis that coffee bean waste and starfish crumb could be acted as undesirable cues for sea urchin.

The results were further confirmed from the similar set of experiment in a large tank carried out with 40 sea urchins. The results were further analyzed by more detailed dissection of their distribution patterns (Figure 7) as determined by an attraction towards materials toward a center zone with cues and a repelling activity toward outer zone quite distance from cues as defined. Results are shown in 2D graph in account with 2 designated zones and 3D graph bars (Figure 8) further elaborated distribution pattern in the tank in different hypothetical cells. Out of 40 sea urchins, an attraction response was identified by 16 sea urchins reaching to agar containing sea weed as depicted at zone 'A' of Figure 8 A and zone '3C' of Figure 8 B.

Sea urchin showed an evading response against coffee waste as per the bar 'E' in Figure 8 C indicating almost 38 sea urchin out of 40 sea urchin were stayed away from tank middle zone as a negative chemotaxis response towards cues. It was also noticed that only three sea urchins out of 40 sea urchin were found in the middle region against starfish. Then, about 37 sea urchins were found from the corners which is away from the stimulant. A much higher proportion of sea urchins was found from the center directly exposed to an agar containing

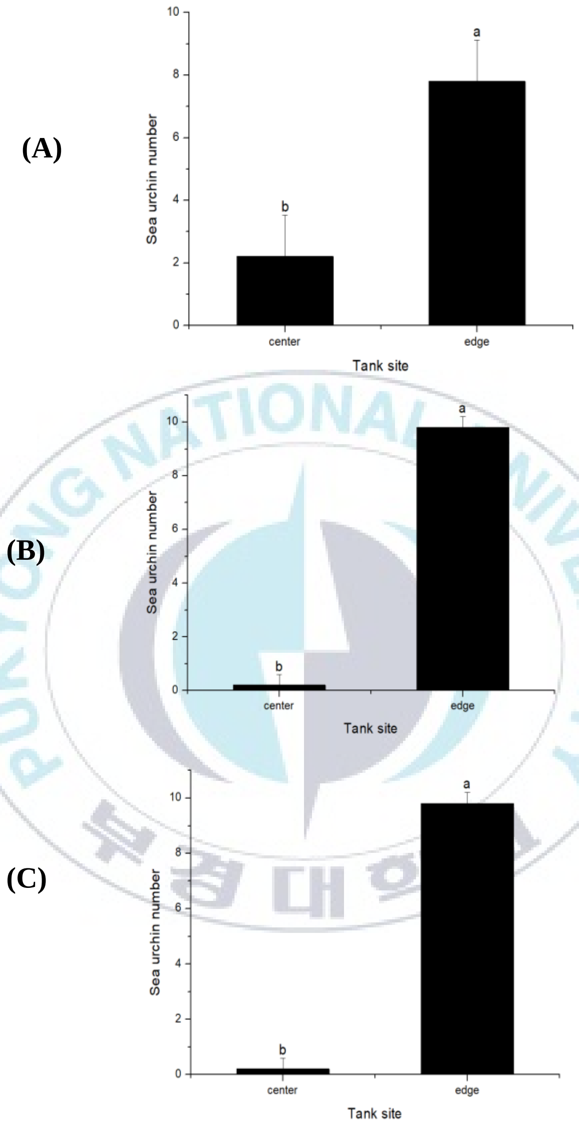

Figure 6: Number of sea urchins found in the center zone of tank and edge zone of tank of the small tank upon exposure to agars containing seaweed (A), coffee bean waste (B) and starfish crumb (C).

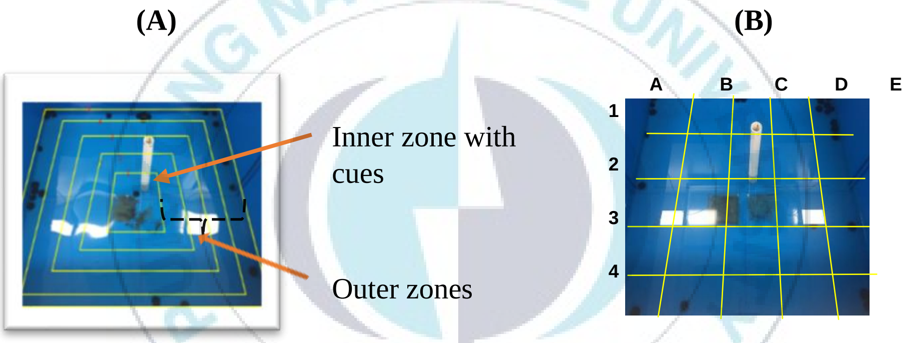

Figure 7 Photographs showing distribution of sea urchins upon exposure to an agar containing coffee bean waste located at the center of the large tank (2 m x 2m). Number of sea urchins found in the designated areas were counted.

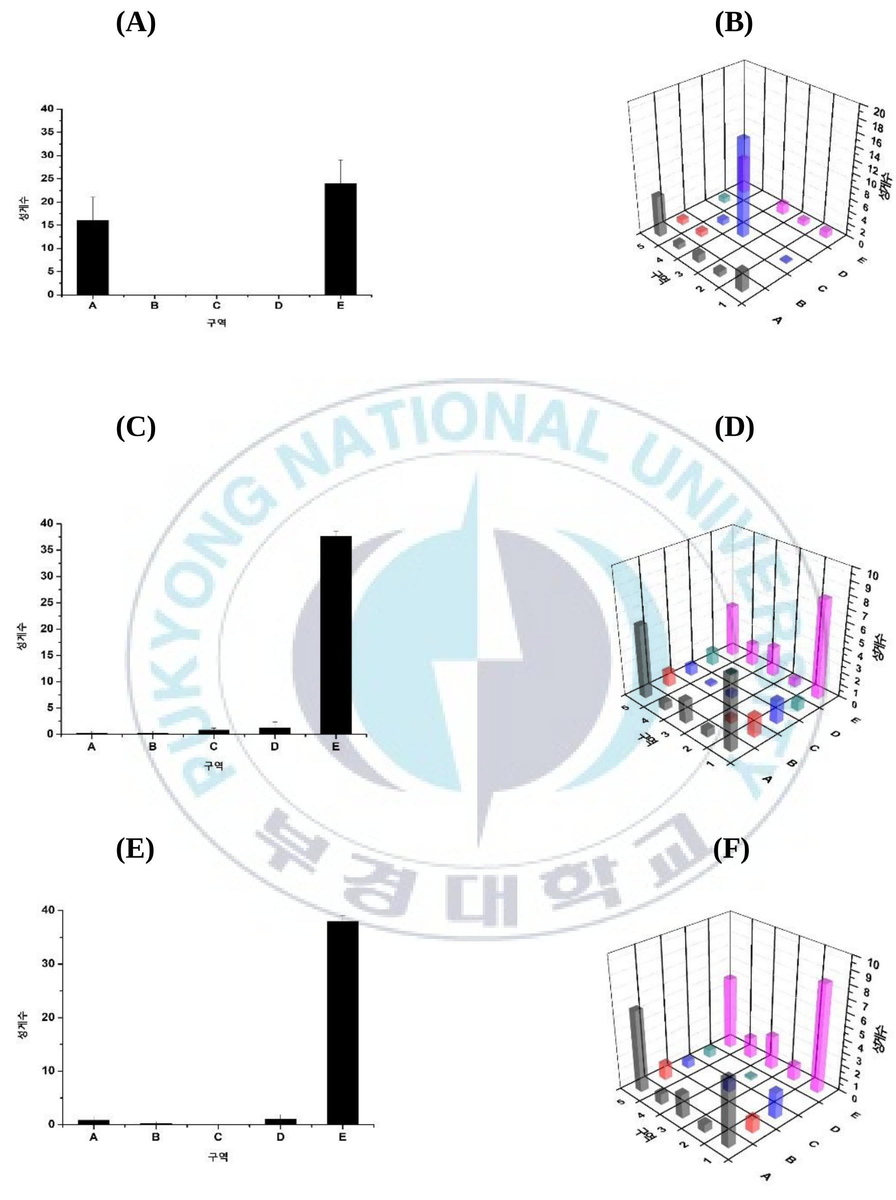

Figure 8: Number of sea urchin distributed along the large tank upon exposure to agars containing seaweed (A & B), coffee bean waste (C & D), and starfish crumb (E & F) for 1 day. Areas were designated as in Fig. 7 A and B, respectively.

kelp but not for agars containing starfish and coffee been waste. The results further confirmed an attracting activity of seaweed but repelling activity of coffee bean waste and starfish against sea urchin.

3. Analysis of rock bream movements

3.1 Behavioral monitoring system of rock bream movement

Video-based tracking system was also tested for its applicability for monitoring the movement of fish. This was tested with rock bream, one of the main aquaculture species in Korea, in different, rectangular and Y-shapes of tanks. Rock breams were starved for 24 hours before the behavioural analysis and therefore, one tank out of two stock tanks fed every next day in order to make ensure providing starved fish every day (24 hr lagging period). Air stones were removed during video recording and tank was aerated about 10 min time period in between control trial and treatment trial to provide similar oxygen condition. Experiment conditions were maintained more or less similarly in all of the trials to avoid any possible discrepancy or biasness.

Illumination on the experimental tank was optimized considering two factors. First, considered the fish not to be being stressed or over stimulated by light. Accordingly, fish were already acclimatized with a similar light condition and light intensity were optimally reduced by increasing the sensitivity of camera and using EthoVision software adjustments. LED (light emitting diode) light were compatible with the camera and fluorescent light was suggested that not suitable due to its blinking nature. Even though the software was capable of supporting with detection of fish from various backgrounds white colour was used for tank inside to satisfy with a low light intensity requirement enabling detection of rock bream fish easily because they have dark stripes on whitish to greyish body. The tank wall height over the 15 inches of water column of the rectangular tank mostly was useful to blocking the visual interaction with the external environment of the tank by fish. The tank was kept in a separated room to minimize undesirable sounds, visual or light disturbances that can directly affect fish behavioural movements.

Tested materials were prepared as a pellet by using a mixture of cellulose and corn flour. Upon testing the ability to form a pellet, 1:9 ratio of corn flour: cellulose was seemed to be the best in forming a pellet that is strong enough to form a pellet and not freely dissolved in water system. Therefore, all the material was prepared by absorbing the water containing the tested material onto a cellulose mixture pellet. To find an optimum amount of the tested materials, pellets containing various amounts of tested materials of 0.02 g, 0.2 g, and 2 g were prepared. Behavioural response of fish was first tested in a rectangular tank where the cellulose pellet was incorporated into a container with testing materials. A plastic container with penetrating slits over the top surface of the lid were employed to detect the signalling from the olfactory but not from visual signalling. Thus, in order to provide gradual diffusion of chemical cues for extended duration of time, cellulose pellets were visually concealed by placing in a container. Further enclosing the material in tea bags was helpful to detect the response against chemical cues.

Preference to the materials were analysed to examine whether there is any bias to the zone in a tank, control experiment was carried out with an empty container or with a container with a similar amount of cellulose pellet free of materials. Treated zone (hypothetical zone) was designed to place containers with materials which are incorporated to cellulose pellets, and opposite zone (distance between two containers ~80 cm) was used to place a container with cellulose pellet without any material.

To test the applicability of the system for identifying a degree of positive or negative attraction for chemical cues, randomly selected 3 individual fish were acclimatized for 15 min in a rectangular tank (Figure 9). Each experimental trial was recorded for 15 min just after the control experiment done with empty containers in both zones followed by another recording for 15 min upon addition of the testing materials (Figure 3). From the 15 min duration of videos which were taken upon exposure to chemicals in rectangular tank 5 min (3^{rd} to 8^{th} min) of period was selected for determining the degree of zones preference in the presence of cues in one or two designated zone on the tank. Response for the materials were analysed by tracking movement during the initial 2-3 min time slot just after exposure to cues as fishes showing frequent moving between containers kept in the two zones (control zone and treatment zone). Therefore, duration and frequency of

Figure 9: Rock bream searching the source of odourent pecking on ground and awaiting around the container with material cue. Fish are showing innate food searchin behaviours even in experimental tank and frequency of such habits were inclease with chemicals.

fish detected in the designated zones during 5 min was selected for comparing differences in preference.

The results from the control experiment showed little bias (Figure 10) toward a specific zone as defined in Figure 2 from the control trial. Each material was tested alone and together in combination for comparing the level of attraction. For experiments carried out with Y-shape tank, all trials were done with two cues at a time in the absence of control trial and one trial were done with rotating 2 cues position to understand the attraction degree and zone bias effects etc. Preference toward chemicals were analysed by duration and frequency of fish detected from the zones (Figure 2) around the chemicals. Difference in response of the fish for different cues was also recognized by means of activity parameters including its velocity, and distance moved using a video tracking system.

Y-shape tank experiment result was also recorded for 15 minutes and captured images were analysed for their preference toward different chemical cues in two different structurally designed tank arms of the tank. Preference for chemical cues and degree of preference were suggest to be interpreted by using heat map, duration, frequency and other quantitative and qualitative fish movement behaviour parameters. Qualitative behaviours such as quick rotations and pecking on grounds and corners frequently and spatial food search movements were observed by observer just for understanding the activities with the exposure to different chemical cues using video clips.

3.2 Identification of substances attracting rock breams

To compare the degree of attraction towards materials from different bait candidates such as rock worms, commercial bait worms, kill were tested with sea urchin internal tissues. Fish feed and powdered barley were used as positive and negative controls respectively. It was also tested to identify whether sea urchin tissues have the bait properties as rock bream. Preference toward the chemicals were analyzed by frequency and duration data acquired by tracking rock bream movement by EthoVision video tracking software. Preference to various chemical cues were analyzed by their duration and frequency of rock bream found around the zone containing the materials. Out of materials tested including sea urchin, rock worm, bait worms, feed, krill, and barley, commercial feed showed a significant difference from its preference to barley kernel (Figures 11 and 12). Further preference in duration zone (%) and preference in frequency (%) of krill and sea urchin are higher than the rock worm in this trial. Heat maps were generated for tracing the movements patterns responding to different materials cues as shown in Figure 13. In each heat map zone two (Z2) is considered to be the zone the given treatment in the treatment trial and z2 of the fish feed trials showed higher attraction towards the treated zone by the red in color with high intensity. Zone 2 in the presence of rock worm, sea urchin and krill also denser in color (hue and saturation) than that of the opposite control zone of each tests. The results from rectangular tank seems to provide useful information for its

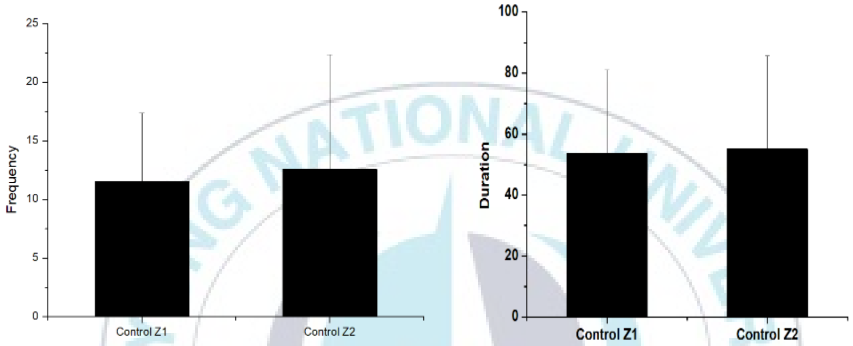

Figure 10: Frequency and duration of rock bream detected in the zone 1 and zone 2 in the control experiment carried out with an empty container at both zones.

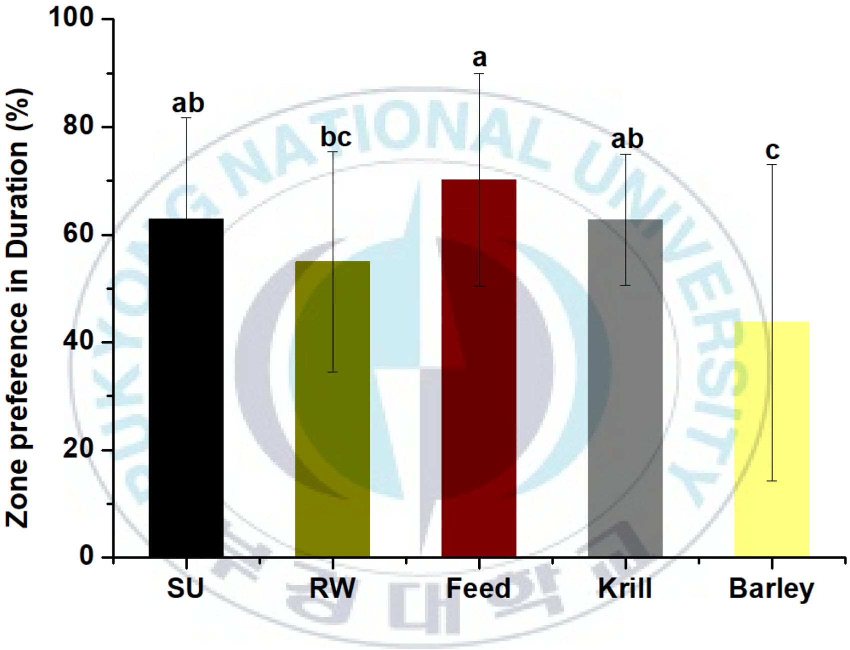

Figure 11: Behavioral response of rock bream upon addition of pellets containing tested materials. Zone preference was determined by preference to the zones containing sea urchin (SU), rock worm (RW), commercial feed (Feed), krill bait (Krill) and barley kernel (Barley).

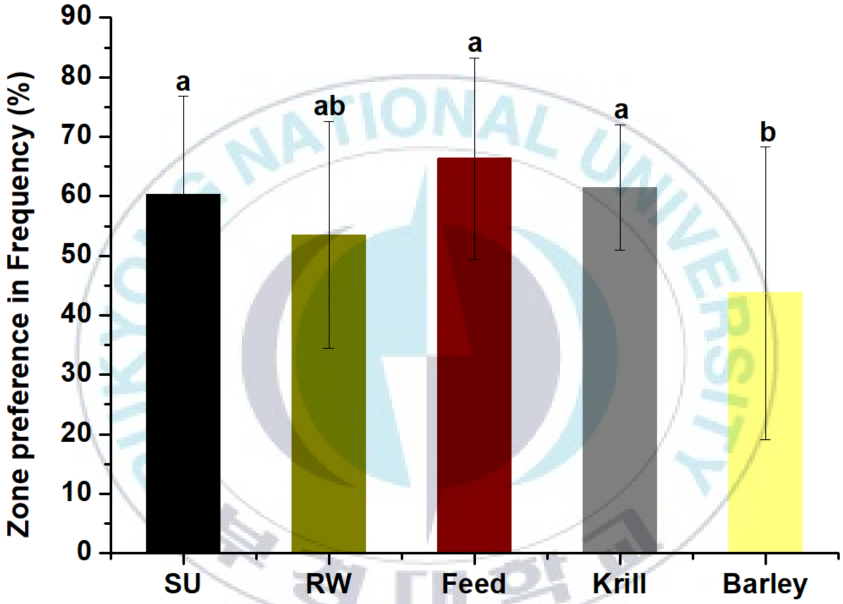

Figure 12: Behavioral response of rock bream upon addition of pellets containing tested materials. Zone preference was determined by frequency (%) of rock bream detected in the zones with pellets containing sea urchin (SU), rock worm (RW), commercial feed (Feed), krill bait (Krill) and barley kernel

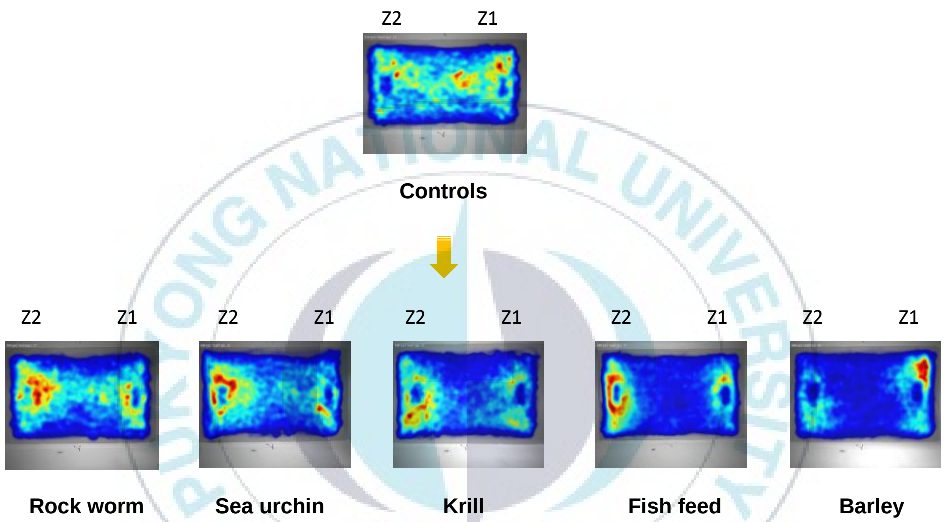

Figure 13: Heat maps acquired from fish movement recording in 5 min duration against different cues, 0.2g of freeze-dried materials/1g cellulose pellets. Right side of the heat map is the zone containing the testing material and left side is the control zone.

(A)

(B)

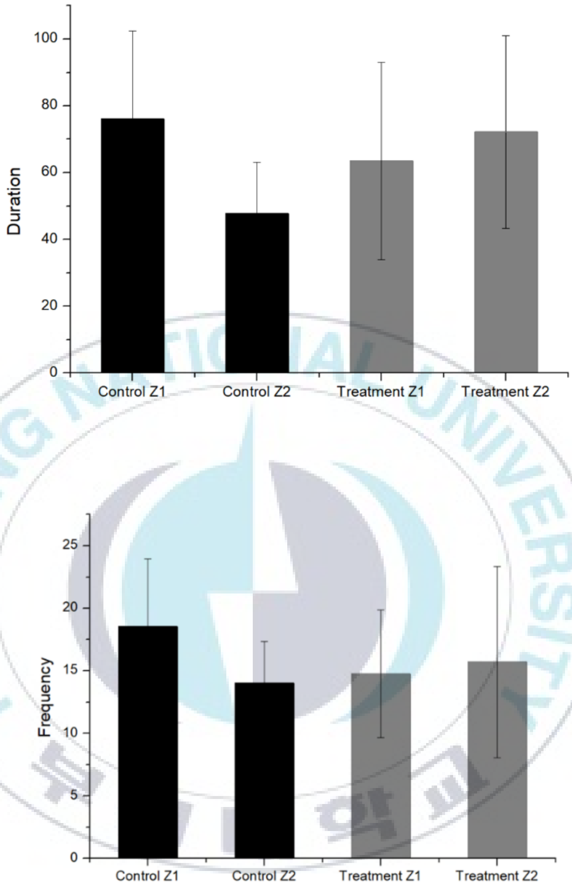

Figure 14. Duration (A) and frequency (B) of fishes detected from the zones around pellets containing rock worm (Z1) and sea urchin (Z2). Control experiment was carried out with an empty container in both zones

applicability in automated aquaculture systems to monitor the aquatic animals through the behavioral response window.

According to the graphs number 11 to 12 in the result section it would suggest clearly that rock bream juveniles was attracted to the zone treated with fish feed, sea urchin, rock worm, and krill as per the higher comparative percent values showed for duration, and frequency. Further, sea urchin, rock worm, and krill values lie in between positive fish feed treatment and barley negative treatment. The reason we choose fish feed as a positive cue is because they already fed with same feed and fish may be much familiar with that smell.

It would also be better to mention here that some fish combinations were unsuccessful for getting data due to begin to fighting each other specially with the exposure to cues. It may be a sort of innate competitive behaviour. Then the zone biasness from the beginning also would be resulted a complex situation when consider zonal factor point of view. Further sometimes when they get stimulated to cues at the zone with material moved other side and was keep pecking on the other container and other corners predicted as sign of finding visual cue but leads to give deviated results. That may be an innate behaviour reflecting from their natural habitat, once stimulated trying to find food from rocks spaces, caves, ditches/trenches, bushes, corals or holes etc. So, it is important to consider those complex behaviours before trying to compare frequency and duration data against the different materials.

However, due to a miner affect or in the absence of other clues they showed the duration and frequency data as an attraction or avoidance behavior. The method of preparation of test cues may plays remarkable effect on their sensitivity and response. Probably food cues with fermented tissues and combination and composition of different chemicals may mimic undesirable odors for fish. It also better to highly consider on the texture and quality of test sample in the chemical composition, concentration and diffusion rate (from pellets) point of view.

Considering the complexity of fish's movement behaviors against the simple rectangular structure of the tank further analysis of the comparison were proposed to repeated in a different facility. Thereby, large size Y tank were used to design a simple test to inter compare results of duration and frequency data of rock bream movement and suggested to showed as a response of attraction movement. Analysis was also carried out in the presence of rock worm and sea urchin cues simultaneously in 2 different zone of a treatment trials of a test (Figure 14). The result showed a slightly higher degree of preference toward a sea urchin extract as compared to that of the rock worm.
Even though the duration and frequency values are not always significantly difference at the cue source zone when compared to control experiment within a considered time slot, the behavior should not be interpreted as the general tendency to indiscriminately avoid the odor of any familiar or unfamiliar cues. Perhaps the positive stimulation and attraction would appear as increase in activity level by means of mobility, distance moved, velocity or any other swimming parameter due to zone biasness, visual cues and an unknown factor or behavioral complexity.

Barley usually use as a visual attractant during fishing activities and also use as ingredient in feed manufacturing. The velocity and distance moved parameters were generally increased with exposure to sea urchin, rock worm, krill, and fish feed as compared to control experiments. However, some of these parameters are inter related. For instance, distance moved deemed to be inversely proportional to the duration parameter in zones. Distance moved theoretically may proportional to the velocity.

In order to confirm the result showing an attraction and compare the efficacy between materials, Y-maize experiments were carried out with two materials positioned at both ends, respectively, in the tank. Movements of 5 fishes acclimated were traced upon exposure to materials followed by heat map analysis (Figure 15). Comparison of the heat map signals indicated that fish feed showed the strongest preference as compared to that of the sea urchin as consistent with the previous result from a rectangular tank experiment. Barley showed the lowest response among the tested materials. This was followed by krill showing a slightly stronger preference than sea urchin. A bait worm showed an efficacy that is a more or less similar to that of sea urchin though rock worm showed a slightly lower attraction efficacy.

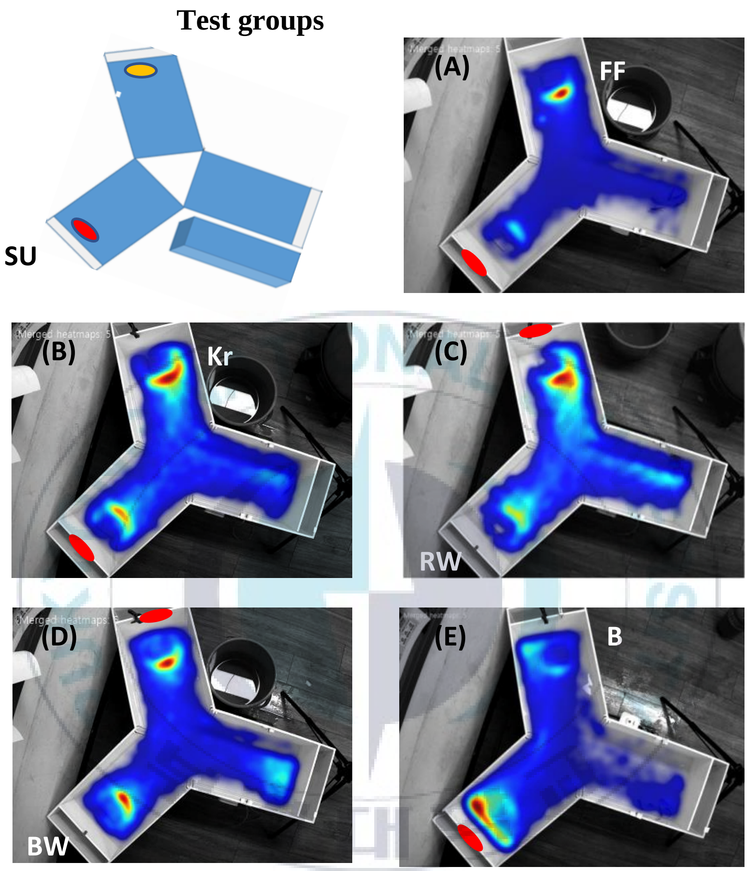

Figure 15: Heat maps acquired from fish movement in Y-maize test carried out with 5 rock breams upon exposure of two materials as depicted. Position of sea urchin located at one side of the arm is marked as a red circle and other testing materials including feed-FF (A), krill-Kr (B), rock worm-RW (C), bait worm-BW (D), and barley-B (E) were located at the end of the other arm.

IV. Discussion

1. Sea urchin behavioral response

Marine forest is important for environment as a primary producer providing habitats for fish. To preserve such an ecologically important forest, it is critical to reduce organisms destroying sea grass beds in the oceanic environment. Sea urchin has been known to be one of the organisms devastating the implanted and natural sea grass forest. Since the physical removal of sea urchin by scuba divers is a time-consuming process, to repel those invading species from marine forest has been sea grass beds would be considered as an alternative and solution.

Chemotaxis is a natural phenomenon that cells or organisms guide their movements according to a chemical gradient in their environment. Chemosensory systems response in predator avoidance (Wirtz & Duarte 2012) has been shown for several sea urchin species. The green sea urchin, *Strongylocentrotus droebachiensis*, showed instantaneous behavioral responses to waterborne chemosensory cues from two durophagous predators (N.T. Hagen et al., 2002). However, asteroids, ophiourids and echinoids, exhibit defensive behavioral responses in the presence of chemical stimuli from predators or injured conspecifics (Rodriguez and Ojeda 1998; Rosenberg and Selander 2000). To develop a chemotactic response-based strategy to prevent approaching of pirate animals to the marine forest, it is critical to have information about materials repelling the target organisms. This can be facilitated by developing a behavioral analysis system for monitoring sea urchin movement against chemical cues. Various materials tested should be obtained in a large amount with a cheaper price to be applicable to the field. These include coffee bean waste that is freely available in a large amount and starfish of which its use can be beneficial to pirate animal removal project. Sea urchin movement was analyzed in the presence of materials including seaweed, starfish, and coffee waste. In the presence of undesirable materials sea urchin seems to be perform diverse patterns of responses including a covering behavior but not in our experimental condition tested. Analysis of the sea urchin distributed against the testing materials indicated a clear attraction toward the kelp but a repelling activities against coffee bean waste and starfish crumb.

Several studies suggest that green sea urchin *S. droebachiensis* responds to chemosensory (Mauzey et al. 1968; Mann et al. 1984; Vadas et al. 1986; Scheibling and Hamm 1991). Conflicting evidence suggests that *S. droebachiensis* does not respond to tactile stimuli from the echinivorous seastar *Crossaster papposus*, but does responded to the presumably non-echinivorous seastar *Leptasterias polaris* (Legault and Himmelman 1993). These may be important for designing, managing and interpreting results taken from experiment such as movement behavioral studies. Interestingly, we observed during this experiment that some of sea urchins were moving along edges or corners while some them become dormant enabling us to predict as escaping, camouflage, and defensive behaviors in the presence of predator starfish or industrial coffee bean waste suggest may be due to unfavorable cues or mimic alarm signals. Manual removal or killing of invading sea urchins on the field is a risky and time-consuming operation (Eklöf, J. S., 2008) and these natural/industrial products based chemical cues would be helpful to implement removal operations of different size of sea urchins from biologically sensitive beds such as implanted seaweeds. Therefore, we suggest to consider for applying of both attractive (seaweed), and repulsive (starfish/coffee waste) cues simultaneously to engender chemical gradient in between distance locations to divert evading sea urchin movement pattern along such a chemical gradient. This suggests to use seaweed as attractant substance, and coffee bean waste and starfish as repellent substances for controlling relevant sea urchins.

2. Rock bream behavioral response

Food search behavior in fish responding to chemical compounds is important for development of fish baits (lure). Sense of sight, smell and taste in fish leads to stimulate the response for the baits. A fish's highly tuned sense of smell is mainly based on a set of well-developed nostrils located on the snout. Generally, it is accepted that certain chemical compounds, such as amino acids, peptides, fatty acids, bile acids in combination with other cues, could mimic a food source, feeding activity or predation and drive fish away from adjacent the waters or attract accordingly. This study focused on fish behavioural analysis of fish responding to the bait-like chemical cues. It is important to understand the physiology associated with a response to stimuli eliciting an attracting and repelling response in fish to develop a chemotactic-based strategy to control the fish population. Thus, could be approached by examining the behaviour aspect such as swimming speed and movement patterns. Other key objectives of this study are to provide a basis for developing a quantitative monitoring system that will be useful for an automation of the aquaculture industry.

Rock bream *Oplegnathus fasciatus* is an economically important fish species also known as striped beakfish and barred knifejaw fish. This study is focused on understanding its chemotaxis-based physiology associated with its catching. Experiments were designed to find chemical cues and materials eliciting an attracting response by rock bream in different shape tanks. Y maize choice tank was also employed to compare the level of attraction by chemical cues and provided a detailed interpretation on attraction and repulsion responses.
Rock bream behavioral experiments were carried out to examine attraction/repulsion responses of the fish analyzed by automated video tracking software. A series of different preliminary tests were carried out to find an optimum experimental condition and to compare the efficacy of the materials used as commercial baits including rock worm and krill and applicability of the sea urchin. Rock bream showed attractant motivational response for sea urchin, rock worm, krill, and commercial fish feeds in contrast to barley showing little preference.

The stud can be extended to plume-search as gradient search or upstream search, and bait search for visualizing source cue associated with an innate behaviour in fish. During the experiment we also observed food searching behaviours such as searching movements of food with frequent rotations, pecking or digging at bottom or small spots, following other fish competitively, quick rotations, stop swimming and waiting followed by rotations and some other unidentified different swimming patters upon exposure to test materials. While the surveillance and escaping movements was observed from rock bream juvenile in the presence of small amount of aesthetic agent, a clear coughing and evading movements followed by a gathering in the opposite zone, was observed upon higher dose treatment, and employed as an e avoiding behaviour. Behavioural analysis under such conditions will provide a valuable information needed to develop an automated aquaculture monitoring system.

The rock bream behavioral experiment was carried out to propose a system to analyze attraction/ repulsion behavioral responses and substances that attract/ repel fish. Accordingly, fish were exposed to predetermined selected cues in different tank set ups and behavior analysis done with using automated video tracking software. Basically, we did series of different preliminary test to determine an experimental set up and cues. The experiments of attraction/ repulsion on sea urchin and rock worm cues tested repeatedly basically with 0.2g and 1g fresh tissues. Then, tested with 0.02g, 0.2g, and 1g freeze dried tissue samples seeking a clear conclusion. However, some other sources of cues such as fish feed, krill, barley were tested with an idea of comparing further for attraction/ repulsion data each other to extrapolate the feasibility of identifying behavioral response of fish through this tracking model and to understand up to what extend our attraction/ repulsion outcomes would be confident.

Rock worm and krill are common baits specimen species and sea urchin is a food component of rock bream in their natural habitat. Krill meal also a feed ingredient (Suresh et al., 2011). Considering the suitable concentration in pellets for rock worm and sea urchin during preliminary studies 0.2g of freeze dried materials were used in the overall comparison test. The odors from food substances could be attain the threshold at a certain concentration, and such components would probably be held at attractive capacity. However, rock bream fish showed attractant motivational response for sea urchin, rock worm, krill, and commercial fish feeds. In contrast none zone preference to control trails plus against barley seeds. This would clearly explain the behavioral differences exhibited towards the materials.

Plume-search as gradient search or upstream search, and bait search for visualizing source cue would be associated as an innate behaviour in fish. During the experiment we also observed food searching behaviours such as searching movements of food with frequent rotations, pecking or digging at bottom or small spots, following other fish competitively, quick rotations, stop swimming and waiting followed by rotations and some other unidentified different swimming patters when exposed to food born chemical cues of test materials. In contrast surveillance and escaping movements was observed from rock bream juvenile in the presence of 2 mL 2pheynoxyethiline in 1g of cellulose pellets. When they directly expose to 5 mL of 2pheynoxyethiline by injecting through a plastic tube from the same treatment zone side, coughing and evading movements was showed clearly. Then they were mainly gathered in the opposite zone to the treated zone. Test with 2pheynoxyethiline was done during preliminary studies to see what sort of the avoiding behaviour would be shown within our experimental facilities.

This study also focused on fish behaviour for the bait cues, and such a behavioural response probably may reflect as natural pray-search behaviour in a feeding situation (Rune Vabø, et al., 2004). Generally, in connection with the response exhibited by the fish, the tendency to attract to food sources born chemical flumes could have the function of stimulation for food finding and feeding. Detection of food, mates, and avoidance of predator in animals guided with orientation strategies (Atema et al., 1988) and visual, sound, and, odour may be used to follow the stimuli. Simple aspect of the behaviour such as swimming speed can be readily recorded (Løkkeborg S. et al. 2000, 2002) and more complicated patterns of movement could not easily be analysed and describe.

Due to diffusion of the chemicals through the entire experimental tank in a short period, a sensory adaptation of the fish, saturation of olfactory surfaces, a habituation to the stimulation, a resumed exploratory activity considering the responses shown by the tested fish in relation to the different chemical situation, it appears that fish behavior is basically dissimilar to the odors of different materials. Therefore, the degree of attraction and repellence proposed to determine after considering many more qualitative or quantitative behavior as much as possible.

One of the aim of fish behaviour research is to suggest a method and cues for identifying the suitable stimulator material which attract fish for the capture purpose. Thereby, when learning deeply the first step will be the study in sensory physiology for input information (Arimoto, T. 1997). Underwater visibility is very poor in many fishes giving them good sense that the smell is vital in allowing fish to find food. The olfaction may be a good criterion for finding hidden prays and food from long distances. When reach the high degree of olfaction fish may be expecting visual cues of food source to feed on quickly.

Usually fish can also sense many of the chemicals we add to baits to make them more appealing. If a new food with a unique smell is introduced to a fishery and the fish have learned that it's a food, they will follow that smell to find the source. Inevitably, sea urchin smell may have they already being innately identified as a sound food odorant. But this can trigger food finding or other kind of behaviours in some of other fish.

The attraction effect can be expected through the artificial stimuli such as bait if the stimulation was properly done in the best intensity against the ambient environment, and the directional movement as the convergent activity toward the stimuli source will be released accordingly (Arimoto, T. 1997). However, considering all such information including test on baits for fishing of the rock bream, identification of attraction/repulsion response could be assessed through studying their movement behaviors to test chemicals.

Food search behavior in fish that elicit in response to chemical compounds is important factor for development of fish baits (lure) (Sevein et. Al, 2013). For instance, the choice of type of natural bait might affect the probability that fish are hooked in deep-body locations (Butcher, P.A., Broadhurst, et al. 2006). Sense of sight, the sense of smell and taste in the fish leads to stimulate fish to be baited and trap as a result of the motion, color, shape and odor of the bait. For instance, bait type may affect fish attraction owing to catch from fishing trap and hook and line and such a scientific information specially on reef fish would widely be valuable (Umar Tangke, et al. 2018).

The sense of sight, sense of smell and taste in the fish leads to stimulate fish as a result of the motion, color, shape and odor of the bait. For instance, bait type may affect fish attraction owing to have a good catch from fishing trap and hook and line and such a scientific information specially on reef fish would widely be valuable (Umar Tangke[1], et al. 2018). A fish's highly tuned sense of smell is mainly based on a set of well-developed nostrils located on the snout. Generally, it is accepted that certain chemical compounds, such as amino acids, peptides, fatty acids, bile acids in combination with other cues, could mimic a food source, feeding activity or predation and drive fish away from adjacent the waters or attract accordingly. Most of our test cues would be containing bunch of stimulatory cues. But certain chemicals and compositions of chemicals may be characteristics and helpful to identified by fish in details. Those things may be associated with innate behaviors, genetic information, previous experience, social and environmental affect etc.

According to Braubach, O. et al, 2011, when exposed to food-associated odorants (i.e., amino acids, and possibly nucleotides), zebrafish said to be exhibit appetitive olfactory behaviors, such as an increased rate of swimming, for instance, distance traveled with frequent turns (>90◦). In general, olfactory discrimination capabilities of fish can be predicted by chemotropic of amino acids, peptides and fatty acids on the olfactory bulb. Attractions and repulsion elicit the locomotive responses in fish in directional ways (Arimoto, T. 1997). Avoidance or attraction depend on receptor activation, exposure history of the species to be shown adaptive response, concentration gradient from chemical plume to be orient in proper direction (Little and Brewer 2001).

In the case of understanding and analysing fish movement behaviours we mostly were referred to previous studies. There are several studies which have demonstrated chemically stimulated rheotaxis in fish (Fernö *et al*., 1986; Løkkeborg, 1998). In unpolluted water fish avoid from detrimental chemicals they detect before become toxic. Restless behaviours and none directional movement encompasses as alarm response. Avoidance response of fishes associated with refusal of to enter freely of treated water zone (Robert C. et al.,1967). Fish may move out as a repellent response for undesirable cues or enter freely when chemical act as attractant to an area of treated water (Robert C. et al.,1967). Due to the variable level of information available to the fish, different prey-search stages are probably guided by different behavioral strategies (Løkkeborg and Fernö, 1999). It is unlikely to be the smell itself that a fish is attracted to, more the connection between the smell indicating the presence of food. The certain copper compounds, such as copper sulfate and copper acetate, in combination with other ingredients, could mimic a dead shark and drive live sharks away from human beings in the water. (www.supportoursharks.com). Due to consistent sensitivity to numerous contaminants swimming responses have been used in automated biomonitoring systems (Miller et al. 1982; Smith and Bailey 1988). Avoidance behavior would be a combination of many behaviors that may culminate in a single endpoint (Kane et al. 2005).

Spatial search pattern in search of food source by fish could influenced by environmental factors of the ocean such as temperature, bottom topography, social interactions, day-night differences, predation, and other olfactory stimuli (Rune Vabø, et al., 2004). Rune Vabø, et al. studied to evaluate the ability of different search strategies in finding odor plumes and bait in order to improve the understanding of fish movement dynamics towards baited gear, and also described that gradients of olfactory cues can be traced from often long distances. It has been proposed in blue crabs and that animals can localize the olfactory source by up-current movement combined with spatial sampling to resolve the position in the plume (Webster and Weissburg, 2001).

When a single excitatory extract is analyzed and tested behaviorally in more than one species, the different test species may respond to different substances in the same extract. A study of Carr, et al., with different fish species, the major stimulants in the squid extract had been different. The stimulatory capacity of most extracts is due to a mixture of substances acting in concert rather than to a single dominant substance. Mixtures were necessary to yield synthetic stimulants with stimulatory capacities similar to those of the natural extracts. (Carr, et al, 1986). In the case of each extracts described here, the principal stimulants of feeding behavior are may be common metabolites of low molecular weight that include amino acids, quaternary ammonium compounds, nucleosides and nucleotides, and organic acids (Carr, W. E. S. et al.,1986). Such information could be possible to investigate using composition analysis and fish physiology studies. In some species of crustaceans, there are examples in which particular extracts or biological fluids contain attractants or feeding stimulants that are high-molecular-weight substances, such as proteins (Can. and Gurin, 1975; Zimmer-Faust et al., 1984).

Chemicals such as amino acids are found in food items and some leak into the water, giving a scent trail for the fish to follow. In fact, natural foods like worms and snails, which have no real smell to us, actually ‘leak’ low level amino acids into the water which fish can locate. Salmonids (salmon and trout) have a limited smelling range but cyprinids (carp, roach, barbel, bream etc) have greater sensitivity to amino acids. They are far more likely to be stimulated by baits and additives with natural amines including amino acids. Recent research on amino acids and their use as attractants has shown that, for most species, there is no single amino acid that gives the best result.

In most investigations to date, generalist consumers have been studied in flumes or other lab settings to determine how they navigate to body fluids of prey or to more water-soluble compounds such as peptides (Hay, M. E. 2009). The effects of these metabolites on feeding as opposed to attraction is less well investigated. In contrast, most studies on specialist consumers have focused on secondary metabolites (often lipid-soluble terpenoids) stimulating feeding once prey have been contacted.

Numerous aquatic organisms show alarm responses to body fluids of conspecifics (reviewed by Smith 1998, and by Ferrari et al. 2010). Therefore, it is very important to handle fish with a great care. In most investigations to date, generalist consumers have been studied in flumes or other lab settings to determine how they navigate to body fluids of prey or to more water-soluble compounds such as peptides (Hay, M. E. 2009). The effects of these metabolites on feeding as opposed to attraction is less well investigated. In contrast, most studies on specialist consumers have focused on secondary metabolites (often lipid-soluble terpenoids) stimulating feeding once prey have been contacted. Sea urchin also may have range of cues such as metabolites, peptides, lipid soluble or lipid base substance that can specifically be identified by the rock bream fish with regards to their predator -prey relationship. Studies on physiology of fish and chemical composition analysis and physiology of sea urchin would be necessary to describe further to explain such a consequence.

3. Olfaction and chemical stimulation in fish

Two pair of paired nostril structure including olfactory glands in the snout of the fish facilitated the olfaction in fish. Olfaction detect from long distance have a functional mechanism which enables them to locate odor sources. Fish chemo-reception sensory take place in olfactory rosette and mouth, barbs, lips, fins (taste buds), would be vital stimuli for olfaction and taste sensation (Pitcher, T.J.1993). This stimulus is responsible for temporally, and spatial disturbance in the natural environment which can create the unstable condition in fish forcing them the secondary response for approach or avoidance.

Natural or synthetic feeding attractants included stimulatory compounds that elicit the food search behavior in target fish species, and the development of alternative baits to replace tradition fish baits is seems to be not an easy task (Sevein et al. 2013). Even though vision is important in pray capture, initial stimulation could be occurred through chemical clues. Chemical compounds that elicit food search behavior tend to be differ from species to species could applied for developing specific attractant in the manufacture of baits (Sevein et al. 2013). Understanding of the modalities how the fish detect, approach and locate a source of food odor which stimulate olfaction affect feeding, and baiting activities have been studied with some of previous researches.

Chemical stimulus does not have directional clues fish also use additional information to locate odor source as visual and acoustic signals (Sevein et al. 2013). Basically, crustaceans and fish used chemically stimulated rheotaxis to locate source of odor (Carton and Montgomery 2003; Lokkeborg et al. 2010). For instant, Squid extracts on various fish species had been studied, and robot (*Scopthalmus maximus*), rainbow trout and plaice (*Pluronectus platessa*) had shown different for feeding attractant (Adoron and Mackie 1978; Mackie 1982). Sablefish (*Anoplopoma fimbria*) had been capable of sense the squid baits at a distance of five hundred meters (lokkeborg et al. 1995). That means such a fish has some kind of functional mechanism for locating source of odor (Sevein et al. 2013). Chemical stimulus last for a long period and cod fish locate baits from 700m and locate bait 7h after (Sevein et al. 2013, Lokkeborg 1998) stimulus. Chemical stimulus generally disperses over long range of distance.

The response threshold of fish for total dissolved amino acids also had been drop down with food deprivation up to 3,000 times lower level (Pawson 1977).

Further, swimming speed, turning rate and the duration of response to bait odor had increased with food deprivation in sablefish (Lokkeborge et al. 1995; Stoner and Sturm 2004) emphasize the stimulation boost consequences with starvation. However, the temperature also can have significant effects on activity, swimming speed, feeding and other basic behaviors to be baiting in fishing (Stoner and Sturm 2004; Lokkeborge et al. 2010).

Low molecular weight substances such as amino acids, peptides, prostaglandins, steroids and bile acids stimulate feeding behavior in fish (Carr and Derby 1986; Hara 1992, 2011). Amino acids would be stimulating food search behavior either by single or in combination of them (Hara 2011). Fish said to be having very high ability to discriminate among different amino acids (Friedrich 2006). In regards to aspects of food search behavior, few studies have been carried out to determine response thresholds. Olfactory thresholds measured behaviorally in animals (Firestein, S. 2001). Gradient search suggested as alternative mechanism to locate source of odors (Webster and Weissburg 2001; Carton and Montgomery 2003). In addition to olfactory organs, lateral line may also be assisting in food-search. Thus, olfaction response of fish for different food born chemical cues expected to study by this research using vision-based monitoring system.

4. Factors affecting behavioral response

- Fish size and age
- Fish number used to track at a time
- General health status and activity level of specimen fish
- Cultured/grown environment and stock tank conditions
- Tracking tank- shape, color, water depth & volume and external or internal visual cues and objects movements
- Water qualities (salinity, temperature, DO, Ammonia, clarity)
- Water changing strategy during tracking
- Fishes feeding status
- Visual effect/ Social interactions/ acclimatizing/ previous experience of fish
- Innate, stereotypical or stress behaviors of fish
- Individual behaviors of fish
- Digging and pecking at the ground and pecking at corners up on stimulation and zone biasness
- Docile fish under certain conditions becoming likely to fight or bully others
- Other external disturbances
- Fish handling techniques
- Tracking time of the day (diurnal biological affect)
- Material type, material composition and attractive/ repulsive material inclusions & rates
- Amount of material use (weight) per trial

- Method of preparation of materials
- Material setting method and container properties
- Tracking set up design including light intensity
- Tracking plan and criteria (tracking time duration, slot and gaps between 2 tracking)
- Random errors due to latency time of tracking software and uncertainty of fish movements plus human errors
- Any other unidentified factors

5. Conclusion

Summing up, sea urchin can be repelled using, starfish, coffee waste and can be attracted with using sea weeds. Certain materials from sea urchin can be used as a bait for fishing and sea urchin material flavors deemed to be act as attractant can be formulated with rock bream feed. Studying the fish behavior movement trajectories would provide a new tool to analyze fish behavioral responses to use them as a bio indicator species and applying them in bioinformatics etc. Eventually, we propose to use behavioral responses of fish as parameters for developing a video tracking monitoring system coupled with bio-sensory and bio indicator system for introducing an innovative artificial intelligence (AI) based automated aquaculture monitoring system.

V. Abstract (Korean)

둥근성게 *Strongylocentrotus nudus* 와 돌돔 *Oplegnathus fasciatus* 의

행동 분석을 이용한 유인 및 기피물질 탐색

Siriya Kankanamage Tilan Chamara Duminda

부경대학교 대학원 수산생물학과

어류 행동 모니터링은 환경변화와 관련된 어류 생리의 다양한 측면의 이해에 중요하다. 비디오 트래킹을 이용한 수생동물의 움직임 분석 가능성을 탐색하기 위하여, 둥근 성게 *Strongylocentrotus nudus* (무척추동물 종)와 돌돔 *Oplegnathus fasciatus* (척추동물 종)을 대상으로 행동 반응을 유발하는 물질 투여 후 이동 패턴의 영상을 기록하였다. 성게의 이동은 해조류, 커피 찌꺼기 및 불가사리등의 물질 노출 시 행동 반응을 조사하였다. 대부분의 성게는 해조류에 대해서 높은 선호도를 나타내는 반면에, 커피 찌꺼기 및 말린 불가사리를 포함한 물질에 대해서는 회피 행동을 나타내었다. 이러한 결과는 바다의 보호구역에 커피 찌꺼기와 같은 폐기물을 재활용 투여함으로서 성게와 같은 해중림 파괴 생물을 효과적으로 제어 할 수 있음을 시사한다. 어류의 움직임을 분석하는 비디오 트래킹 시스템에서는 낚시용 미끼로 사용되는 물질과 시판 사료를 포함한 화학적 물질 중 돌돔을 유인하는 물질을 확인하였다. 각 화학적 물질의 성능 분석은 마쇄한 바위털갯지렁이, 청개비, 성게 조직, 크릴새우, 압맥, 어류 사료를 셀룰로오스와 녹말가루를 첨가한 고형물로 제조하여 분석하였다. 각 물질에 대한 선호도는 자동화된 비디오 트래킹 소프트웨어를 사용하여 각 물질 주위 일정 영역에서의 출현 빈도 및 시간을 이용하여 분석하였다. 성게가 바위털갯지렁이 보다 높은 유인 효과를 나타내는 것으로 보아 성게를 돌돔 낚시에 미끼로 사용이 가능할 것이다. 본 연구에서 성게와 어류의 행동을 분석하기 위해 사용된 비디오 트래킹 시스템은 양식 시설의 자동 모니터링 시스템 개발에도 이용 가능할 것이다.

References

Alós, J., Arlinghaus, R., Palmer, M., March, D., & Álvarez, I. 2009. The influence of type of natural bait on fish catches and hooking location in a mixed-species marine recreational fishery, with implications for management. Fisheries Research, 97(3), 270–277.

Arimoto, T., 1997. Fish behaviour approach for improving trawl gear selectivity. In Proceeding of the Regional Workshop on Responsible Fishing, Bangkok, Thailand, 24-27 June 1997, pp. 251-255.

Atema, J. 1980. Chemical senses, chemical signals and feeding behavior in fishes, pp. 57–101, *in* J.E. Bardach, J.J. Magnuson, R.C. May, and J.M. Reinhart (eds.). Fish Behavior and Its Use in the Capture and Culture of Fishes. International Center for Living Aquatic Resources Management, Manila, Philippines.

Blaser, R.; Gerlai, R., 2006. Behavioral phenotyping in zebrafish: Comparison of three behavioral quantification methods. Behav. Res. Methods, 38, 456–469.

Braubach, O.; Wyeth, R.; Murray, A.; Fine, A.; Roger, P.C., 2011. A Simple and Effective Method to Condition Olfactory Behaviors in Groups of Zebrafish. In Zebrafish Neurobehavioral Protocols; Kalueff, A.V., Ed.; Springer Science Business Media: Berlin/Heidelberg, Germany, Volume 51, pp. 85–97.

Butcher, P.A., Broadhurst, M.K., Brand, C.P., 2006. Mortality of sand whiting (*Sillago ciliata*) released by recreational anglers in an Australian estuary. ICES J. Mar. Sci. 63, 567–571.

C. Harrold, J. S. Pearse, 1987. Echinoderm Studies 2, 137.
C.A. Douglas, 1976. Availability of drift materials and the covering response of the sea urchin *Strongylocentrotus purpuratus* (Stimpson) Pacific Science, 30, pp. 83-89.

Cachat, J.; Canavello, P.R.; Elkhayat, S.I.; Bartels, B.; Hart, P.; Elegante, M.F.; Beeson, E.C.; Laffoon, A.; Haymore, W.M.; Tien, D.H.; et al., 2011. Video-

Aided Analysis of Zebrafish Locomotion and Anxiety-Related Behavioral Responses. In Zebrafish Neurobehavioral Protocols; Kalueff, A.V., Ed.; Humana Press: New York, NY, USA, Volume 51, pp. 1–14.

Carr, W. E. S., & Derby, C. D.,1986. *Chemically stimulated feeding behavior in marine animals. Journal of Chemical Ecology, 12(5), 989–1011.*

CARR, W.E.S., and GURIN, S., 1975. Chemoreception in the shrimp, Palaemonetes pugio: Comparative study of stimulatory substances in human serum. Biol. Bull. 148:380-392.

Carter, C. G., Houlihan, D. F., McCarthy, I. D., & Brafield, A. E.,1992. Variation in the food intake of grass carp,*Ctenopharyngodon Idella* (Val.), fed singly or in groups. Aquatic Living Resources, 5(3), 225–228.

Castilla JC, Crisp DJ., 1970. Responses of Asterias rubens to olfactory stimuli. J Mar Biol Assoc UK 50:829–847.

Clark, D., Lamare, M., & Barker, M., 2009. Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. Marine Biology, 156(6), 1125–1137.

D. Barnes, A. Crook, 2001. Quantifying behavioural determinants of the coastal European sea urchin *Paracentrotus lividus*, Marine Biology, 138, pp. 1205-1212.

D.W. James, 2000. Diet, movement, and covering behaviour of the sea urchin *Toxopneustes roseus* in rhodolith beds in the Gulf of California, Mexico, Marine Biology, 137, pp. 913-923.

Dayton PK, 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecol Monogr 45:137–159.

Doving, K. B., and Lastein, S., 2009. The alarm reaction in fishes–odorants, modulations of responses, neural pathways. *Ann. N. Y. Acad. Sci.* 1170, 413–423.

Duggins DO., 1983. Starfish predation and the creation of mosaic patterns in a kelp-dominated community. Ecology 64:1610– 1619.

Dumont, C. P., Drolet, D., Deschênes, I., & Himmelman, J. H., 2007. *Multiple factors explain the covering behavior in the green sea urchin, Strongylocentrotus droebachiensis. Animal Behavior, 73(6), 979–986.*

Eklöf, J. S., de la Torre-Castro, M., Gullström, M., Uku, J., Muthiga, N., Lyimo, T., & Bandeira, S. O. (2008). *Sea urchin overgrazing of seagrasses: A review of current knowledge on causes, consequences, and management. Estuarine, Coastal and Shelf Science, 79(4), 569–580.*

F. Huntingford, M. Jobling, S. Kadri, 2012. Conclusions: Aquaculture and Behavior. Aquaculture and Behavior. Wiley-Blackwell. Aquaculture and Behavior, pp. 322-332.

F. Huntingford, S. Kadri, M. Jobling, 2012. Appetite and feed intake Aquacult. Behav., pp. 183-210.

Fangmeier, M.L.; Noble, D.W.A.; O' Dea, R.E.; Usui, T.; Lagisz, M.; Hesselson, D.; Nakagawa, S., 2018. Computer Animation Technology in Behavioral Sciences: A Sequential, Automatic, and High-Throughput Approach to Quantifying Personality in Zebrafish (*Danio rerio*). Zebrafish, 15, 206–210.

FAO. (2016). Food and Agriculture Organization Fisheries Statistics.
Fernö A., Solemdal P., Tilseth S., 1986. Field studies on the behaviour of whiting (Gadus merlangus L.) towards baited hooks, Fiskeridirektoratets Skrifter Serie Havundersøkelser, vol. 18, pg. 83-95.

Firestein, S., 2001. How the olfactory system makes sense of scents. Nature, 413, 211–218.

Firestein, S., 2001. How the olfactory system makes sense of scents. Nature, 413(6852), 211–218.

H.S. Alzubi, W. Al-Nuaimy, J. Buckley, I. Young, 2016. An intelligent behavior-based fish feeding system IEEE., 10.1109/SSD.2016.7473754.

Hagen NT, Mann KH., 1994. Experimental analysis of factors influencing the aggregating behavior of the green sea urchin Strongylocentrotus droebachiensis (Mu¨ller). J Exp Mar Biol Ecol 176:107–126.

Hay, M. E., 2009. Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. Annual Review of Marine Science, 1(1), 193–212.

Hurk, R. V. D., and Lambert, J. G. D., 1983. Ovarian steroid glucuronides function as sex pheromones for male zebrafish, *Brachydanio rerio*. *Can. J. Zool.* 61, 2381–2387.

I.-S. Kwak, T.-S. Chon, H.-M. Kang et al., 2002. "Pattern recognition of the movement tracks of medaka (*Oryzias latipes*) in response to sub-lethal treatments of an insecticide by using artificial neural networks," Environmental Pollution, vol. 120, no. 3, pp. 671–681.

Kalueff, A.V.; Gebhardt, M.; Stewart, A.M.; Cachat, J.M.; Brimmer, M.; Chawla, J.S.; Craddock, C.; Kyzar, E.J.; Roth, A.; Landsman, S.; et al., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish, 10, 70–86.

Kane, A. S., Salierno, J. D., & Brewer, S. K., 2005. Fish models in behavioral toxicology: Automated techniques, updates and perspectives. In G. K. Ostrander (Ed.), Methods in aquatic toxicology, Boca Raton: Lewis Publishers, pp. 559–590.

Kane, A. S., Salierno, J. D., Gipson, G. T., Molteno, T. C. A., & Hunter, C., 2004. Avideo-based movement analysis system to quantify behavioral stress responses of fish. Water Research, 38(18), 3993–3999.

Kermen, F.; Franco, L.M.; Wyatt, C.; Yaksi, E., 2013. Neural circuits mediating olfactory-driven behavior in fish. Front. Neural Circuits, 7, 62.

Kobayashi N, 1984. Marine ecotoxicological testing with echinoderms. In: Persoone G, Jaspers E, Claus C (eds) Ecotoxicological testing for the marine environment. State University of Ghent and Institute Marine Scientific Research, Bredene, pp 341–405.

L. Rogers-Bennett, 2007. in Edible Sea Urchins: Biology and Ecology, J. M. Lawrence, Ed. (Elsevier, Amsterdam, Netherlands,), pp. 393–425.
Lang C, Schroeter SC., 1976. Change in sea urchin populations after the destruction of kelp beds. Marine Biology 36: 321-326.

Lawrence 2007- Lawrence JM., 2007. biology and ecology, 2nd edn. Elsevier, Amsterdam.

Lawrence JM., 1975. On the relationship between marine plants and sea urchins. Oceanogr. Oceanography and Marine Biology - An Annual Review 13: 213-286.

Lawrence JM., 1991. A chemical alarm response in *Pycnopodia helianthoides* (Echinodermata: Asteroidea). Mar Behav Physiol 19:39–44.

Legault C, Himmelman JH., 1993. Relation between escape behavior of benthic marine invertebrates and the risk of predation. J Exp Mar Biol Ecol 170:55–74.

Liley NR., 1982. Chemical communication in fishes. Can J Fish Aquat Sci 39:22–35.

Lindsay, S. M., and Vogt, R. G., 2004. Behavioral responses of newly hatched zebrafish (*Danio rerio*) to amino acid chemostimulants. *Chem. Senses* 29, 93–100.

Little, E. E., & Brewer, S. K., 2001. Neurobehavioral toxicity in fish. In D. Schlenk & W. H. Benson (Eds.), Target organ toxicity in marine and freshwater teleosts, London: Taylor and Francis, pp. 141–176.

Løkkeborg S, Ferno¨ A, Humborstad O-B., 2010. Fish behavior in relation to longlines. In: He P (ed) Behavior of marine fishes: capture processes and conservation challenges. Wiley, Oxford, pp 105–141.

Løkkeborg S., Skajaa K., Fernö A., 2000. Food-search strategy in ling (Molva molva L.): crepuscular activity and use of space, Journal of Experimental Marine Biology and Ecology, vol. 247, pp. 195-208.

Løkkeborg S., 1998. Feeding behaviour of cod Gadus morhua: activity rhythm and chemically mediated food search, Animal Behaviour, vol. 56, pp. 371-378.

Løkkeborg S., Fernö A., Jørgensen T., 2002. Effect of position-fixing interval on estimated swimming speed and movement pattern of fish tracked with a stationary positioning system. In: Thorstad E.B., Fleming I.A., Næsje T.F. (eds) Aquatic Telemetry. Developments in Hydrobiology, vol 165. Springer, Dordrecht.

M.-J. Bae and Y.-S. Park, 2014. "Biological early warning system based on the responses of aquatic organisms to disturbances: A review," Science of the Total Environment, vol. 466-467, pp. 635–649.

Mann KH, Wright JLC, Welsford RW, Hatfield E.,1984. Responses of the sea urchin Strongylocentrotus droebachiensis (O.F. Mu¨ller) to water-borne stimuli from potential predators and potential food algae. J Exp Mar Biol Ecol 79:233–244.

Marks, C.A.; Cheng, K.; Cummings, D.M.; Belluscio, L., 2006. Activity-dependent plasticity in the olfactory intrabulbar map. J. Neurosci. 26, 11257–11266.

Martin Føre, K. Frank, T. Norton, E. Svendsen, J.A. Alfredsen, T. Dempster, H. Eguiraun, W. Watson, A. Stahl, L.M. Sunde, C. Schellewald, K.R. Skøien, M.O. Alver, D., 2018. BerckmansPrecision fish farming: a new framework to improve production in aquaculture Biosyst. Eng., 173, pp. 176-193.

Mas-Muñoz, J., Komen, H., Schneider, O., Visch, S. W., & Schrama, J. W., 2011. Feeding Behavior, Swimming Activity and Boldness Explain Variation in Feed Intake and Growth of Sole (Solea solea) Reared in Captivity. PLoS ONE, 6(6), e21393.

Mauzey KP, Birkeland C, Dayton PK, 1968. Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. Ecology 49:603–619.

Mayo P, Mackie AM., 1976. Studies of avoidance reactions in several species of predatory *British seastars* (Echinodermata: Asteroidea). Mar Biol 38:41–49.

Miklavc, P.; Valentinˇciˇc, T., 2012. Chemotopy of amino acids on the olfactory bulb predicts olfactory discrimination capabilities of zebrafish Danio rerio. Chem. Senses, 37, 65–75.

Miller, D.C., Lang, W. H., Graeves, J. O. B., and Wilson, R. S., 1982. Investigations in aquatic behavioral toxicology using a computerized video quantification system. In Aquatic Toxicology and Hazard Assessment: Fifth Conference, STP 766. J. G. Pearson, R. B. Foster, and W. E. Bishop (eds). American Society for Testing and Materials, Philadelphia, PA.

N., H., Å., A., & O., S., 2002. Alarm responses of the green sea urchin, Strongylocentrotus droebachiensis , induced by chemically labelled durophagous predators and simulated acts of predation. Marine Biology, *140(2), 365–374.*

Ole B. Stabel, NT Hagen, A. Anderson, 2020, Alarm responses of the green sea urchin, Strongylocentrotus droebachiensis, induced by chemically labelled durophagous predators and simulated acts of predation, Marine Biology, researchgate.net, 140(2):365-374

Pansch C, Schaub I, Havenh and J, Wahl M., 2014. Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. Glob. Change Biol. 20, 765– 777.

Parker DA, Shulman MJ., 1986. Avoiding predation: alarm responses of Caribbean sea urchins to simulated predation on conspecific and heterospecific sea urchins. Mar Biol 93:201– 208.

Peacor SD, Werner EE., 2001. The contribution of trait-mediated indirect effects to net effects of a predator. Proc. Nat. Acad. Sci. USA. 98:3904–8.

Pfeiffer W., 1963. Alarm substances. Experientia (Basel), 19:113–168.
Phillips DW., 1978. Chemical mediation of invertebrate defensive behaviors and the ability to distinguish between foraging and inactive predators. Mar Biol 49:237–243.

Pitcher, T .J., 1993. Behavior of Teleost Fishes (2nd ed.), Chapman & Hall, Chpt. 3, pp.715.

Pratama Diffi Samuel 1 *, Dewa Gede Raka Wiadnya 2 , Bagyo Yanuwiadi 1, 2017. Species and Abundance of Sea Urchins (*Diadematidae*) on Different Environmental Pressure Conditions, THE JOURNAL OF TROPICAL LIFE SCIENCE, VOL. 7, pp -149-150.

Qin, L., Zhu, B.-W., Zhou, D.-Y., Wu, H.-T., Tan, H., Yang, J.-F., … Murata, Y., 2011. Preparation and antioxidant activity of enzymatic hydrolysates from purple sea urchin (Strongylocentrotus nudus) gonad. LWT - Food Science and Technology, 44(4).

Ranåker, L., Nilsson, P. A., & Brönmark, C., 2012. Effects of Degraded Optical Conditions on Behavioral Responses to Alarm Cues in a Freshwater Fish. PLoS ONE, 7(6).

Robert C. Summerfelt and William M. Lewis, 1967. Journal (Water Pollution Control Federation) Vol. 39, No. 12, pp. 2030-2038.
Rodriguez SR, Ojeda FP., 1998. Behavioral responses of the sea urchin *Tetrapygus niger* to predators and food. Mar Freshw Behav Physiol 31:21–37.

Rosenberg R, Selander E., 2000. Alarm signal response in the brittle star *Amphiura filiformis*. Mar Biol 136:43–48.

Rune Vabø, Geir Huse, Anders Fernö, Terje Jørgensen, Svein Løkkeborg, Georg Skaret, 2004. Simulating search behaviour of fish towards bait, *ICES Journal of Marine Science*, Volume 61, Issue 7, Pages 1224–1232.

S. Silvia, M. Riccardo, L. Barbara, G. Ivan, B. Maura, S. Roberta, *et al.*, 2016. Influence of seasonal and environmental patterns on the lipid content and fatty acid profiles in gonads of the edible sea urchin *Paracentrotus lividus* from Sardinia, Marine Environmental Research, 113, pp. 124-133.

Scheibling RE, Hamm J., 1991. Interactions between sea urchins (Strongylocentrotus droebachiensis) and their predators in field and laboratory experiments. Mar Biol 110:105–116.

Schroeter SC, Dixon J, Kastendiek J., 1983. Effects of the starfish *Patiria miniata* on the distribution of the sea urchin *Lythechinus anamesus* in a southern Californian kelp forest. Oecologia (Berl) 56:141–147.

Smith RJF., 1992. Alarm signals in fishes. Rev Fish Biol Fish 2: 33–63.
Snyder N, Snyder H., 1970. Alarm response of *Diadema antillarum*. Science 168:276–278.

Smith, E. H., and Bailey, H. C., 1988. Development of a system for continuous biomonitoring of a domestic water source for early warning of contaminants. In Automated Biomonitoring: Living Sensors as Environmental Monitors D. S. Gruber, and J. M. Diamond (eds). Ellis Harood Publishers Ltd. Chichester UK.

Speedie, N., and Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behav. Brain Res.* 188, 168–177.
Spence, R.; Gerlach, G.; Lawrence, C.; Smith, C., 2008. The behavior and ecology of the zebrafish, Danio rerio. Biol. Rev. Camb. Philos. Soc., 83, 13–34.

Stacey, N. E., and Kyle, A. L., 1983. Effects of olfactory tract lesions on sexual and feeding behavior in the goldfish. *Physiol. Behav.* 30, 621–628.

Stoner AW, Sturm EA., 2004. Temperature and hunger mediate sablefish (Anoplopoma fimbria) feeding motivation: implications for stock assessment. Can J Fish Aquat Sci 61:238–246.

Suresh, A. V., Kumaraguru vasagam, K. P., & Nates, S. (2011). Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. Aquaculture, 319(1-2), 132–140

Thiel M, Breithaupt T., 2011. Chemical communication in crustaceans: research challenges for the twenty-first century. In press: Breithaupt T, Thiel M (eds) Chemical communication in crustaceans. Springer, New York, pp 3–23.

Umar Tangke, Sitkun Deni and Asri Aunaka, 2018. The Influence of Using Bait Types to the Number and Composition of Fishing Traps Catch in South Ternate Waters, IOP Conf. Series: Earth and Environmental Science 175, pp 1-8.

Vadas RL, Elner RW, Garwood PE, Babb IG., 1986. Experimental evaluation of aggregation behavior in the sea urchin Strongylocentrotus droebachiensis. A reinterpretation. Mar Biol 90:433– 448.

Valentincic, T., 2004. Taste and olfactory stimuli and behavior in fishes. In The Senses of Fish; Emde, G., Mogdans, J., Kapoor, B.G., Eds.; Kluwer Academic Publishers: Boston, MA, USA.

von Frisch, K., 1941. Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Z. Vgl. Physiol.* 29, 46–145.

Webster D.R., Rahman S., Dasi L.P., 2001. On the usefulness of bilateral comparison to tracking turbulent chemical odor plumes, Limnology and Oceanography, vol. 46. pp. 1034-1047.

Whitlock, K.E., 2006. The sense of scents: Olfactory behaviors in the zebrafish. Zebrafish, 3, 203–213.

Wirtz, Peter Duarte, Joana, 2012. Alarm reaction and absence of alarm reaction to simulated predation on conspecifics in two temperate sea urchin species, Arquipelago Life Mar Sci. 29:59-62.

Xia, C., Fu, L., Liu, Z., Liu, H., Chen, L., & Liu, Y., 2018. Aquatic Toxic Analysis by Monitoring Fish Behavior Using Computer Vision: A Recent Progress. Journal of Toxicology, 2018, 1-2.

Xu, D., Shen, K.-N., Fan, Z., Huang, W., You, F., Lou, B., & Hsiao, C.-D. (2016). The testis and ovary transcriptomes of the rock bream (*Oplegnathus fasciatus*): A bony fish with a unique neo Y chromosome. Genomics Data, 7, 210–213.

Y. Liu, S.-H. Lee, and T.-S. Chon, 2011. "Analysis of behavioral changes of zeebrafish (Danio rerio) in response to formaldehyde using Self-organizing map and a hidden Markov model," Ecological Modelling, vol. 222, no. 14, pp. 2191–2201.

Yukio Agatsuma, 2007. Developments in Aquaculture and Fisheries Science, Chapter 21 Ecology of *Strongylocentrotus nudus,* Volume 37, pp 443-457.

Zhao, S., Cheng, Q., Peng, Q., Yu, X., Yin, X., Liang, M., … Jia, W., 2018. *Antioxidant peptides derived from the hydrolyzate of purple sea urchin (Strongylocentrotus nudus) gonad alleviate oxidative stress in Caenorhabditis elegans. Journal of Functional Foods, 48, 594–604.*

ZIMMER-FAusT, R.K., MICHEL, W.C., TYRE, J.E., and CASE, J.F., 1984. Chemical induction of feeding in California spiny lobster, Panulirus interruptus (Randall): Responses to molecular weight fractions of abalone. J. Chem. Ecol. 10:957-971.02

Websites;

Rainer Froese, Emily Capuli, Cristina Garilao and Daniel Pauly, *Oplegnathus fasciatus* (Temminck & Schlegel,1844), 2020 June 20, https://www.fishbase.de/Summary/SpeciesSummary.php?ID=6532&AT=Rock+bream

Ryan Kempster, Shark Repellents, 2020 June, http://www.supportoursharks.com/en/Education/Shark_Repellents.htm

Acknowledgement

I would like to thank the following people who have helped me undertake this research: My supervisor Prof. Jong-Myoung Kim, for his enthusiasm for the project, for his support, encouragement and patience;

Rest of the professors of the The Department of Marine Biology, Pukyong National University including prof. Chang-Hoon Kim, prof. Jongwhan Park, prof. Choi Yun Hee, prof. Seung-Pyo Gong, prof. Nam Gyu Park for their kind guidance and support.

The staff of the Pukyong National University, All the laboratory funding projects, and members and staff of the Suhyp Scholarship Programme of the Republic of South Korea, officials and members of the Korea Fisheries Resources Agency FIRA and National Fisheries Research and Development Institute NFRDI , Busan and the fisheries community of Korea, and the Ministry of fisheries and Aquatic Resources Development of the Democratic Socialist Republic of Sri Lanka, for various inputs throughout this MSc programme.

Special thanks to Hon. Minister and Hon. former Minister, Hon. State Minister and The Secretary and former Secretaries and all the staff members of the The Ministry of fisheries and Aquatic Resources Development of the Democratic Socialist Republic of Sri Lanka, Mr. Monty Ranathunga, Prof. JMPK Jayasinghe, Prof. WMTB Wanninayaka, Rear-admiral Ranjith Senadheera, Dr. Kim Younsook, Mr. Ajith Kumara Aluthgedera, Mr. Shiran Chamika, Ms. Nilushi Bamunuarachchi, Mr, Young Hyun Jee of Suhyp and Academic and non-academic staffs of the Wayamba University of Sri Lanka for their blessings and kind support.

My lab mates Mr. Yeo-Reum Kim, Mr. Sung-min Bang, Mr. Sung-youn, Ms. Mi-jin, Mr. Bokki, Ms. Geong-myo, Ms. Regina, and Mr. Jae hoon – I simply couldn't have done this without you, special thanks. My sister Ms. Nadeesha Sandamali, borther Mr. Nilan Sri Kankanamge and all my dear friends helped me in numerous ways during this long journey of life.

And to my parents, Mr. S.K. Lal Dharmasiri and Mrs. Kusuma Yakandawala who set me off on the road to this MSc a long time ago.