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Effect of Several Seaweeds on Gut Regeneration of the Sea cucumber, *Apostichopus japonicus*

by

Hashim C. Muumin

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

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해삼 *Apostichopus japonicus* 창자

재생에 미치는 해조류의효과

Advisor: Prof. HONG, Yong-Ki

by

Hashim C. Muumin

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Hashim C. Muumin

Approved by :

(Chairman) Prof. CHANG Young Jin

(Member) Prof. KIM Hyun-Woo

(Member) Prof. HONG Yong-Ki

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Chapter I

Effect of Several Seaweeds on Gut Regeneration of the Sea cucumber,

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Hashim C. Muumin

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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Abstract

In the present study we examined the importance of Seaweed in alteration of *Apostichopus japonicus* (Selenka) body parts. Sea cucumber of Size 29.0±11.0gm and 10.0±3.0cm were fed powder of 14 different Seaweed species in 14days after 7days of recovery from evisceration which was induced by injecting 0.7M of KCl which is 4% of their body weight. After total 21days of culturing, body weight, body length, gut weight growth rate and gut length growth rate were evaluated in each group. The results showed that *Laminaria japonica, Ulva pertusa, Lomentaria catenata* and *Gelidium amansii* have higher effect in weight gain ranged between 33±4.2gm and 25.75±2.05gm while *Sargassum honoreri* and *Enteromorpha linza* showed the least weight gain of 20.3±3.76gm and 11.0±0.0gm respectively. *Laminaria japonica, Lomentaria catenata, Ulva pertusa* and *Scytosiphon* *lomentaria* showed increased body length effect ranged between 14.1±1.3cm and 11.0±0.5cm while *Carpopeltis comea, Hypnea pannosa* and *Sargassum hemiphyllum* showed the least effects ranged between 9.4±0.85 and 9.0±1.0cm. The gut weight to body weight ratio was significantly higher in *Ecklonia cava, Undaria pinnatifida* and *Gelidium amansii,* which ranged between 0.1085±0.01mg/gm and 0.0512±0.01mg/gm while significantly lower ratio was observed in *Ulva pertusa, Hypnea pannosa* and *Lomentaria catenata* which ranged between 0.0165±0.004mg/gm and 0.0121±0.0034mg/gm. In the contrary, gut length to body length ratio was significantly higher in *Undaria pinnatifida, Sargassum thunbergii* and *Ulva pertusa*, which ranged between 1.0513±0.16mm/cm and 1.022±0.265mm/cm but *Ecklonia cava, Sargassum hemiphyllum*, *Chondrus ocellatu*s and *Gelidium amansii* showed little effects ranged between 0.9005±0.16mm/cm and 0.813±0.05mm/cm. *Scytosiphon lomentaria, Laminaria japonica*, *Sargassum honoreri, Carpopeltis comea, Lomentaria catenata, Ecklonia cava* and *Hypnea pannosa* showed least effects ranged between 0.5859±0.04 and 0.3651±0.03mm/cm. These suggest that Seaweeds can play important role in improving the quality and quantity of Sea cucumber body parts including guts which can be utilized as nutritional source.

Key words: *Apostichopus japonicus*, Sea cucumber, Evisceration, Gut regeneration, Nutritional source.

1. Aquaculture of Sea cucumber

Global aquaculture production is still continued to grow in recent years (FAO, 2012) especially in developing countries where marine aquaculture sector is leading (Troell *et al.,* 2009). Rapidly Coastal aquaculture investments draws attention since there is promising opportunity for large-scale expansion of the aquaculture industry (Troell *et al.,* 2009). Hence production of crustaceans, shellfish and fish has to increase to satisfy the growing demand and compensate for the reduced capture from overexploited fisheries (Bosma and Verdegem, 2011).

There are about 1,400 species of Sea cucumber worldwide with more than 66 species harvested commercially (FAO, 2011; Purcell *et al.,* 2012). The tropical species belongs to families Holothuriidae and Stichopodidae while temperate species to the family Cucumariidae as an important commercial species in Northeast Asia and Far East (Russia, China, Japan and Korea) (Purcell *et al.,* 2012).

Sea cucumbers are commonly harvested worldwide due to the important of their body wall (beche-de-mer), ovaries and the intestines (Purcell, 2010; Toral-Granada et al. 2008; Akamine, 2004), where they believed to have aphrodisiac, curative (in wound, impotence, constipation, frequent urination and stomach ulcers), and medicinal properties as tonic and pain killer (Purcell, et al., 2012; Purcell 2010; Toral-Granada et al. 2008; Chen, 2004; Choo, 2004).

Holothurians in most cases are consumed raw in domestic markets with vinegar and soy sauce (Akamine, 2011) while dried products are consumed globally (Purcell, *et al.,* 2012; Yuan, *et al.,* 2010;) are also economically valuable (Akamine, 2011; Purcell 2010; Toral-Granada et al. 2008; Akamine, 2004), which are commonly exported to Asia (Giraspy, and Grisilda, 2008; Purcell, *et al.,* 2012), some American and European countries (Ferdouse, 2004; Gamboa *et al.,* 2004). This led to worldwide declines in stocks of tropical Sea cucumbers which have encouraged aquaculture, stock restoration and enhancement programs (Conand, 2004).

Nowadays, tropical Sea cucumber mariculture become potential industry contributing to natural population replenishment (Purcell, *et al.,* 2012), since methods of juvenile rearing and hatchery techniques are well established (Purcell *et al.,* 2012; Purcell 2010; Toral-Granada *et al.,* 2008Pitt *et al.,* 2004).

The world production through Sea ranching, cages, pond culture and other farming systems are now reaches about three times of wild harvest by dry weight (Purcell *et al.,* 2012). The future perspective of Sea cucumber industry will be sustainable if we will follow holistic approach while culture operations are economically practicable and cost-effective (Ren, *et al.,* 2012).

The purpose of this chapter is to review the existing potential technical methods of aquaculture and production of Japanese sea cucumber, *Apostichopus japonicus*. This includes broodstock selection and spawning, larval and juvenile culture, diets and feeding, diseases and parasites, methods of farming and general management systems of Sea cucumber culture.

1.1. Distribution

Japanese Sea cucumbers (*Apostichopus japonicus*) occurs in shallow coastal waters, in the intertidal zone to 80 m depth in northern regions of Pacific coastal waters of Japan, north eastern China, and Korean peninsula, Sakhalin, Kuril Islands and Alaska (FAO, 2011; Purcell *et al.,* 2012).

Apostichopus japonicus are normally appear in red, green or black in colour, their colours means a lot in business and believed to have their own unique tastes (Kan-no and Kijima, 2003). They are found to appear in different habitats due to water temperature, salinity, substratum and attachment sites, for instant red type inhabits gravel bed while green and black types inhabit sand–muddy bottom inshore (Purcell *et al,*. 2012). This might have resulted in reproductive isolation although there is no clear genetic independence of the red types explained due to only their differences of microhabitat (Kan-no and Kijima, 2003).

1.2. Production

World production of more than 58 species of commonly traded Sea cucumber reaches 100,000 tons of wet weight per year which is more than 16times in 2000s with worth's of more than 130Millions USD (Purcell *et al.,* 2012; Purcell, 2010; Vannuccini, 2004). China only produced 102159tons from Aquaculture in 2009 (Huiling, 2010) and being with his counterpart Hong Kong the largest importers subsequently they consume and re-export half of their imports to European, North American and the United Arab Emirates (UAE) markets (Purcell, 2010; Ferdouse, 2004). In comparison with other traded species, Japanese Sea cucumber *Apostichopus japonicus* are leading in the market price which reaches up to about US$2950 kg^{-1} dried though prices vary with the quality and places (Purcell, Hair and Mills, 2012; Purcell *et al,* .2012).

2. Biological knowledge

Apostichopus japonicus can reach up to more than 30 cm in length and 200g weight with 110g as their size at maturity. Previous studies explained Sea cucumber exhibit ranges of colours in their dorsal and ventral parts from grey, olive green, blue, brown (red) and black (Yamada *et al,.* 2009; Choo, 2008; Yanagisawa, 1998). They have quadrilateral symmetry from anterior to posterior ends with 20 tentacles at ventral mouth and no teeth at terminal Anus (Purcell *et al.,* 2012).

Numerous tube-feet arranged rows on abdominal surface while spiny and supporting rods on dorsal part are rare in their body wall (Purcell *et al.* 2012; FAO, 2011). They have convex area behind the mouth, known as the gonophore (YSFR, 1991) which is a reproductive organ although it is difficult to identify their sex by external appearance (Purcell, 2010). Chen, 2003 noted that each individuals alteast have one genital pore with rare cases of 2 or 3 pores as their spawning functions.

They have longitudinal digestive tract winding two times in their body cavity, with tentacles, mouth, pharynx, oesophagus, stomach, intestine, general cloacal cavity and anus as their main digestive organs (FAO, 2011). They also use respiratory trees and skin as their main respiratory organs (YSFR, 1991).

3. Hatchery production

Knowledge of production is well known worldwide, almost most of hatcheries focused on juveniles of more commercially valuable Sea cucumber species, temperate species, *Apostichopus japonicus*, and tropical species, *Holothuria scabra* (Yuan *et al.,* 2010; Liu *et al.,* 2009; Dong *et al.,* 2008a; Dong *et al.,* 2008b; Giraspy and Ivy, 2008; An *et al.,* 2007; Yuan *et al.,* 2006; Pitt *et al.,* 2004; Huiling *et al.,* 2004; Battaglene and Bell, 1999; Yanagisawa, 1998). The main progresses of seed production have been achieved in the 1980s by using wild broodstock (Zhang and Liu, 1993; Sui, 1985;), however getting viable broodstock being major problem (Handoko, 2002) and repetition of same parents being avoided for better production (Yanagisawa, 1998). Seed production reaches more than 70 billions in 2007 in China while in Japan reaches 2 557 000 in 1994 in only one Institution (Huiling, 2010; Yanagisawa, 1998). This means seed productions have been successful and remarkably increased to satisfy the growing demand although production now should focus on Value addition of Sea cucumber products for maximum profit.

4. Broodstock conditioning and spawning

Artificial production normally starts with Broodstock collection with natural fully matured stocks by farmers or local collectors (FAO, 2011). They are raised by feeding dried brown Algae such as *Eisenia bicyclis* and *Undaria pinnatifida* upto 5 months to increase their Gonad Index (GI) which is a good criterion for assessing spawning time (Yanagisawa, 1998). In spawning season more than 10 million egges can be released by healthy female *Apostichopus japonicus* with respect to body weight (Ito and Kahawara, 1994b; Chen, 2003). This species mature and spawn naturally along the coast from March to May while their maturation are judged by their egg diameter (>150 um) and sperm formation in the gonads (FAO, 2011; Ito and Kitamura, 1998; Ito and Kawahara 1994).

Natural Spawning is induced by heating the gonads and oocytes in sea water (+5˚C) at sizes of 20 and 140 µm respectively within a month. Also spawning can be stimulated through drying and introducing powerful jet water for few minutes which results sperm releasing for male followed by releasing eggs for female after sometime (Ito and Kitamura, 1998; Yanagisawa, 1998). Many artificial spawning methods have been developed and used but the above mentioned are the best and easy to adopt (Chen *et al.* 1991; Kishimoto and Kanatani, 1980; Maruyama, 1980).

4.1. Larval culture

Hatched larvae are collected through frame fitted screens made by polyethylene or polypropylene or Algal polythene sheets coated with algal extractand and raised in tank at density of 0.5 indls/ml. They fed on fresh phytoplankton 5×10^3 to 5×10^5 cells/ml of *Dunaliella euchlaia*, *Chaetoceros muelleri*, *Dicrateria zhanjiangensis, Chlorella sp. Chaetoceros gracilis, Pavlova lutheri* and *Isochrysis galbana* either mixed or single species although better growth rate has been reported by feeding *Isochrysis galbana* 2×10^4 to 3×10^4 (Pangkey, *et al.*, 2012; Chang and Chen, 2008; Yanagisawa, 1998; Ito and Kitamura, 1998). It takes 20 days to 2 months to reach Juvenile stage (10mm) after fertilization although variation depends on types of feed they were given (Chang and Chen, 2008; Ito and Kitamura, 1998; Yanagibashi *et al.* 1984).

4.2. Juvenile rearing

Rearing Sea cucumber Juveniles needs careful treatment when transferred to tank. They fed on diatoms to 20 mm for 4 months before being transferred to growout although may take up to 6 months in unfavourable conditions (Chang and Chen, 2008; Chen, 2001). The ideal temperature for their surval is 10-26˚C although best growth performance has been reached at 15-18˚C

(Zamora and Jeffs, 2012; Wang, and Cheng, 2011; Sui, 2011; Chen, 2001). Copepods being the major problem at this stage, trichlorofonic chemical are used for their elimination. They grow up to an average of 80 mm (max. 150 mm) in one year (Ito, and Kitamura, 1998) in either farming methods including pond culture, pen culture, bottom culture, and land-based intensive culture (Wang, and Cheng, 2011; Yuan *et al.,* 2006a; Chen, 2004; Chen, 2003). Artificial supplemented feed have been used for years for Sea cucumber aquaculture where dried powder of macroalgae such as *Ulva pertusa, Enteromorpha spp,Undaria pinnatifida, Eisenia bicyclis, Ecklonia cava, Chondrus occellatus, Gelidium amansii, Porphyra tenera, Codium fragile*, *Thallus laminariae*, *Sargassum thunbergii*, *Sargassum fusiforme, Sargassum polycystum, Laminaria japonica, Zostera marina* as the main ingredients of the formulated diet (Yuan, *et al.* 2013; Xia, *et al.* 2011; Sui, 2011; Slater *et al.,* 2009; Yuan *et al.,* 2006; Yanagisawa, 1998). Although many macroalgae species have been used, better understanding may provide useful information for the development of the aquaculture of this species (Zamora, and Jeffs., 2011), since commercial feeds may help to increase production in a hatchery and may also result in a decline (Dabbagh, *et al,.* 2012).

4.3. Disease and parasites

Disease is the main problem affecting Sea cucumber aquaculture Industry. They are facing different diseases in juvenile stage and at growout stages and most those diseases are related with bacteria, virus, parasites and fungus (Wang *et al.* 2004; Hamel *et al.* 2001). Clinical signs of infections include lesions around mouth or anus, stomach oedema, whitish ulcerations on the skin or papillae, excessive mucus on the body, skin discoloration, and changes in behaviour and appearance (Purcell, and Eeckhaut, 2005). Although these are true biotic diseases but illnesses can be from chemical or environmental stressors beyond limit such as excess aeration, inappropriate temperatures, pH, Salinity cause illness and fasten death in Sea cucumber (Li, *et al.,* 2008; Wang *et al.* 2004; Hamel *et al.* 2001).

Holothuroids are reported to provide biotic substrates to microscopic organisms as a source of food and/or as a source of developmental stimuli to complete their life cycle (Eeckhaut *et al,* 2010). They are releasing chemicals as their defensive mechanisms from other organisms but still they can be attacked directly or indirectly by Bacteria, Protozoa and Metazoans. Platyhelminthes, Mollusca and Arthropoda are the taxa that include the highest numbers of holothuroid parasites but low attention has been devoted to holothuroid-associated gregarines and probable the number of infesting species recorded in the future will be higher than that of metazoan taxa (Eeckhaut *et al,*. 2010).

Bacterial diseases in *Apostichopus japonicus* has been studied over recent years where skin ulceration disease found to spread very quick and affect two thirds of the juveniles within a short period with massive death after first symptoms of its detection (Zhao, *et al.* 2012; Zheng, *et al.* 2011; Wang, *et al.* 2011; Liu, *et al.,* 2010; Li, *et al.,* 2008; Wang, *et al.* 2007). The clear sign of the disease is appearance of mouth tumidity, skin ulceration, enlarged ulcers and massive death. So far there are three species identified as pathogenic to Sea cucumber which are *Shewanella marisflavi, Vibrio splendidus* and *Pseudoalteromonas tetraodonis* (Zhao, *et al.* 2012; Zheng, *et al.* 2011; Wang, *et al.* 2011; Liu, *et al.,* 2010; Li, *et al.,* 2008; Wang, *et al.* 2007).

Protozoa are ciliates living in the digestive tract and in the respiratory trees of holothuroids have been reported by Barel and Kramer (1977). Sea cucumber are very sensitive to gregarine infestations since they have six complex developmental stages, sporocysts, sporozoites, trophozoites, gamontes, gametocysts and sporogonia. *Boveria labialis* are Protozoans found in respiratory trees of the Sea cucumber, *Apostichopus japonicus* (Long*, et al.,* 2006).

Platyhelminthes are among parasites found in Sea cucumber aquaculture and simply known as parasite Platyhelminthes which were observed in the skin, subcutis and even deep tissues of Sea cucumber. This parasite temporarily named as *A. yapomcws-platyhelminth* and *Rhabdocoela* are also included in *Apostichopus japonicus* infecting groups (Eeckhaut et *al.,* 2004). Annelida are commensal polychaetes in Sea cucumber which are simply attached to the surface by their parapodia although are considered totally harmless to their hosts (Eeckhaut *et al.,* 2004). Britayev and Lyskin (2002) mentioned *Gastrolepia clavigera* as the commonest Indo-Pacific polychaete found to feed on spicules and integuments of their hosts that means symbiotic polychaetes could be true parasites, concurrently play roles in retarding growth and weaken health of Sea cucumber (Eeckhaut *et al.,* 2004).

Mollusca group have some effects in aquaculture of *Apostichopus japonicus* with four Bivalvia lives in their cloaca or digestive wall of synaptid while 33 Gastropoda are known to infest them by wounding and penetrates in respiratory tree, digestive tract or coelom using their proboscis (Jangoux, 1990). *Megadenus cantharelloides* found to feed on dermal tissue of digestive wall of *Stichopus chloronotus* (Humphreys and Lützen, 1972) which gives warning sign that the same species can affect *Apostichopus japonicus* as they belong in the same family. (Eeckhaut *et al.,* 2004)

In arthropods 12 pinnotherid crabs were reported to affect in most cases wounding holothurians whereby most of them live in respiratory trees or in digestive tract forexample *Pinnotheres decanus,* is rarely found in the coelomic cavity while *Lissocarcinus orbicularis* often found on the host's body surface. In contrary there are four classes of copepods that live symbiotically with holothurians and 85 cases their association have been described so far (Eeckhaut *et al.,* 2004).

Pisces are affected by holothurin (toxic) but pearl fish (Carapidae, Ophidiiformes) are living with different invertebrates by making protective nacreous substances (Eeckhaut *et al.,* 2004; Ballard, 1991). Also *Carapus spp.* and *Encheliophis spp* are usually found in the respiratory trees or in the coelomic cavity and hence considered as true parasites, feed on the internal tissues of their hosts (Markle & Olney, .1990).

4.4. Diseases and Control Measures

Sea cucumber diseases lack detailed information on appropriate control measures however different studies have been done to some extent on control measures in general (Zheng *et al.,* 2011; Lavitra *et al.,* 2009; Li, 2008; Eeckhaut *et al.,* 2004). Currently, acute peristomal oedema disease which is caused by virus (*Corona viridae*) has no treatment. On the contrary bacterial diseases such as rotting edge, Stomach ulceration, off plate syndrome, Skin ulcer disease and ulceration syndrome which are caused by *vibrio spp* and unidentified spp have no guarantee measures. This is to say the diseases might not be controlled or might be controlled by antibiotics such as Terramycin, acheomycin, Kanamycin, Neomycin, Norflaxacin, Ciproflaxacin, Azithromycin and TMP+SMZ (Trimethoprim+ Sulfamethoxazole). For research level seems work although it is not advisable and impractical for commercial aquaculture. Furthermore for fungal, protozoan and Platyhelminthiasis diseases has no treatment other than harvesting and disinfecting the farm. Copepod predation from *Microsetella* spp and *Tigriopus japonicus*, trichlorphon is useful if permitted but also advised to eliminate copepods population through filtration in 45 µm mesh (Li, *et al.,* 2008; Eeckhaut *et al.,* 2004).

5. Sea farming

There are three methods (Chen, 2003) used for farming Sea cucumber: pond culture, pen culture, and sea ranching or sometimes called bottom culture. Recently another two methods emerge which are co-culture (IMTA) and Cage culture in abalone cage reported (Xing *et al.,* 2012; Sun, 2010), thus make five methods of aquaculture of Sea cucumber. Stock enhancement is not exactly aquaculture but leads to increase production of Sea cucumber in sustainable manner and compensate for the reduced capture from overexploited fisheries (Bosma and Verdegem, 2011). Since bottom culture needs lower investment but leads to higher return, therefore this culture accounts for 75 % of total area of culture (Chen, 2004). The following are currently existing Sea cucumber aquaculture systems.

5.1. Pond grow-out

In recent years, farming Sea cucumber in ponds (eathern or Cofferdam Culture) has become very popular in China (Sun, 2010; Chen, 2003). The pond conditions are ideal for culture Sea cucumber in site since they enjoy their necessity in the substrate as well as protection from predators (Chen, 2003). Normally stones, tiles, bricks or non toxic materials (around 2 250 m^3 per hectare) are introduced and arranged carefully in the ponds before introducing seawater for the intension of making artificial home for Sea cucumber. 10 g seed weight of Sea cucumber can reach mean weight of 150 g within five months of the same year (Chen, 2003).

5.2. Co-culture

Integrated multi-trophic aquaculture (IMTA) has the potential to reduce environmental impacts and increase effectiveness of intensive monoculture systems (Neori *et al.,* 2004). It has been adopted internationally (Ridler *et al.,* 2007; Troell *et al.,* 2003; Chopin *et al.,* 2001) and shown that aquaculture derived nutrients are able to be removed by seaweed biofilters (Buschmann *et al.,* 2008). Sea cucumbers are detritus feeders in sediment (Kitano *et al.,* 2003) which found to be a good candidate for co-culture in IMTA systems with either cultured finfish (Ahlgren, 1998), shrimp (Purcell *et al.,* 2006; Pitt *et al.,* 2004), bivalves (Paltzat *et al.,* 2008; Slater and Carton, 2007; Zhou *et al.,* 2006) or abalones (Kang *et al.,* 2003).

Apostichopus japonicus can change aquaculture waste into good products through IMTA system, for instant they reduce inorganic matter through feeding organic matter when cultured with Abalone while improving growth and survival of both animals (Kang *et al.* (2003). Also they grow faster and survive well under Sea urchin cage (Ito, 1995), hanging scallop lantern net (Zhou *et al.* 2006) and fish cages (Yokoyama, 2013).

Their good growth and survival under the suspended culture is due to conditions of high salinity, low temperature, and oxygenation as well as being away from benthic predators (Hu *et al.,* 2010; Dong *et al.,* 2008; Yamamoto *et al.,* 2003; Hatanaka *et al.,* 1994) and also they have abundant supply of food originating from fish feed, bivalve and fish feaces (Yokoyama, 2013; Zhou *et al.* 2006; Kang *et al.*, 2003; Ito, 1995).

5.3. Farming in sea pens

Pen is a fixed enclosure in which the bottom is the bed of the water body (Kutty, *et al.,* 1987). Normally in this culture at high tide, the seawater naturally flows into the pond by means of gravity; at low tide, the water in the pond is about 80-100cm in depth. This method saves a great deal of energy that would otherwise be used for pumping water; meanwhile, incoming seawater brings organic debris into the ponds which provide a good food sources for the Sea cucumber. Therefore, this is low cost and highly efficient model, but is done in limited area along the coast (Chen, 2004).

5.4. Cage farming

Coastal environment will be recorved if future aquaculture investment focus on offshore and reduce stress along the shore areas. The aquaculture of Sea cucumber in Abalone caged has been successfully reported in recent years where by cages are supended from rafts and anchored with longlines in sand sea bottom (Xing, *et al.,* 2012; Tuwo, 2004;). They feed on fermented *Laminaria japonica* and *Undaria pinnatifida* and showed 100% survival (Xing, *et al.,* 2012) which is practically good for the commercial aquaculture of the Sea cucumber. The Cage farming of Sea cucumber is advantageous due to its accessibility, cost-efficiency, relative protection against storms and predation (Xing, *et al.,* 2012).

5.5. Sea ranching

Sea ranching of Sea cucumber was initiated by Yellow Sea Fisheries Research Institute in 1980 (Chen, 2003) after experimental researches in Japan in late 1930s (Sui 1988), Hebei province in 1950s (Zhang, 1958) and Shandong Province in 1970s (Zhang and Liu, 1998) and has become very popular in recent years (Giraspy and Ivy, 2005). Sea ranching is more reasonable compared with aquaculture in terms of investment and water management although proper site selection, good environmental condition, monitoring predators and appropriate sizes are needed for successful for sea ranching. The previous studies confirmed that sea ranching is an efficient model for recovering, increasing resources and relieve burden on natural resources (Purcell, Hair, and Mills, 2012; Olavides, *et al.* 2011; Bell, *et al.* 2008; Chen, 2004).

5.6. Stock Enhancement Program

The overfishing for beche-de-mer created more attention in managing them sustainably due to exploitation of many species of Sea cucumber (Dance, *et al.* 2003). Advancement of aquaculture technology shorten time for production and rising of juveniles for stock enhancement (Battaglene, 1999; Bell and Gervis, 1999), It has been practiced in Japan since the 1980s (Masaki *et al.,* 2007; Tanaka, 2000) and gaves promise through increasing productivity close to carrying capacity of the habitat (Doherty, 1999; Munro and Bell, 1997;). In few cases released population found to decrease rapidly due to dispersion and/ or mortality (Masaki *et al.,* 2007; Tanaka, 2000). Therefore, enough information is needed to identify exactly areas of releasing healthy seeds to minimize their mortality as well as frequent monitoring for causes of their dispersions.

6. Management issues

The potential benefit of a restocking program for Sea cucumber can realized through effective management of the released animals (Strehlow, 2004) although effective management plans for Sea cucumber fisheries are uncommon (Toral-Granda, *et al.,* 2008) due to unrealistic analytical models for managing Sea cucumber resources (Hassan, 2005). In most cases, overfishing resulted due to management problems (Altamirano *et al.* 2004; Conand 2004) such as inappropriate regulations, weak enforcement, inadequate collection and management of statistics (Rasolofonirina, 2007). Sea cucumber aquaculture is currently considered to be the best solution to manage exploitation (Lavitra, 2008), since all potential methods and techniques of production, operational management and processing of Sea cucumber are in place.

6.1. Threats of Exotic Species and Pollution

Exportation of exotic species from their origin and hatchery operation is very crucial in aquaculture and it is likely can results genetic risks. Limited genetic diversity can alter next generation in aquaculture or wild populations through restocking programs (Eeckhaut *et al.,* 2012; Léonet *et al.,* 2009; Bell *et al.,* 2005). Most of the literature focus on genetic risks of stocking hatchery-reared marine fish (Hindar *et al.,* 1991; Le Vay *et al.,* 2007), there fore more studied are needed to reveals the important of ecosystem survices and their potential risks for proper management of Sea cucumber fisheries and aquaculture programs (troell *et al.,* 2009). There are common risks from aquaculture that we need to be aware off such as habitat alternation, excessive water consumption, organic pollution, eutrophication, chemical contamination, infectious diseases, genetic risk of escaped culture animals, and depletion of wild fish stock for seeds and fish feed (Leung and Dudgeon, 2008; Phillips and Subasinghe, 2008).

7. Conclusions

Aquaculture of Sea cucumber is a promising business all over the world leading to maximum profit and can potentially be done socio-economical and eco-friendly ways since maximum knowledge of systems and whole process are in place for quite some time. The truth will remain that there are some sea weed species which are plenty until now and have never been used or have been used to small extent but if we use for Sea cucumber culture will increase production with very low costs of feeds. There have been a lot of dreams in management actions so called effective management actions involving local community, Governments, NGO's, Regional and

International organizations but still aquaculture will remain the best solution of over-exploitation of Sea cucumbers all over the world and at all levels although is expected to remain focused on the high-value species (Purcell, Hair, and Mills, 2012).

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Chapter II

Effect of Several seaweed on Gut Regeneration of the Sea cucumber, *Apostichopus japonicas*

Abstract

In the present study we examined the importance of seaweed in alteration of *Apostichopus japonicus* (Selenka) body parts. Sea cucumber of Size 29.0±11.0gm and 10.0±3.0cm were fed powder of 14 different seaweed species in 14days after 7days of recovery from evisceration which was induced by injecting 0.7M of KCl which is 4% of their body weight. After a total 21days of culturing, body weight, body length, gut weight growth rate and gut length growth rate were evaluated in each seaweed group. The results showed that *Laminaria japonica, Ulva pertusa, Lomentaria catenata* and *Gelidium amansii* have higher effect in weight gain ranged between 33±4.2gm and 25.75±2.05gm. Meanwhile, *Sargassum honoreri* and *Enteromorpha linza* showed the least weight gain of 20.3±3.76gm and 11.0±0.0gm respectively. *Laminaria japonica, Lomentaria catenata, Ulva pertusa* and *Scytosiphon lomentaria* showed increased body length effect ranged between 14.1±1.3cm and 11.0±0.5cm while *Carpopeltis comea, Hypnea pannosa* and *Sargassum hemiphyllum* showed the least effects which ranged between 9.4±0.85 and 9.0±1.0cm. The gut weight to body weight ratio was significantly higher in *Ecklonia cava, Undaria pinnatifida* and *Gelidium amansii,* which ranged between 0.1085±0.01mg/gm and 0.0512±0.01mg/gm while significantly lower ratio was observed in *Ulva pertusa, Hypnea pannosa* and

Lomentaria catenata which ranged between 0.0165±0.004mg/gm and 0.0121±0.0034mg/gm. In the contrary, the gut length to body length ratio was significantly higher in *Undaria pinnatifida, Sargassum thunbergii* and *Ulva pertusa*, between 1.0513±0.16mm/cm and 1.022±0.265mm/cm range. However, *Ecklonia cava, Sargassum hemiphyllum, Chondrus ocellatus* and *Gelidium amansii* showed little effects ranged between 0.9005±0.16mm/cm and 0.813±0.05mm/cm. *Scytosiphon lomentaria, Laminaria japonica, Sargassum honoreri, Carpopeltis comea, Lomentaria catenata, Ecklonia cava* and *Hypnea pannosa* showed least effects ranged between 0.5859±0.04 and 0.3651±0.03mm/cm. These suggest that Seaweeds can play important role in improving the quality and quantity of Sea cucumber body parts which can be utilized as good nutritional source.

Key words: *Apostichopus japonicus*, Sea cucumber, Evisceration, Gut Regeneration, Nutritional Source.

1. Introduction

The ability of echinoderms to repair their injured or autotomized body parts has been well known (Hyman, 1955; Candia, 2006). They can completely discard most of their internal organs and rapidly regrow them asexually (Mashanov and Garcia-arraras, 2011). This occurs when they face any physical, chemical or environmental stresses and under threat of predation (Smith and Olds, 2011). Sea cucumbers, or holothurians, are exclusively marine invertebrates classified in this phylum Echinodermata; class Holothuroidea (Mashanov and Garcia-arraras, 2011).

Then, they are characterized by a relatively long life span, estimated at about four to ten years (Storelli *et al.,* 2001). They constantly renew cells in their adult tissues, including the digestive tube (Mashanov *et al.,* 2004) and regenerate the same structure multiple times over their lifetime (Mashanov *et al.,* 2010). Visceral regeneration in Sea cucumbers provides a promising system for fundamental questions of regenerative biology (Mashanov and Garcia-arraras, 2011). Gut regeneration in Sea cucumbers points to a connection between development and regeneration (Smith and Olds, 2011).

Furthermore, Sea cucumber is one of the sea treasures which have been used not only as luxury food for certain countries but also as medicines (Pangkey *et al.,* 2012). Sea cucumber's diet comprises of mainly inorganic compounds, organic detritus of macroalgae, decaying animals, and microorganisms (Dar and Ahmad, 2006). Powdered macroalgae and sea mud have been used as the main components of formulated feeds in semi-intensive sea cucumber culture systems (Liu *et al.,* 2010). In north of China *G. lemaneiformis*, *S. thunbergii, S. polycystum, Z. marina, U. lactuca* and *L. japonica,* are the most common seaweeds used in feeding cultured sea cucumbers (Xia *et al.,* 2012; Gao *et al.,* 2011; Seo *et al.,* 2011; Liu *et al.,* 2010; Yuan *et al.,* 2006). *Apostichopus japonicus* is the most culture species in China due to its high quality meat and the success of the method used for its culture (Purcell *et al.,* 2012; Purcell 2010; Toral-Granada et al. 2008; Huizeng, 2001).

Knowledge to feed larvae and juvenile sea cucumbers is well known, while the feed for the rearing (grow-out) of sea cucumbers, yet to be studied in detail (Pangkey *et al.,* 2012). Therefore, the present study is designed to evaluate the effect of several seaweeds on the rate of gut regeneration and potential significance in the future production of Sea cucumber products.

2. Materials and Methods

2.1. Stimulation methods

Sea cucumbers of same size (i.e. 29.0±11.0gm and 10.0±3.0cm) were brought from Namcheong fish Market, Busan and Acclimatized for one day in the Aquarium. Several physical and chemical stimulation methods were used to induce evisceration safely and use them for feeding experiments. In case of chemical methods, Potassium chloride, Sodium chloride, urea, methanol, ethanol, ammonia, chloroform, concentrated sea water and distilled water were injected into sea cucumbers' body at different concentration via coelom. In case of physical methods, stimuli such as sonication, shaking, electric shocks and forceps were used through changing their different working parameters including time, revolution, strength and volts.

Sea cucumbers were induced evisceration by injecting 0.7M KCl (4% of body wet weight) into the coelom (modified from Tan *et al.,* 2008) and eviscerate after few minutes later then were transferred to glass aquarium (55×30×35 cm, water volume of 200 L) after taking all body measurements (Fig. 2 and 3).

2.2. Seaweed Materials

The different Sea weeds and sand were collected from Gijang-Gun beach, Busan. Sand was washed and sieved to grain size between 250 and 400 µm while seaweed were dried, grinded into fine powder of 250 and 400 µm random sizes. Sand and seaweed powder were mixed at equal volume of 25ml (1:1 ratio) and sea water was added to mix thoroughly into a thick paste to avoid size stratification of grains. The paste made into 2cm pellets before being rapidly frozen at −20 °C. (Fig 4)

2.3. Rearing condition and feeding

The aquarium condition was maintained at salinity ranged between 30 to 32 PSU, pH ranged between 7.8 to 8.2, at temperature of 14±0.4 °C, Light at 14D: 10L and a dissolved oxygen level >6ppm. Ammonia was less than 0.3 mg/L. The flow rate of recirculating aquarium was 3L/min. Eviscerated sea cucumbers were randomly distributed into rectangular boxes which were then transferred to glass aquarium (55×30×35 cm, water volume of 200 L) with 5 individuals per box. The eviscerated sea cucumbers were left for seven days of wound healing and recovery (Mashanov and Garcia-arrara, 2011). The pellet seaweed feed directly fed to the animals after seven days of recovery and after every 48hours with the amount of 3% of body wet weight. Remained feeds were collected and measured. Similarly feaces and debris were removed an hour before new feeding.

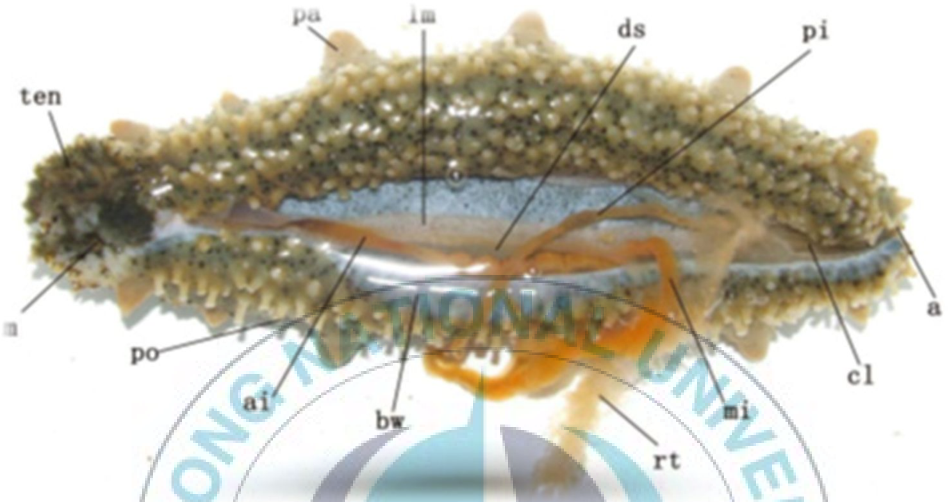

Figure.1. Internal parts of Sea cucumber: m=mouth, po=podia, ai=anterior intestine, bw=basement wall, rt=respiratory tree, mi=middle intestine, cl=cloaca, a=anus, pi=posterior intestine, ds=dysentery, lm=longitudinal muscles, pa=papila, ten=tentacle (Sun, 2010).

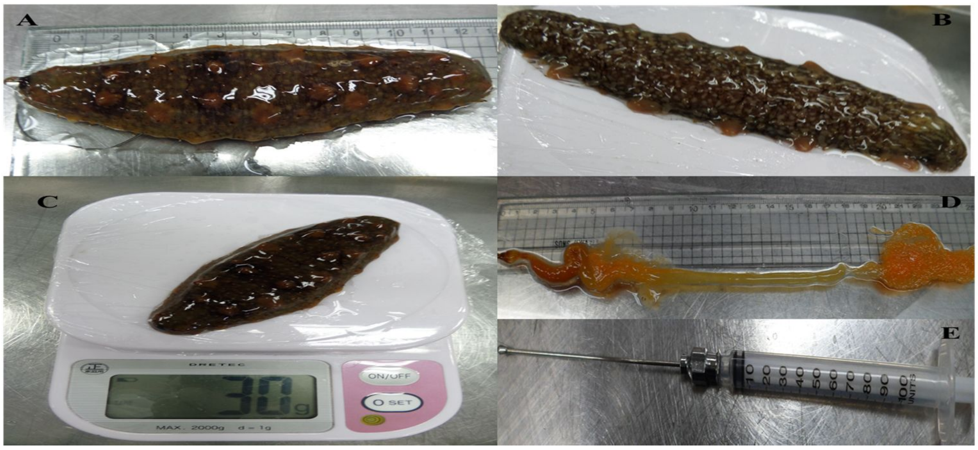

Figure.2. A: Sea cucumber during length measurement, B: Ventral side of Sea cucumber. C: Weighing balance and ruler for measuring weight, D: Gut during length measurement and E: 1ml syringe for injecting KCl into the coelom.

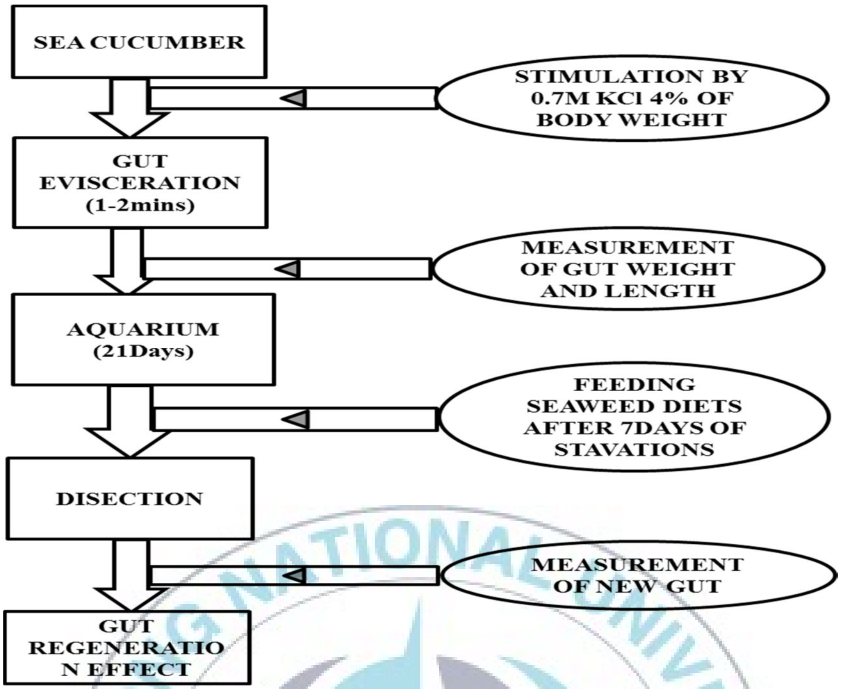

Figure.3. Procedures used for inducing evisceration and Gut regeneration of Sea cucumber, *Apostichopus japonicus*.

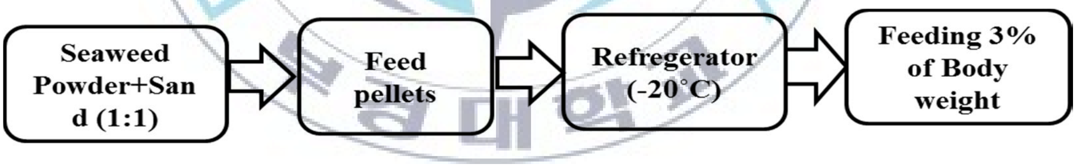

Fig.4. Procedures used for seaweed feeds for feeding experiment.

Figure.5. Mixture of Seaweed powder and sand during feed preparation for feeding.

2.4. Statistical analysis

Data was presented as Mean±SEM. Statistical comparison of the mean values were performed by an analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS software version 17.0 (SPSS Inc., Chicago.IL,USA). *P*-values < *0.05* were considered as statistically significant. Microsoft excel package 2010© also used for drawing Charts.

3. Results

3.1. Effects of different stimulation methods

The effect of both chemical and physical stimulation methods of releasing guts in sea cucumber is reported in table 1. According to the trend of the effects potassium chloride >sonication>distilled water>methanol>electric shocks>sodium chloride >forceps pinch>ethanol>shaker. Significant differences among all stimulation methods were observed (p<0.05).

Table.1. Inducing evisceration by different stimulation methods in Sea cucumber

Type of stimuli and quantity applied	Percentage released	Percentage survival
0.35M KCl (2-24%)	48/48=100	48/48=100*
NaCl (4%)	1/9=11	4/9=44.4
Methanol (1/10 th)	1/7=14.3	4/7=57
Ethanol (1/10 th)	0/7=0	3/7=42.86
Chloroform (1/10 th)	2/7=28.6	0/7=0
Distilled Water (4%)	5/7=71.4	7/7=100**
Sonication (5mins)	7/12=58	7/12=58***
Forceps pinch (Gentle-Strong)	0/7	7/7=100
Electric shock (4.5-12V, 5mins)	7/21=33.3	0/21=0
Shaker (247rpm/1hour)	0/3	3/3=100

3.2. Sea cucumber Measurements

The Body weight of Sea cucumber (29.4±9.46gm) and Body length (11.32±1.971cm) were presented in Fig. 5. Significant correlations were observed between body length and wet weight of Sea cucumber *Apostichopus japonicus* (R^2 =0.7724, fig. 6). The gut weight and body weight of sea cucumber were presented in fig. 7 which shows little significant correlations (R^2 =0.7999, fig. 7). Meanwhile gut weight and body weight showed little significant correlation (R^2 =0.7541 fig. 8). On the contrary, the relationship between gut lengths and body weight showed the least correlation values of 0.7015 (fig. 9), followed by relationship between gut length and body length which have correlation value of 0.5258 (fig. 10)

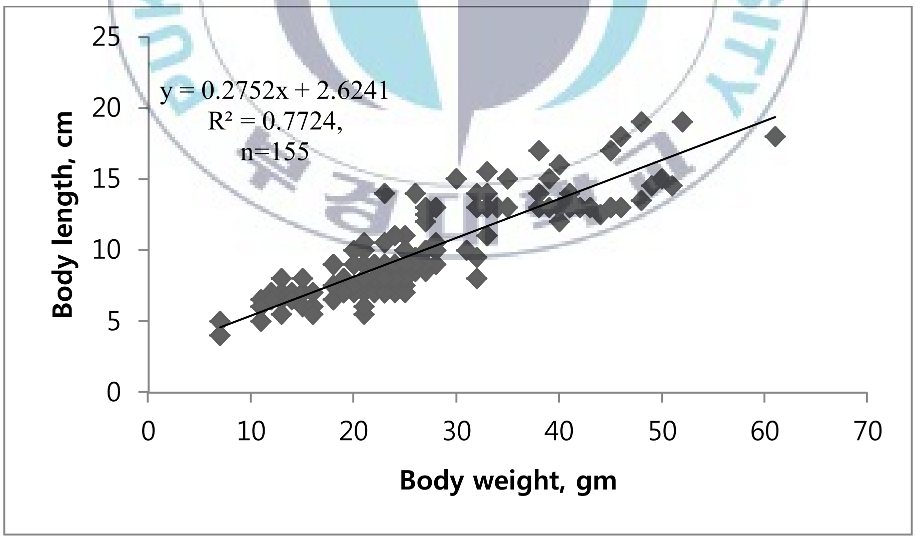

Figure.6. the relationship between body length (cm) and body weight (gm) of sea cucumber, *Apostichopus japonicus*.

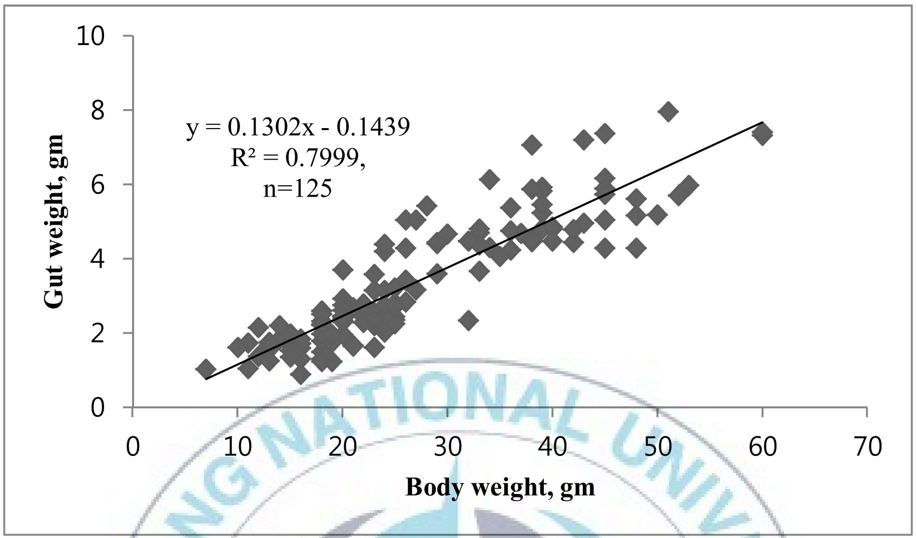

Figure.7. the relationship of gut weight and body weight of Sea cucumber, *Apostichopus japonicus*.

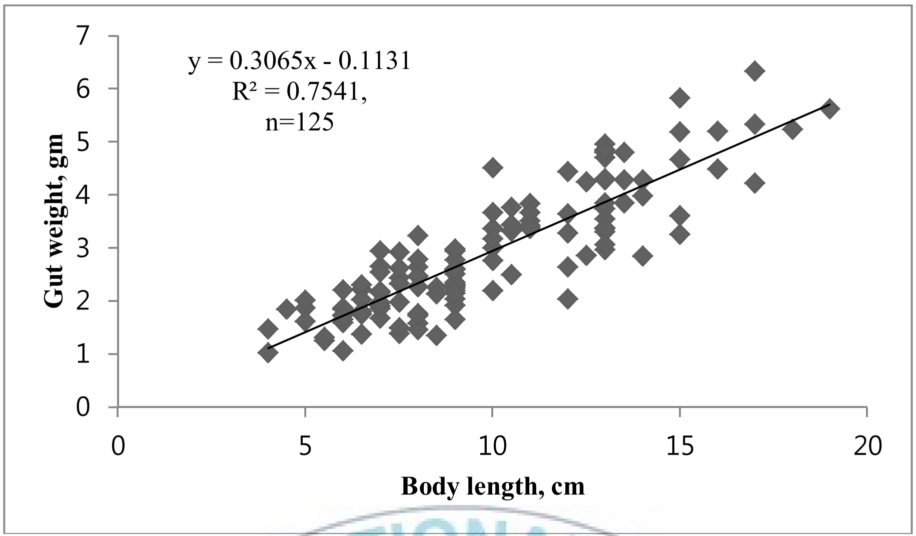

Figure.8. the relationship between gut weight and body length of Sea cucumber, *Apostichopus japonicus*.

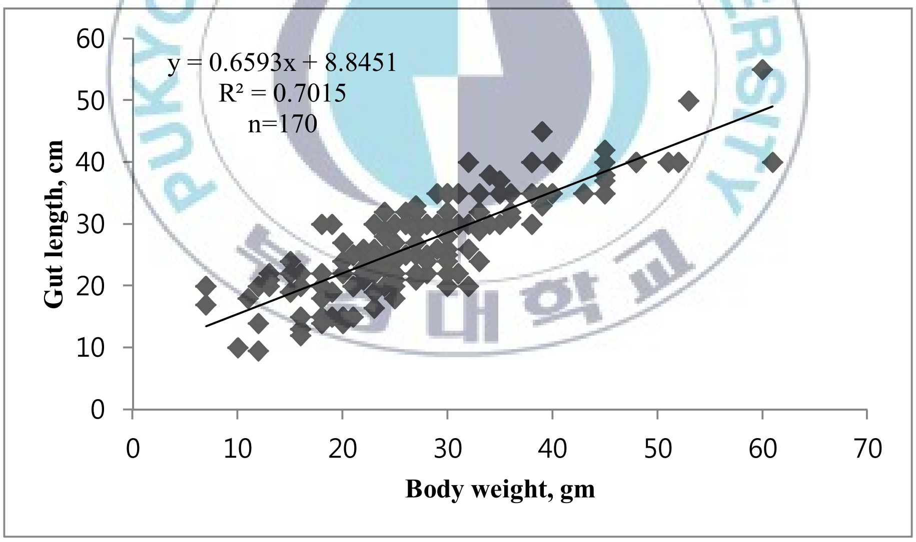

Figure.9. the relationship between gut length and body weight of Sea cucumber, *Apostichopus japonicus*.

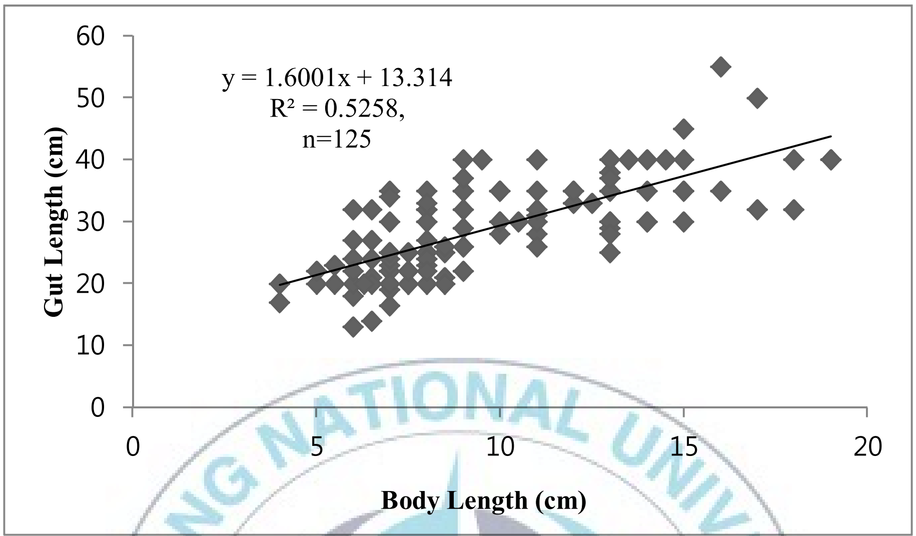

Figure.10. the relationship between gut length and body length of Sea cucumber, *Apostichopus japonicus*.

Table.2. Anova table shows significant differences of seaweed feeds in body weight, body length, gut growth ratio and gut length ratio of Sea cucumber, *Apostichopus japonicus* after 21days of feeding trial

	Body weight (gm)	Body length (cm)	Gut growth (Milligram/gram)	Gut length ratio (Millimeter/centimeter)
P value	0.011	0.003	0.0001	0.0001

3.3. Effects of seaweed on the sea cucumber's body

The different seaweed feeds had shown significant differences among groups of *Apostichopus japonicus* after 14days of feeding trials ($p<0.05$, Table 2 and 3). The body weight showed significant differences where *Laminaria japonicus, Ulva pertusa, Lomentaria catenata* and *Gellidium amansii* showed higher body weight gain of 33±4.12gm, 29.25±4.9gm, 27.5±6.1gm, 25.75±2.05gm respectively. Meanwhile little weight gain effects was observed upon feeding with *Scytosiphon lomentaria, Undaria pinnatifida, Chondrus ocellatus, Hypnea pannosa, Sargassum thunbergii* and *Ecklonia cava* 22.2±3.6gm, 22±4.6gm, 22±4.06gm, 20.3±3.76gm, 19.5±3.4gm and 18.25±2.2gm respectively. The least body weight gain was observed upon feeding with *Sargassum honoreri* and *Enteromorpha linza* with coresponding values of 13.67±1.86gm and 11.0±0.0gm. These values are below control group without feeding while *Laminaria japonica* was observed to be higher than that of before feeding group (Figure. 11).

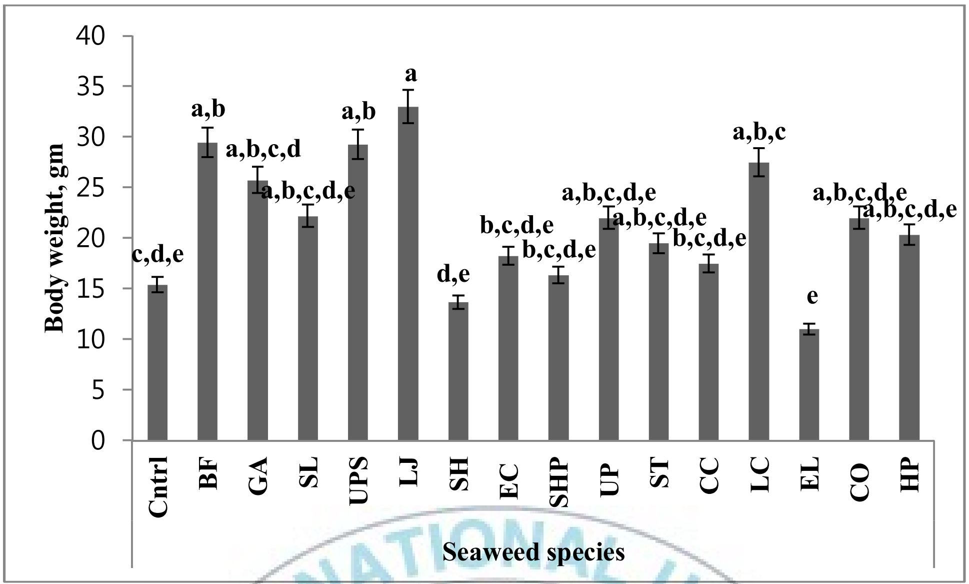

Figure.11. Body weight of *Apostichopus japonicus* expressed in gram (gm) after feeding with different seaweed species fof 14days and 7days recovery (Total 21days). Small letter above bars refers to significant differences among groups ($p<0.05$, $n\leq5$, fig. 11 and table 2).

The body length of sea cucumber showed significant differences among each group, *Laminaria japonica, Lomentaria catenata, Ulva pertusa* and *Scytosiphon lomentaria* showed higher values of 14.1±1.3cm, 12.5±1.4cm, 11.75±1.1cm and 11±0.5cm respectively. Meanwhile, *Ecklonia cava, Gellidium amansii, Sargassum thunbergii, Undaria pinnatifida* and *Sargassum honoreri* showed little body length increament of 10.875±0.9cm, 10.875±0.83cm, 10.625±0.4cm, 10.0±1.0cm and 9.5±0.3cm respectively while *Hypnea pannosa* and *Sargassum hemiphyllum* showed least results of 9.0±1.0cm and 9±0.6cm respectively (Figure. 12).

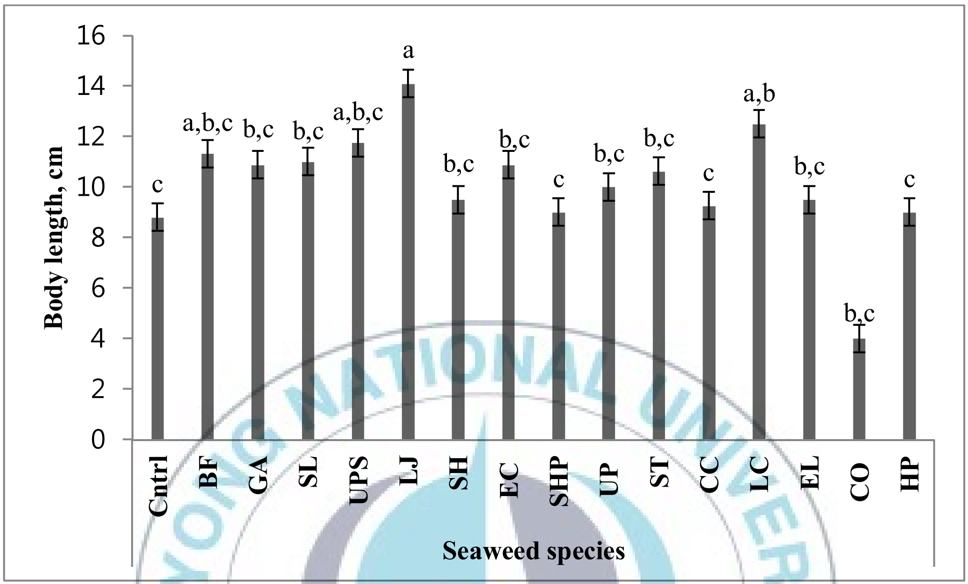

Figure.12. Body length of *Apostichopus japonicus* expressed in centimeter (cm) after feeding different seaweed species for 14days and 7days of recovery (totally 21 days). Small letter above bars refers to significant differences among groups ($p<0.05$, $n\leq5$, fig. 12 and table 2).

Gut growth ratio expressed in milligram per gram of body weight of sea cucumber were significantly different among the groups after 21 days of feeding trial, the results as presented in table 3 and Fig. 13. The highest gut growth ratio was shown by *Ecklonia cava, Undaria pinnatifida, Gellidium* *amansii, Sargassum thunbergii* and *Scytosiphon lomentaria* and followed by gut ratios upon feeding with from *Sargassum hemiphyllum, Chondrus ocellatus, Enteromorpha linza, Sargassum honoreri* and *Laminaria japonica*. The least ratio was observed upon feeding with *Carpopeltis comea, Ulva pertusa, Hypnea pannosa and Lomentaria catenata.* The value for *Lomentaria catenata* was below control group before feeding while, *Ecklonia cava* was above the value before feeding (P<0.05, Mean±SE, Figure. 13&16 and table 2&3).

Table.3. Gut growth expressed in milligram per gram (mg/gm) of body weight (Mean±SEM) relative to Seaweed species after 21 days of feeding trial

Seaweed Species	Gut growth in mg/gm (Mean±SEM)
Gellidium amansii (GA)	0.0512±0.005***
Scytosiphon lomentaria (SL)	0.0476±0.01****
Ulva pertusa (UPS)	0.0165±0.004
Laminaria japonica (LJ)	0.0234±0.005
Sargassum honoreri (SH)	0.0239±0.005
Ecklonia cava (EC)	0.1085±0.013*
Sargassum hemiphyllum (SH)	0.0475±0.014****
Undaria pinnatifida (UP)	0.063±0.007**
Sargassum thumbergii (ST)	0.0476±0.01****
Carpopeltis comea (CC)	0.0219±0.006
Lomentaria catenata (LC)	0.0121±0.003
Enteromorpha linza (EL)	0.0244±0.003
Chondrus ocellatus (CO)	0.0268±0.005
Hypnea pannosa (HP)	0.0156±0.003
Before feeding (BF)	0.101± 0.005*
Control Group CNTR(Without feeding)	0.0148±0.002

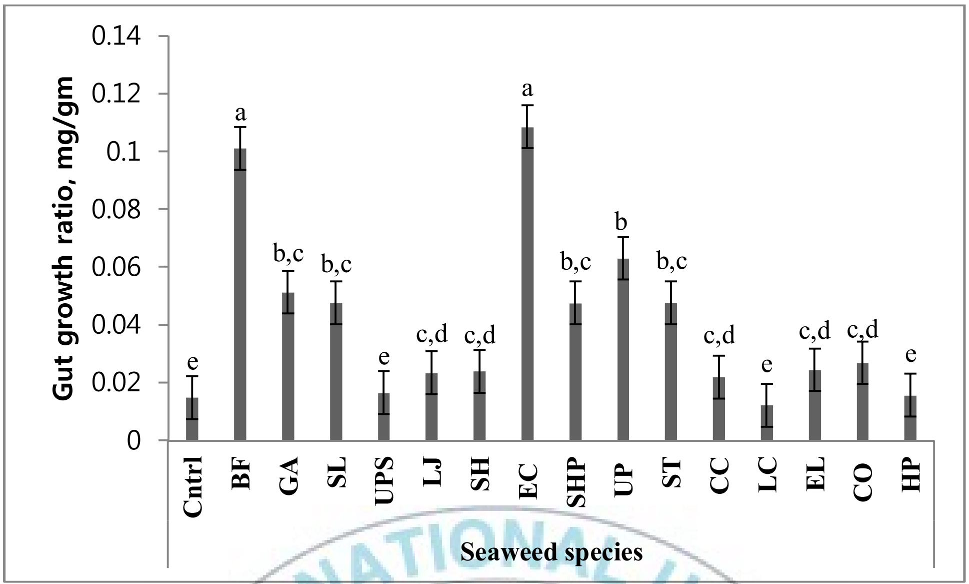

Figure.13. Gut weight ratio (mg/gm) in different Seaweed species after 21 days of feeding trial. Small letter above bars refers to significant differences among groups (p<0.05, n≤5. fig. 13 and table 2&3).

Then gut length growth in milimeter per centimeter (mm/cm) of body length have showed significant difference among treatment groups after 21days of feeding. Highest results were observed from *Undaria pinnatifida, Sargassum thumbegii* and *Ulva pertusa* with results of 1.0513±0.2mm/cm, 1.0366±0.3mm/cm and 1.022±0.3mm/cm respectively. Meanwhile, *Ecklonia cava, Sargassum hemiphyllum, Chondrus ocellatus, Gellidium amansii, Laminaria japonica* and *Sargassum honoreri* have shown little increament in the gut length values of with 0.9005±0.16mm/cm, 0.887±0.113mm/cm, 0.8834±0.113mm/cm, 0.813±0.05mm/cm, 0.6372±0.07mm/cm and 0.6271±0.1mm/cm respectively. *Scytosiphon lomentaria, Hypnea lomentaria, Carpopeltis comea, Enteromorpha linza* and *Lomentaria catenata* showed the least growth ratios of 0.5859±0.04mm/cm, 0.5568±0.04mm/cm, 0.3772±0.04mm/cm, 0.3722±0.07mm/cm and 0.3651±0.03mm/cm respectively. Some seaweed species including *Scytosiphon lomentaria, Laminaria japonica, Sargassum honoreri, Carpopeltis comea, Chondrus ocellatus, Lomentaria catenata, Enteromorpha linza* and *Hypnea pannosa* showed results below control group without feeding ($P<0.05$, Figure. 14&16, Table. 2).

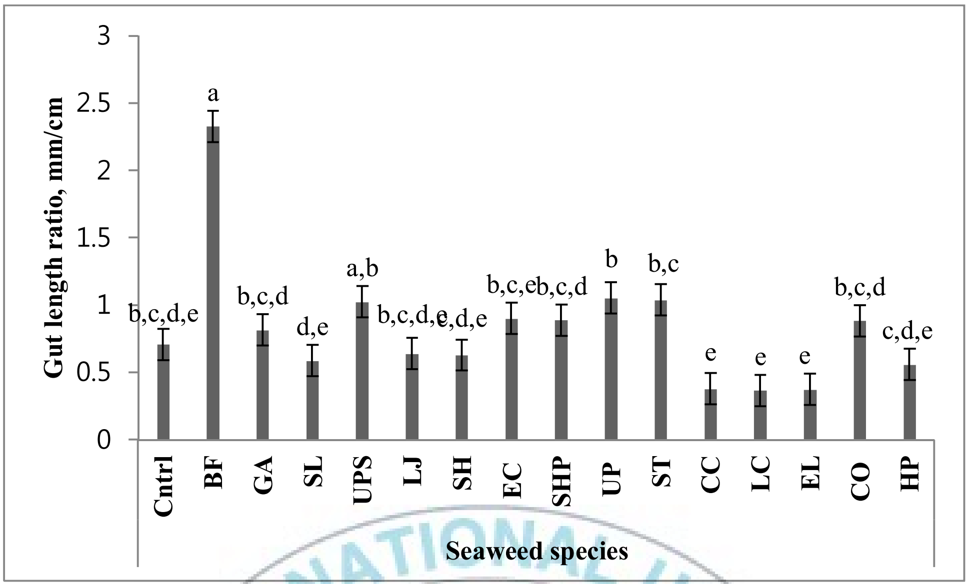

Figure.14. Gut length ratio (mm/cm) of body length) in different Seaweed species after 21 days of feeding trial. Small letter above bars refers to significant differences among groups ($p<0.05$, $n\leq5$. fig. 14 and Table. 3).

Survival rate was higher in all groups after 21days of feeding (60-100%) except in few seaweed groups like *Enteromopha linza, Sargassum hemiphyllum* and *Carpopeltis comea* in which survival rate was 20% (Figure. 14).

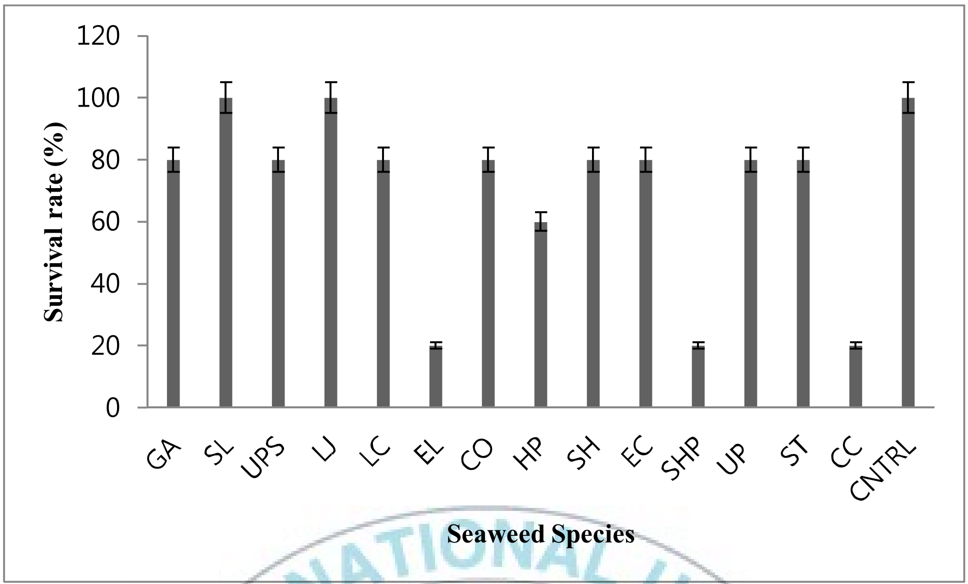

Figure.15. Survival rate of *Apostichopus japonicus* (%) in different seaweed species after 21 days of feeding trial. (p<0.05, n≤5).

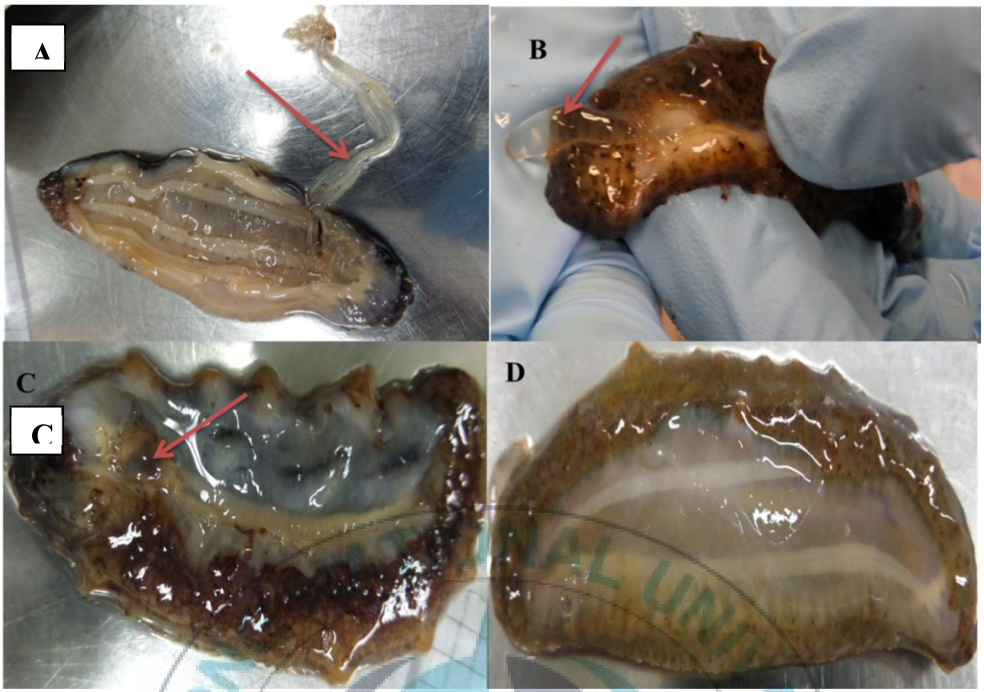

Figure.16. Gut regenerated after 21days of feeding (ABC) in dissected sea cucumber, *Apostichopus japonicus* arrows shows the different sizes of guts with respects to Seaweed species fed and D is disected sea cucumber from vetral side (P<0.05).

4. Discussion

Holothurians are known for gut evisceration when faces any kind of disturbances (Vandenspiegel, *et al.* 2000; Garcia-arraras and Greenberg. 2001; Mashanov, *et al.* 2010). The present study demonstrates the effects of various seaweeds in gut growth after applying effective and safe evisceration method before feeding the various seaweeds. The guts evisceration was induced by chemical and physical means. The criteria used for effectiveness of the induction were the evisceration rate, intensity of stimuli and survival of the animals after evisceration. Rapid evisceration after inducing stimulus made collection of time taken to release, identification of the intensity of stimulus and distinguishing the harmlessness of the stimulus toward the animals after keeping them for atleast one week.

In chemical stimulation KCl at 0.35M showed similar results with previous studies (Byrne, 1986; Tan *et al.,* 2008; Sun., *et al.* 2011; Yuan., *et al.* 2012; Zang *et al.*, 2012) where KCl shows good performance with 100% (Table. 1) which led to evisceration as well as well being of the animals after more than four days compared with other stimulation sources, narcosis effect of

KCl might be the reason of its effectiveness. Similarly distilled water shows good results as that used by Shukalyuk and Dolmatov, 2001; and Odintsova *et a.l,* 2005 with good survival rate as KCl while NaCl showed similar results as Byrne, 1986 with no evisceration, and chloroform, methanol and ethanol led no or less evisceration with survival rate between 0-57% (Table. 1). The death of animals was between 2 to 4days after evisceration which might be due to lethal effects of these chemicals.

Physical induction by sonication showed better evisceration and survival rate. Also the results for pinching by forceps in the present results was different with that of Vandenspiegel, *et al.,*(2000) in which 15 and 300 tubules were released through gentle and strong pinching simultaneously (Table. 1). The same case appeared in electric shocks stimulus probably due to weak voltage (24V) used as mentioned by Byrne (1986) when voltage amount increased (30-100V) induce evisceration. This might be due to disturbances they got in their body although strong electric shocks fasten their death within three days with long period of unconsciousness. Shakers led no to evisceration probably due to weak revolution per minutes (rpm) used which might not be enough to induce evisceration (Table. 1). All successful stimuli led to eviscerate all gut except cloaca as been observed through scarifying animals after evisceration by dissecting as explained previously (Byrne, 2001 and Mashanov, 2010). In the present study KCl and sonication were considered as effective and safe for the animal survival; hence throughout the feeding experiments KCl was used.

Length weight relationship is used for determining the sexual marturity of Sea cucumber as well as determination of exploitation in of these resources at particular area (Pitt *et al.,* 2004; Al-Rashdi, *et al.,* 2007). In the present study length weight relationship showed slightly weak significant regression coefficient. Meanwhile, the gut weight to body weight relationship, gut weight to body length relationship, gut length to body weight relationship and gut length to body length relationship showed slightly weak significant regression coefficient (fig. 6,7,8,9,10). These could be due to extendable and contration of the animal which led to give these results since the method of measurement used is not advanced as that used in previous studies (Yamana and Hamano, 2006), althought these results are differ with previous studies (Pitt *et al.,* 2004; Al-Rashdi, *et al.,* 2007) which were positive. In case of Sea cucumber gut measurement (fig. 6,7,8,9,10), there was slightly significant regression could be due to collection from the wild environment since they have tendancy of releasing their guts and regrow again atleast four times throughout their life time (Mashanov *et al.,* 2011, Mashanov et *al.,* 2010; Mashanov *et al.,* 2004).

In the present study, the main target was gut weight and gut length regenerated after feeding several seaweed powders although other measurements were considered too such as body wet weight and body length in 21 days. The result of weight gain showed *Laminaria japonica* has greater effect (figure. 11). Xia, *et al.* 2012 reported similar results compared with other seaweed. Although body sizes maters in growth rate but *Ecklonia cava, Undaria pinnatifida, Ulva pertusa, Sargassum spp, Gelidium amansii* showed little effects as previous studies (Yanagisawa, 1998; Yuan, *et al*., 2006, Xia, *et al.,* 2012). In the present study body weight was abnormal compared to healthy sea cucumber as in previous studies. This is might be due to the phase of true regeneration and absence of effective digestive function which is in most cases lead to weight loss followed by gaining in growth phase (Vandenspiegel, *et al.* 2000; Yuan *et al.* 2006).

In contrast the present study length of Sea cucumber body in this study has been increased after 21 days feeding trial which is also explained by Garcia-arraras and Greenberg. 2001; Mashanov *et al.,* 2011, they explained that recovery stages starts in wound closure, body reorganization and later significant extention of length. Again *Laminaria japonica* showed higher body length increament and *Sargassum hemiphyllum* showed the least (figure. 12). Meanwhile the remained seaweeds species showed medium results as indicated in the body weight gain effects. These results are similar with the findings of Yanagisawa, 1998; Yuan, *et al.* 2006 and Xia, *et al.* 2012. This might be due to their preferences as *Apostichopus japonicus* prefers *Laminaria japonicus* and *Sargassum species* (Xia, *et al.* 2012).

The regeneration and autotomy of sea cucumber body parts have been studied for many years and 21days is confirmed to be enough to regenerate fully the gut in Sea cucumber (Mashanov and Garcia-arraras, 2011). In the present study, different seaweeds has showed significant variation in gut regeneration, in which *Ecklonia cava*, *Undaria pinnatifida* and *Gellidium amansii* showed highest results of 0.1085±0.01mg/gm, 0.063±0.01mg/gm and 0.0512±0.01 respectively while the least values of 0.0121±0.003mg/gm, 0.0156±0.003mg/gm and 0.0165±0.004mg/gm were reported by *Lomentaria catenata, Hypnea pannosa* and *Ulva pertusa* respectively (Table. 3). The remaining eight species showed values ranges in between. These results could be due to the presence of bioactive compounds in particular seaweed species as confirmed that are primary sources of bioactive compounds to different marine animals (Blunden, 2001; Venugopal, 2009; Guerard, *et al.* 2011).

Furthermore, gut length ratio has shown significant variation between treatments which similarly reported due to rapidly increasing in regenerating cells in gut tissues (Shukaluk and Dolmatov, 2001) as well as no increase in number of cells have been observed by Odintsova, *et al.* 2005. In this study *Undaria pinnatifida, Sargassum thunbergii* and *Ulva pertusa* showed the highest results of 1.0513±0.16mm/cm, 1.0366±0.15mm/cm and 1.022±0.265mm/cm respectively and *Ecklonia cava*, *Sargassum* *hemiphyllum, Chondrus ocellatus* and *Gelidium amansii* showed little length increamnent of 0.9005±0.16mm/cm, 0.887±0.113mm/cm, 0.8834±0.113mm/cm and 0.813±0.05mm/cm respectively. Meanwhile *Scytosiphon lomentaria*, *Laminaria japonica, Sargassum honoreri, Carpopeltis comea, Lomentaria catenata*, *Ecklonia cava* and *Hypnea pannosa* showed the least on gut length ratio (Fig. 14 and Fig. 16). These differences could be due to cell dedifferentiation as explained previously some tissue will grow long while other shorter and irregular shape although in few cases found to lose at all (Shukalyuk and Dolmatov, 2001; Odintsova *et al.,* 2005; Mashanov, *et al.,* 2011) on the other hand might be bioactive compound present in different seaweed species.

Survival rate in aquaculture can be altered by physical and chemical parameters in water (An *et al,*. 2007; Wang *et al,*. 2007; Zamora and Jeffs, 2012) but in this study *Laminaria japonica, Scytosiphon lomentaria,* Control groups, *Chondrus ocellatus, Gellidium amansii, Sargassum honoreri, Sargassum thunbergii, Ecklonia cava, Ulva pertusa, Undaria pinnatifida, Lomentaria catenata, Sargassum hemiphyllum* and *Hypnea pannosa* have showed good perfomances between 60-100% survival rate while *Enteromorpha linza* and *Carpopeltis comea* showed the least survival rate of 20% each (Figure. 15). This could be due to turbidity caused by seaweed and water circulation as have been explained by Wang *et al.,* (2007) that poor growth associated with lower water exchange rates, poor light penetration and nutrient concentrations.

5. Conclussion

The Sea cucumber *Apostichopus japonicus* viscera (guts) and gonads are among the favoured sea food in Japan, Korea and China which fetches higher prices currently. Seaweeds are known for providing important bioactive compounds. Using seaweed powder for improving the quality of Sea cucumber guts and body is likely a promising idea in Sea cucumber industry. In this study different seaweed showed significant different effects in particular areas which pave the way for future studies in proteiomic studies in Seaweed and Sea cucumber for better future in Medicinal, Pharmaceautical and Biotechnological backgrounds.

In the present study, different seaweeds showed significant differences in rate of gut regeneration the ratio of gut length to body weight, in body weight and length gain. These basic datas will pave the way to further investigate the effects of specific bioactive compounds from different seaweeds on the quality and safety of sea cucumbers.

In case of stimulation method, this study found also Potassium chloride is the best simulation method; this will also give inlight for commercial production of sea cucumber although more studies are needed to investigate the safety on KCl to human or should focus on distilled water which also showed good results.

The present study shows *Undaria pinnatifida* and *Ecklonia cava* are the best seaweed regarding the effects in terms of gut regeneration, gut length, gut weight respectively while *Laminaria japonica* is the best for both body length and body weight gain, this is information will help farmers when they want to focus on production of Sea cucumber products.

Regeneration of sea cucumber body parts has been studied for many years but yet no studies focus on the important of Seaweed in improving regeneration. Meanwhile there are some studies mentioned the important of bioactive compound in speed up physiological activities to marine animals such as metamorphosis. By relying of those studies, this study and chemical analysis of compound present in seaweed might give better results for pharmaceutical or medicinal development.

Future perspectives of the present study should focus on analysis of bioactive compound present in Seaweed species and Sea cucumber's body. More studies also should focus on mechanism of regeneration of sea cucumber body parts. These will pave a way for more in revolution of developmental biology, Medicinal and Biotechnological backgrounds.

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