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Status of Marine Environment of Olive Flounder, *Paralichythys olivaceus*, Culture Ground in Jeju-do

- Focus on *Kudoa septempunctata* positive and negative farm -

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제주도 육상 넙치양식장의 해양환경현황

- 쿠도아 양성양식장과 음성양식장을 중심으로 -

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Abstract

In this study, we monitored the status of marine environment of olive flounder, *Paralichythys olivaceus*, culture ground in Jeju-do, Republic of Korea. It reported the consumption of raw olive flounder meat containing of Kudoa, *Kudoa septempunctata*, could induce vomitting and diarrhea in Japan. The Kudoa is a new *mycosporean* species, researchers found Kudoa from the muscles of olive flounder cultured at western Japan and imported from Jeju-do. We choose two Kudoa positive farms and two negative farms in the mid of concern about the relation between Kudoa and habitat. We found two marine invasive species at the water-pumping seabed at one of the Kudoa positive farm. The concentration of pH and DO at on-growing flounder farms showed the decreasing pattern along side the raceway (influent sea waters > on growing sea waters > outfluent sea waters). The TN and TP values increased gradually following to the raceway (influent sea waters < on growing sea waters < outfluent sea waters). The concentration of COD and SS were in the range of 0.100 − 2.581 $mg \cdot L^{-1}$, 1.00 − 12.70 $mg \cdot L^{-1}$, respectively. The calculated residence time was 4hr 32minutes at F1, 11hr 21minutes at F2, and 9hr 50minutes at F3, respectively. It was calculated same distance of 4 km away from effluent pipes. Although direct relation between *Kudoa* and marine environment could not define well based on this study result, the more studies on marine environmental stressors for olive flounder are required to conduct as a reliable method including socio-economic group and environmental group.

Key words : Olive flounder, Paralichthys olivaceus, Kudoa septempuntata, Jeju-do

Ⅰ. Introduction

Status of flounder − The olive flounder, *Paralichythys olivaceus*, is the most common flatfish species and accounts for 98 percent of the flatfish culture in Republic of Korea(NOAA). It is one of the most important marine aquaculture species, it contributed 51% of the total marine finfish aquaculture production in Republic of Korea in 2013(Kim et al., 2014). It was raised in

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land-based aquaculture in Jeju-do since mid-1980s. The production of olive flounder in Jeju-do accounts for more than 60% of the total production in Republic of Korea(Kim et al., 2014). The Republic of Korean production exceeded Japanese in 1990s, exports to Japan targeting for raw fish market(Kim and Kang, 2011). The flounder culture is based on the hatchery seeds and practiced in the raceway system. This flow-through land-based system is easy to setup and manage the water quality compared to re-circulatory system in the ocean. The total production tends to be under control thanks to the conditioning technology of the broodstock in capacity, but disease control and better broodstocks are on-going subjects in Jeju-do, Republic of Korea.

Disease issue - The several cases of diseases related to the olive flounder reported since late 2000s in Japan. It reported that consumption of raw olive flounder meat containing of high concentration of Kudoa, *Kudoa septempunctata*, induces vomitting and diarrhea through an unknown mechanism(Ohnishi et al., 2013). In the mid of undertaking the identification of *Kudoa septempunctata* which is a new myxosporean species in Japan, researchers found Kudoa from the muscles of olive flounder both cultured at western Japan and imported from Jeju-do, Republic of Korea(Matsukane et al., 2010; 2011). The reasons of this unexpected myxosporean species are very complicated and diverse until now, it caused the concern to aquaculture, commercial fisheries, and fish dealers about transitive relation among host, pathogen, and mitigating environments. The disease of olive flounder rarely results from simple contact between host and potential pathogen. Before fish become sick, mitigating circumstances would be easy to present such as poor water quality, excessive crowding, or similar stressor(Austin and Austin, 2007).

Previous study - To decrease the occurrence of disease outbreaks, flounder culture ground should be managed to minimize the stress to fish. The poor water quality could be the important stressor for cultured fish and could cause many disease outbreaks(Rottmann et al., 1992). For the good water quality, fish farmer consider preventing accumulation of organic debris and nitrogenous wastes, maintaining appropriate pH and proper range of water temperature, and maintaining dissolved oxygen levels (Francis-Floyd, 2009; Barton and Iwama, 1991). Most studies on flounder farm management from disease have been conducted the relationship between hosts and pathogen excluding environmental factors(Han et al., 2006; Jee et al., 2001). Both the status of marine environment itself and an environmental effect on productivity were studied relating with the growth rate of olive flounder, stocking density rate, and survival rate in Jeju-do, Republic of Korea(Eh, 2011). Similarly, the effect of sediment and seawater quality of cultured fleshy prawn, *Penaeus chinensis* was measured focusing on growth in western coastal waters(Kang et al., 2000). As the natural optimum sea water temperature for growth of olive flounder is 15 ℃ to 25 ℃ in Republic of Korea, fish farmers asked technical support for desirable environmental conditions such as control of temperature and appropriate amount of feed(Cho et al., 2006a). However, monitoring of *Kudoa septembunctata* in cultured olive flounder in Jeju-do began in 2012 (Song et al., 2013). Related to the Kudoa host-pathogen study, survey on the marine environment began since 2013.

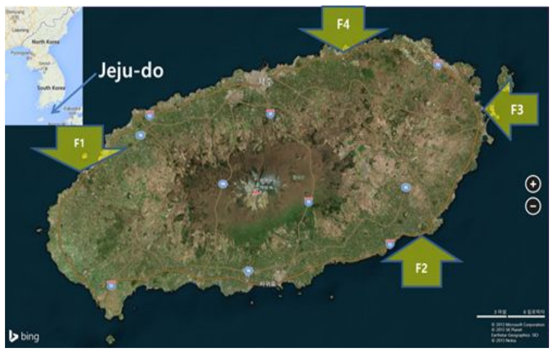
Goal of the study - In the mid of great concern on marine environment of flounder, we choose two

Kudoa positive culture grounds and two Kudoa negative culture grounds. We aim to assess the status of marine environment based on followings: ① Because the typical life cycle of a myxozoan involves 2 alternate hosts, such as vertebrates(mainly fish) and annelid invertebrates which are generally oligochates for freshwater species and polychaetes for marine species(Bartholomew et al. 1997; Benajiba & Marques 1993; Iijima et al., 2012; Uspenskaya 1995; Yokoyama et al., 2012), we classified the polychaete species to assess the benthic community and grain composition in the sediment around influent water bottom. ② A majority of flounder farms use a moist pellet (MP) in Jeju-do, this MP associates with the deterioration of water quality and fish diseases(Cha et al., 2008). To monitor the status of water quality on the raceway of flounder culture ground, we analyzed the variation of water quality following the flow-through of the culture grounds. ③ From flounder farms, organic and inorganic materials including nutrients, feaces, and biological organism flow to the ocean in front of fish farms. We measured the particle residence time by means of remote tracer system in order to define how fast particles spread to the ocean. At present, there are no clearly status result of marine environment to find any relation between pathogen and marine environment of the flounder farms. This study could be the first of its kind for flounder environment as whole.

Ⅱ. Materials and Methods

1. Study sites

The Jeju-do is located at the southern tip of the Korean Peninsula. The Jeju-do is in a temperate region climatically and it shows four distinct seasons with moderately hot summer and cold winter. The sea temperature ranged 13.3 - 26.1 ℃ over the year, which is suitable for the aquaculture of olive flounder. At present, the land-based culture of olive flounder is a major activity with about 300 commercial farms situated along the coastal region of Jeju-do(Song et al., 2013). The sea water is pumped from the open ocean into the reservoirs of fish farms mostly. Most of the farms in Jeju-do, pipes are contacted and it is buried 150 – 200 m away from coastline in order to pump sea water constantly. The diameter of pipe is 500 – 600 mm, usually three or four pipes are buried together. And also sea water is supplied by pumping underground water in the northern part of Jeju-do(NFRDI, 2008). In this study, we choose 4 sampling stations; in the west(F1), south(F2), east(F3), and north(F4). The sea water is pumped from open ocean through pipes in F1, F2, and F3. At F4, it is pulled from underground water. The F1 and F3 are the Kudoa positive farms which confirmed the highly concentration of Kudoa before, while the F2 and F4 are the Kudoa negative farms ([Fig. 1]).



[Fig. 1] The study area in Jeju-do, Republic of Korea ; F1(west, Kudoa+), F2(south, Kudoa-), F3(east, Kudoa+), F4(north, Kudoa-)

2. Sampling strategy

An olive flounder has been rearing in coastal areas all around Jeju-do since mid-1980s. The federation of fishery cooperative in Jeju-do recommended sampling stations considering the representative of region(east - west - north - south Jeju-do), undesirable flounder disease conditions (positive or negative existence of *Kudoa septempunctata* cyst) and sea water pumping. Among them, F1 and F3 were considered as a Kudoa positive farms, while F2 and F4 as a negative farms. The field survey for benthic species and particle track experiment were conducted in August, 2013. And chemical properties such as pH, DO, TN, TP, COD, SS were conducted August and November, 2013. Through the investigation of benthic community structure, we designed the classification of polychaete which could be the potential host before the sea water is pumped into the flounder farms. For the benthic environment sampling, two scuba divers grabbed the sediment samples around pumping pipes(0.15 ㎡). It is located 150-200 m away from coast line. The depths of each site are 15-20m. For the assessment of water quality, we distinguished water samples following raceway in the culture ground(<Table 1>). The first sampling point was reservoirs, and next point was circular tank for small size(fish weight～200g) and market size(fish weight～700g). Last point was the out-going spot from land-based raceway to coastal waters. For analyzing residence time of pollutants from olive flounder farms, we monitored the electronic signal of the particle tracking bottles through wireless telecommunication system. The particle track experiment was conducted separately at effluent sites in 2013.

3. Data analysis

The scuba divers collected the sediment samples per 0.15 ㎡ from three stations except F4, because F4 used the underground water underneath the surface about 40 m depth directly. After sieving through a 1-㎜ mesh sieve, that samples transferred to the laboratory for analysis of benthic community by the taxonomy expert. We monitored the water temperature(Temp, ℃), pH, dissolved oxygen(DO, $mg \cdot L^{-1}$) inside the flounder farm on site. We took water samples to a laboratory in order to analyze the content of chemical characteristics such as total nitrogen(TN, $mg \cdot L^{-1}$), total phosphate(TP, $mg \cdot L^{-1}$), dissolved inorganic nitrogen(DIN, $mg \cdot L^{-1}$), dissolved inorganic phosphate(DIP, $mg \cdot L^{-1}$), chemical oxygen

<Table 1> The information of sampling stations including location, record of Kudoa, method of water pumping, water sampling point on the raceway, benthic sample collection, and residence time experiment.

Station	Longitude	Latitudes	Kudoa	Pumping water	Sampling station in Raceway	Sampling in the Seabed	Tracer
F1	126 10.12 E	33 21.14 N	Positive	Sea waters	In-On-Out	Benthos, grain composition	Effluent site
F2	126 46.54 E	33 18.17 N	Negative	Sea waters	In-On-Out	Benthos, grain composition	Effluent site
F3	126 55.28 E	33 28.08 N	Positive	Sea waters	In-On-Out	Benthos, grain composition	Effluent site
F4	126 41.58 E	33 32.51 N	Negative	Sub-ground	In-On-Out	-	-

demand(COD, mg·L^{-1}), suspended solids(SS, mg·L^{-1}) according to the experimental standards for marine environment. We used the buoy which is allowed to drift with the sea wave from the out-flowing waters of olive flounder farms. This buoy was equipped with tracking device through pulse amplitude modulation(PAM) technology. To communicate between buoy and global positioning system, the wavelength was 824 – 848 MHz of transmitter (Tx)and 869–893 MHz of receiver(Rx). The frequency stability was ± 300 Hz, and the observation error of this global position system is about 5 m - 25 m in the open ocean.

Ⅲ. Results

1. Benthic community

The influent pipes were located on the rocky bottom habitiat at F1 and F2 with the sea depth of 18m and 20m. Near the edge of pipes, there were sandy bottom habitat in which polychaete could live. The sandy habitat existed within 10 m from the influent pipes, thus we collected sediment samples both rocky and sandy bottom at F1 and F2. The influent pipes of F3 imbedded with sandy bottom with the depth of 6 m. In particular, influent pipes of F3 set on top of sandy sediment in semi-closed bay in front of olive flounder farms.

The most abundant species at each sampling sites were polychaete. A total of 33 polychaetes were identified. A total number of species at F1(rocky), F1(sandy), F2(rocky), F2(sandy), F3(sandy) are 11 /0.15 ㎡, 8/0.15 ㎡, 9/0.15 ㎡, 5/0.15 ㎡, 11/0.15 ㎡, respectively. At F1, *Armandia sp.*, *Scoloplos armiger*, and *Spio martinensis* were dominant. At F2, there were not any specific dominant polychaete species. Both F1 and F2, there were not any marine invasive polychaete species. At F3, 11 polychaetes were identified with *Mediomastus sp.* (35%),

<Table 2> Species of polychaetes in Jeju-do, Republic of Korea. Benthic community classified both rocky and sandy bottom habitat at F1 and F2. Near influent pipe of F3, only sandy bottom existed.

Species of polychaete	F1 Ro	F1 Sa	F2 Ro	F2 Sa	F3 Sa	Sum
Amphinomidae sp.			2			2
Aphelochaeta monilaris		1				1
Aquilaspio krusadensis					10	10
Armandia sp.		19				19
Cirriformia tentaculata				1	4	5
Eunicidae sp.			3			3
Flabelligeridae sp.					1	1
Glycera sp.		1		4		5
Halosydna brevisetosa	3					3
Harmothoe imbricata	7					7
Hesionidae sp.	1					1
Lumbrineridae sp.			1			1
Lysidice sp.	4					4
Mediomastus sp.					214	214
Mysta ornate					1	1
Neanthes caudata		1			6	7
Nephtys polybranchia					29	29
Nereidae sp.	1		4			5
Perinereis cultrifera	1					1
Pilargidae sp.				1		1
Pista cristata		1	1			2
Polynoidae sp.	1					1
Pseudopolydora paucibranchiata					325	325
Sabellidae sp.2	3					3
Sabellidae sp.3	1					1
Sabellidae sp1.			1			1
Scolelepis sp.				4		4
Scoloplos armeger		13			9	22
Sigalionidae sp.		3				3
Spio martinensis		18			5	23
Spionidae sp.			1			1
Syllidae spp.	2		9	4	4	19
Terebellidae sp.	1		3			4
Total number of species	11	8	9	5	11	33

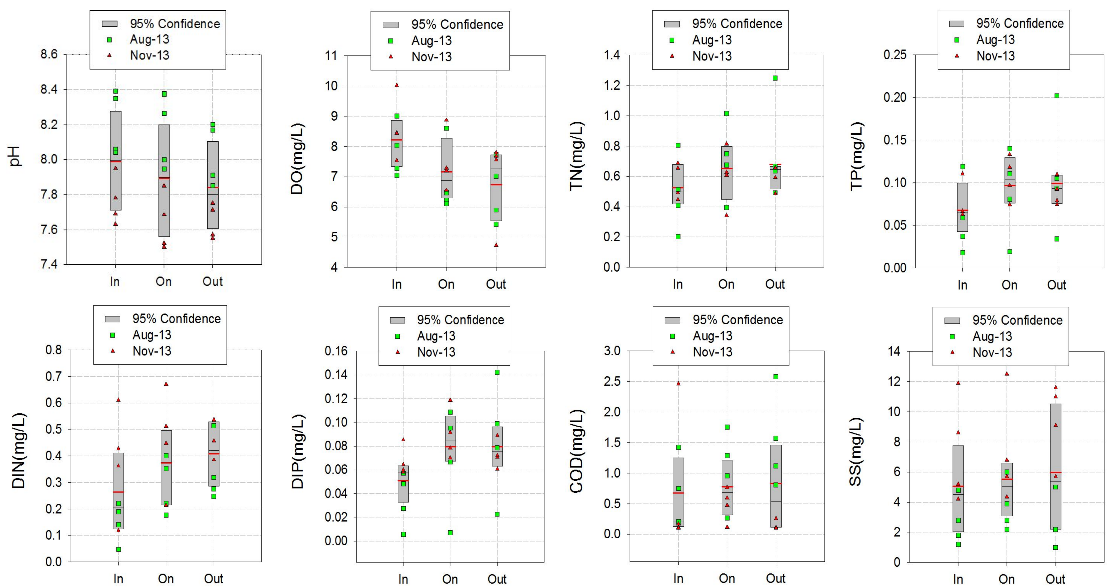
Ro : Rocky, Sa: Sandy

Pseudopolydora paucibranchiata (53%) as a majority species. The *Pseudopolydora paucibranchiata* is considered as a marine invasive species, it could negatively modify marine habitat with its large densities (Molnar et al. 2008). The *Mediomastus sp.* was considered tolerant of pollutants. Therefore, two species represented that sandy bottom habitat near F3 influent pipes was the area of high input of organic matters and polluted areas. From the flounder farms, excessive input of organic matters are considered as the main factors disturbing benthic community.

The grain size in sandy bottom ranged 0.74 – 3.45 Φ, which means it consisted of very fine sand. The sorting skewness ranged 1.34 – 2.16 Φ, which means very poorly sorted. Types of sediment in F1, F2, F3 are S (sand), (g)mS (slightly gravelly muddy sand), gS (gravelly sand), respectively. In particular, the contents of sediment at F3 were gravel (0 %), sand (92.88 %), silt (6.8 %), clay (0.94 %).

2. Seawaters inside culture ground

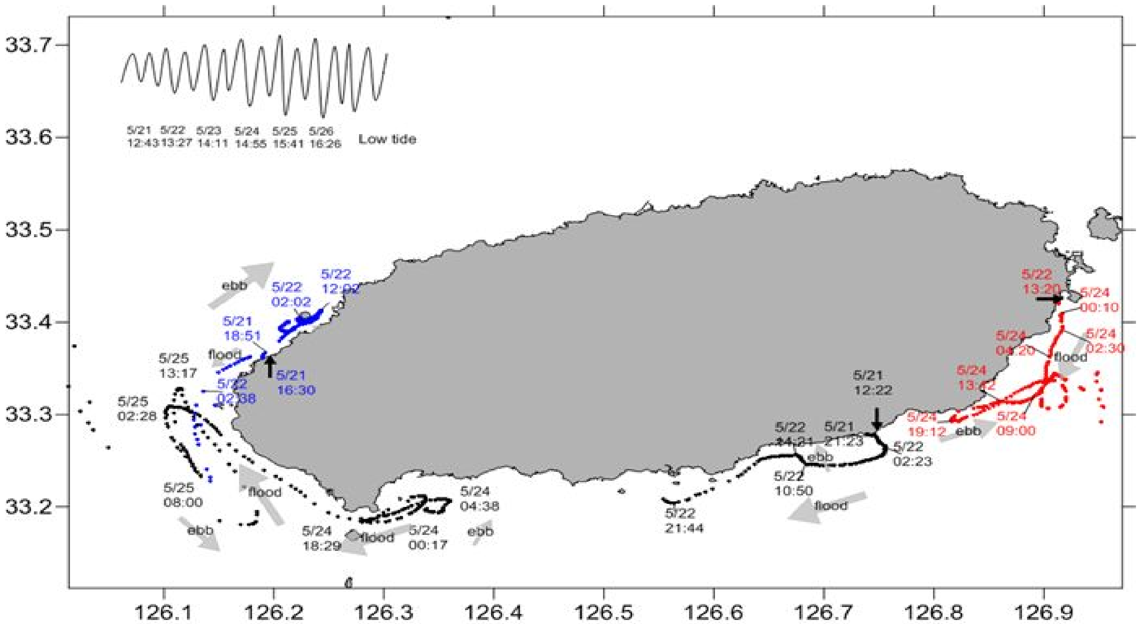
In this study, we analyzed parameter values of water quality based on the raceway locations at the influent point, on-growing point, effluent point of flounder farm. We found no significant chemical concentration and nutrients differences between F1–F4 stations. The sea water pulled from open sea through pipes(F1-F3) and underground water(F4) did not make any significant differences between stations, because water resources showed similar circumstances all over the Jeju-do. Major environmental parameters of water quality analyzed in this study including pH, DO, TN, TP, DIN, DIP, COD, SS ([Fig. 2]).



[Fig. 2] Concentration of water quality parameters : pH, DO, TN, TP, DIN, DIP, COD, SS. The value of "In" means reservoirs of influent water, "On" is the averaged value of water for young and adult fish, and "Out" is the effluents from fish farm. Boxplot visualized the distribution in a set of measured data. In boxplot, red line means the value of median, box means 5th 95th percentiles.

The pH values ranged 7.35 – 8.40, it showed maximum value at ongrowing ground of F1 in August. Its concentration gradually decreased from influent waters to effluent waters. The mean values in August and November were 8.13, 7.67, respectively. The F4 used the underground water for the raceway system, but there were not any significant differences of pH concentration with other stations. On the raceway of culture ground, the value of pH in reservoir(In) was a little higher than in the mid of raceway(On) and effluent waters(Out). The DO values ranged 4.73 – 10.02 mg·L^{-1}, maximum value analyzed at influent water at F2 and minimum values showed at effluent water at F1. The oxygen supplied by the oxygen tank sufficiently at sea water reservior in F1–F4. The values of DO dropped gradually from the reservoirs(In), culture(On), and effluent waters(Out). In general, DO values require at least 5 mg·L^{-1} for respiration and per forming metabolic activities by fish(Lawson, 1995; Phillips et al., 2009). In this study, we found unacceptable saturation of DO at F1 in August, which caused by the increase of nutrients and metabolism at the farms.

The TN and TP values ranged 0.20 – 1.24 mg·L^{-1}, 0.02 – 0.20 mg·L^{-1}, respectively. The mean values of TN were 0.715 of juvenile flounder farm and 0.591 of adult flounder farm, because the young flounder metabolism shows much higher respiration and nutrition rates than adult flounder. The values of TN, TP showed the minimum value at influent waters at F1 and maximum values at effluent waters at F4. According to the raceway, the values of TN and TP increased gradually from the beginning to the end of the system. The values of DIN and DIP showed similar variation pattern with TN and TP([Fig. 3]).



[Fig. 3] The result of residence time in Jeju-do. At effluent pipes near flounder farms, the gear of residence time deployed considering ebb tide. Deployed bottle equipped with remote tracking system transferred out to the open ocean. At F1, the device moved back and forth in the beginning. Then, it moved out quickly following the currents.

The concentration of COD was in the range of 0.100 - 2.581 $mg \cdot L^{-1}$. There were no significant differences in COD concentration between each station, the values increased gradually following to the raceway from the influent waters to effluent waters. The value of SS ranged 1.00 - 12.70$mg \cdot L^{-1}$, most of samples showed highly concentrated values exceeding the 3$mg \cdot L^{-1}$ before feeding which is suggested as a guideline for sustainable environment. We collected samples before feeding though, it exceeded the value of 10 $mg \cdot L^{-1}$ at F1 and F4. We found no significant differences with the values of SS between influent – ongrowing –effluent waters in Jeju-do.

3. Residence time

To assess the effects of waste solids from olive flounder farms, particle residence time was measured using the telecommunication technology developed by the National Fisheries Research and Development Institute(NFRDI, 2012). The calculated residence time was 4 hr 32 minutes at F1, 11 hr 21 minutes at F2, and 9 hr 50 minutes at F3, respectively(Fig. 3). It was calculated same distance of 4 km from effluent pipes. Thus, F1 showed the fastest movement and the next is F3 and F2. F1 was much faster than that of west sea of Republic of Korea, F2 and F3 were similar that of the west sea of Republic of Korea(NFRDI, 2012). The time in all station was faster than that of Masan. In Masan Bay which is located in the southern coastal area of the Republic of Korea, this move back and forth inside the bay, the residence time takes a longer than this study site. The gear of residence time did not show any reciprocation and move following to main currents. Based on these experiment data, the residence time was very fast, so particle from flounder ground could spread very quickly and far away from the source point.

Ⅳ. Discussion

The flounder farmings in Jeju-do had began to make efforts to foster the industry since mid-1980s thanks to the development of the land-based management technology and healthy breeding research. Experiencing the unexpected disease of Kudoa, flounder farmers and local authorities have serious concerns about not only flounder disease and its pathogen, but also the environment inside the culture farm and seabed around the influent waters. Even though there a number of monitoring stations related to marine environment impact assessment from fish farms, they are not even close to olive flounder at all. There has been no way to assess the status of marine environment of flounder farms. In this study, we monitored the marine environment of flounder culture ground in Jeju-do, 2013.

1 Highly concentrated SS

The flounder farms mostly use a moist pellet (MP) in Jeju-do. The MP is a mixture of frozen fish and fishmeal, and it is easily soluble in the waters(Cha et al., 2008). Due to the rapid collapse of MP, the nutrient concentration and metabolism of flounder dramastically increase while feeding. Over-feeding caused the degradation of the water quality in flounder cage generally. The increase of COD and SS associate with the problems such as the deterioration of water quality, outbreak of fish disease, increase the stress on fish(Francis-Floyd, 2009). In a view of pathogen’s side like Kudoa, the more value of COD and SS could make more chance to spread their spore. In this study, the mean value of COD and SS before feeding showed 0.83 mg·L^{-1} and 5.98 mg·L^{-1}, respectively. The mean value of COD did meet the guideline of effluent water quality, but SS exceeded the guideline and approximately two times greater than the critical value. The local authority in Jeju-do has been monitoring the effluent water quality since 2011. The stations choosed randomly almost 10 flounder farms per year. Based on local authority dataset, the average value of COD and SS did meet the guideline. The value of SS from this study was 5 – 20 times higher than the value of local authority(Jeju-do, 2011; 2012; 2013). Only one station in 2011 exceeded the point of guideline analyzed by local authority, but the values of SS in all sampling flounder farms(F1 - F4) from this study exceed the critical point(<Table 3>). For the management of effluent water quality, local authority and organization of marine environment need to collaborate with quality control and quality assurance. When monitoring of the water quality conducts in flounder farms, it needs collecting samples more before and after feeding.

<Table 3> The value of COD and SS from this study and local authority in Jeju-do

Parameter	This study (2013)	Local authority in Jeju-do		
		2011	2012	2013
COD (mg·L^{-1})	0.83 (0.10-2.58)	0.67 (0.00-1.20)	0.33 (0.00-1.20)	0.18 (0.00-0.50)
SS (mg·L^{-1})	5.98 (1.00-12.70)	0.59 (0.20-1.20)	1.23 (0.00-3.40)	0.29 (0.00-2.00)

Source : Jeju-do, 2011; 2012; 2013

2. Relation between Kudoa and marine environment

The biological parameter at bottom seabed near influent pipes, chemical parameters at culture ground, and physical parameter at effluent sea surface were monitored both Kudoa positive stations and Kudoa negative stations. From these results, we tried to find any differences of environmental parameters. It was very complicated and hard to say the relation between Kudoa and environment based on the facts from this study. It is known that the detection of Kudoa in Jeju-do was related to the sampling part from olive flounder like head or tail(Song et al., 2013). Although certain marine environmental conditions could be present for a pathogen existence, finding of relation among host, pathogen, environment was beyond the scope of this study. Through the more understanding of environmental condition, we can get the better understanding, which could be the more unstressful circumstance for the host from the pathogen. We found two marine invasive species at the water-pumping seabed at F3. The excessive input of organic matters from flounder culture grounds were considered as the major factors disturbing benthic community, in particular influent seabed at F3 located in semi-enclosed coastal waters. Because Kudoa can use these invasive species as host (Iijima et al., 2012; Ogawa, 1996), we preserved classified benthic species for genetic detection of Kudoa related to this study(<Table 4>). The more extensive studies about Kudoa host species are required to find the unknown life cycle of Kudoa.

The optimal growth of the flounder documented 15 – 25 ℃(Cho et al., 2006a; 2006b), however water temperature at culture ground ranged 26.4 – 27.2 ℃ at F1. The water temperature is one of the most important influences on the immune system of the fish. The optimal temperature could differ from local circumstances, but it could affect the growth

<Table 4> Observation reselts overview. The colored data means the exceeded value of optimal circumstances of flounder aquaculture.

Content		F1 (Kudoa+)	F2 (Kudoa-)	F3 (Kudoa+)	F4 (Kudoa-)	Optimal criteria	References
Biological (Influent seabed)	Benthic species	19 species of polychaetes	14 species of Polychaetes	11 species 2 invasive species	-	Invasive species	Bax et al., 2003
Chemical (Culture grounds)	Temp	17.6-27.2	18.0-23.2	16.1-18.9	15.2-19.4	15-25 ℃	NFRDI, 2006
	Sal	31.04-34.90	29.03-31.48	30.74-32.32	30.44-30.89	≥ 20 ‰	NFRDI, 2006
	pH	7.75-8.40	7.67-8.35	7.52-8.06	7.35-8.12	6.5-9.0 $mg{\cdot}L^{-1}$	Lawson, 1995
	DO	4.73-8.03	7.74-10.02	6.43-8.45	5.43-8.43	≥ 5.0 $mg{\cdot}L^{-1}$	Phillips et al., 2009
	TN	0.20-0.69	0.41-0.74	0.49-0.78	0.65-1.24	-	Lawson, 1995
	TP	0.02-0.11	0.04-0.20	0.06-0.11	0.07-0.20	≤0.20 $mg{\cdot}L^{-1}$	Jeju-do, 2011
	COD	0.24-1.57	0.11-2.58	0.10-0.80	0.10-1.12	≤2.00 $mg{\cdot}L^{-1}$	Jeju-do, 2011
	SS	1.00-12.70	1.80-9.10	2.00-8.60	1.20-11.00	≤3.00 $mg{\cdot}L^{-1}$	Jeju-do, 2011
Physical (Effluent water)	Residence Time	4h 32min (4km)	11hr 21min (4 km)	9hr 50min (4km)	-	-	NFRDI, 2006

rate and become dangerous situation when it exceeds 28 ℃. In order to minimize the stress and pathogen activities on fish farms, maintaining strategy how to control the good water conditions such as proper temperature, oxygen, pH are needed.

Generally, most of the countries use an average range of between 6.5 – 9.0 as a acceptable pH levels for marine aquaculture(Lawson, 1995). Physical condition such as water temperature, salinity, altitude can also affect dissolved oxygen level. Most of the countries listed below had set greater than or equal to 5 $mg{\cdot}L^{-1}$ as an ideal DO concentration for marine aquaculture(Phillips et al., 2009). In this study, pH levels were acceptable, but DO levels at F1 was lower than 5 $mg{\cdot}L^{-1}$.

The nitrogen enters into the flounder culture system through influent waters, uneaten feeds, and flounder excretion. There is no consensus yet on the permissible levels of total nitrogen(Lawson, 1995), but each country use several parameters such as ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen with different criteria. The phosphorous concentrations is measured by the total phosphorous in this study. The acceptable level of phosphorous shows a little difference in each country, acceptable level of TP is less than or equal to 0.20 mg L^{-1}.

The COD used to measure the amount of organic compounds in flounder culture grounds. The value of COD determines the organic pollutants from unused feed, metabolic wastes in waters. The Jeju-do and other local authorities in Republic of Korea have a required COD standard which less than or equal to 2 $mg{\cdot}L^{-1}$ while normal and 5 $mg{\cdot}L^{-1}$ while feeding(Jeju-do, 2011). In this study, the values of COD were acceptable in F1, F3, F4, but it exceeded at F2.

The SS in water measured as total suspended solids in this study. The value of SS can be an indicator of higher concentration of bacteria, unused feed, metabolic pollutants. The most Asian countries do not have a specific standard for SS, but Jeju-do and other local authorities in Republic of Korea have a required standard which less than or equal to 3 mg·L^{-1} while normal and 10mgL^{-1} while feeding(Jeju-do, 2011). The values of SS exceeded the critical point based on the guideline of effluent water quality at all stations. The excessive feeding within the limited space of fish farms and high stocking density cause the productivity decrease(Salari et al., 2012). The marine environmental impacts are required to assess according to properly improved feed formation and control of stock density.

The limit of this study was the total number of experiment gear for residence time, weakness of bottle, time depending results, etc. We used only 1 gear per station. It has a buoyance tapped by the glass bottle, it could be broken due to the strike the coastal stone. Thus, we need to make a more safe cover for this machine. Even though it shows very strong current and tides in Jeju, we need more experiment depending on the ebb tide and flood tide.

3. Scoring of environmental stressors

When the marine environmental factors are beyond its normal level of tolerance, fish got certain stress and placed in a situation (Francis-Floyd, 2009). The biological factors are equivalent for population density, pathogenic microorganisms, potential host for Kudoa. The chemical factors are relevant for water quality parameters, diet composition, and metabolic wastes. In this study, water temperature and salinity in culture grounds were included in the chemical factors. The physical factors are equivalent for residence time. Before fish become sick or contaminated by pathogen, certain environmental factors are present such as poor water quality, highly concentrated suspended solids, excessive crowding. Although direct relation between Kudoa and marine environment could not define well based on this study result, we could list certain environmental stressors including biological, chemical, and physical group based on results of this study. In order to address the marine environmental stressors, scoring system using geographical information systems have become of increased significance for assessment(Perez et al., 2003; Nath et al., 2000). The more environmental factors we have, we could get the better understanding why and what stressors related to the fish diseases.

<Table 5> The score of marine environmental stressors in Jeju-do(*indicate the double weight considering potential relation between marine invasive species and Kudoa)

Content		F1 (Kudoa+)	F2 (Kudoa-)	F3 (Kudoa+)	F4 (Kudoa-)
Biological group		-	-	☹☹*	-
Sub-score		0	0	4	0
Chemical group	Temp	☹	-	-	-
	Sal	-	-	-	-
	pH	-	-	-	-
	DO	☹	-	-	-
	TN	-	-	-	-
	TP	-	-	-	-
	COD	-	☹	-	-
	SS	☹	☹	☹	☹
Sub-score		3	2	1	1
Physical group		-	-	-	-
Sub-score		0	0	0	0
Total score		3	2	5	1

☹: Indicators exceeded the standard values

In order to give a score of environmental stressor for olive flounder, we combined the weighted linear combination method(Malczewski, 2000). A hierarchical modeling scheme used to identify the environmental stressors. Considering Kudoa, we adopted the weight of suitability class. The maximum score of each group is alloted to four. In case of finding marine invasive species, we gave weight of two as a score of biological group in each species. As these species negatively modifies habitat with large densities and could be the host for Kudoa at bottom seabed(Ohnishi et al., 2013), we highly considered the importance of existence of marine invasive species at influent bottom.

We scored four at F3 based on two invasive species multiply by weight two. In chemical group only four most important parameters such as temperature, DO, COD, and SS were considered to add the score. Because values of salinity, TN, and TP in all stations were acceptable, and scores of TN and TP could add repeatedly with COD just in case. At F1, sum of score was three, because water temperature exceeded 25 ℃ and DO was less than 5 $mg{\cdot}L^{-1}$ and concentration of SS exceeded 3 $mg{\cdot}L^{-1}$ which is the critical point for sustainable management. At F2, the concentration of COD and SS exceeded the standard value, so we scored two. At F3, the value of SS exceeded the guideline and found two marine invasive polychaetae, thus we scored five. We did not find any physical stressors in all stations. The maximum score of environmental stressors was five at F3, and minimum was one at F4. The total score of F1 was score of three and F2 was score of two.

The more studies on marine environmental stressors for olive flounder are required to conduct as are liable method including socio-economic group and environmental group.

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