博　士　論　文

Studies on attachment strengths of *Sargassum horneri* (Turner) C.Agardh to the substrate and its dislodgment forces from the substrate

(アカモクの基質への固着力と基質からの引き剥がし力に関する研究)

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Content

Chapter 1 Introduction

1.1 Geographical distribution and its applications

Sargassum beds play important ecological functions on the surrounding environment in which includes water temperature (**Komatsu et al. 1982; Komatsu 1985; Komatsu et al. 1994**), pH (**Komatsu and Kawai 1986**), dissolved oxygen content of seawater (**Komatsu 1989**), downward illumination (**Komatsu 1989**), water flow (**Komatsu and Murakami 1994**). Among these species, *Sargassum horneri* (Turner) C. Agardh is a very important economic seaweed species Asia scientists felt interests in **(***)** throughout the temporal variations combining with attachment period and floating period. Its considerable wet biomass of individuals at the peak growth provides potential stable industry materials in manufacturing brown seaweed fucoidans and alginic acid of which widely are used in food, medicine and organic fertilizer (**Sun et al. 2008**). In ancient Japan, they are sun-dried and stored as immediate food against natural disasters. Some regions in Japan Northeastern still have diet traditions to process them to food and salt (**Sasaki 2014**).

Also, *Sargassum horneri* they are quite important seasonal ocean habitats for economic fishes, echinoderms and mollusks struggling to live in hard substratum of subtidal zone (**Umezaki 1984**). Their plentiful foliages are natural good habitats for many organisms e.g. caprellids and gammarids (**Imada et al. 1981**), and thus attracting many fish juveniles to enter into the beds to feed on them. In Nanji islands of Zhejiang Province of China, local fishermen call seaweed species *Sargassum horneri* as “Ding Xiang House”, in which “Ding Xiang” is a

Chinese slang in naming fish species Japanese anchovy (*Engraulis japonicus*) juveniles and they often preferring to school in *S. horneri* beds. This species fishing is another important economic income to the local fishery communities in the summer. **Komatsu et al. (2015)** found that *S. horneri* populations were growing bigger in a water depth of 7 m around Sanriku coastlines in Japan Northeastern. Thus, giant marine coastal forests composed of e.g. *S. horneri* populations often are severed as important fish grounds for many economic fishes in feeding, migrating and spawning.

Sargassum horneri in attachment period widely spreads around Asia Northeastern coastlines, including Korean Peninsula, Japanese Archipelago (except for the eastern of Hokkaido and Rykyu Archipelago) and Chinese coastlines (except for Taiwan). Additionally, they, during floating period, form seasonal floating rafts in the East China Sea, especially abundant in March-June of each year (**Segawa et al. 1957; Yatsuya et al. 2005**).

In the recent 30 years, *S. horneri* beds in the China coastlines continue decreasing and even disappear owing to rapid industrial developments and land reclamations of China (**Sun et al. 2008**). Additionally, rises of ocean water temperature are thought as important threats to the formation of floating rafts (**Komatsu et al. 2014**). However, Chinese scientists had been performing some scientific projects in trying to restore or recreate *Sargassum horneri* beds in Zhejiang Province of China.

1.2 Temporal changings of morphological traits of subtidal seaweed *Sargassum horneri* along its life history stages

Sargassum horneri is the annual seaweed species of which the order belongs to Fucales (Family: *Sargassaceae*; Genus: *Sargassum*). They survive in under low tidal line generally from sea surface to depth of 4 m M.L.T., but at a maximum situation they appear in 7 m M.L.T. (**Komatsu et al. 2015**). In Gouqi island of Zhejiang Province of China, maximum depth of this species is in 3.8 m M.L.T. (**Xu 2008**).

Morphological traits of annual species *Sargassum horneri* were described by **Sun et al. (2008)** and in a Chinese book called as "Color Atlas of Zhejiang alga" **(1983)**. The primary lateral stipe (also called as the stem) of the seaweed has an only one, with a longitudinal groove went across the primary lateral stipe midline, generally stem diameter being able to be ~1.5-3 mm. The leaves of 3-7 cm length and 0.5-1.5 cm width singly are feather-form rib and cap, with slender shanks. The vesicle of 0.5-1.5 cm length and 2-3 mm diameter is cylinder-shaped, and both distals of the cylinder are a little more slender, generally they occurring in the axillae or the apical section of the stipe. The receptacle, in a case of the male, generally growing within 4-8 cm length and 1.5-2 mm diameter, and in converse, female commonly remaining 1.5-3 cm length and 2-3 mm diameter, both are short shank. The holdfast is rhizoid forming. The cylinder-shaped lateral branches of a whole thallus commonly splits and elongates to 3-4 times. **Umezaki (1984)** found that leaves of the plant and lateral branches of the primary lateral were produced spirally with a divergence of 2/5, and this pattern did not change for the entire length of the primary laterals, even for the leaves in the lateral branch. The in-between distances among lateral branches of the primary lateral become elongating with the growth.

The growing stages of the seaweed attaching to the substratum according to **Yoshida (2005)** classifications are separated into 4 periods. (1) Early growth period. Sporophytes releasing from their mother frond attach to hard substrata prior to the embryo beginning to sprout out. The frond germinates and begins to produce linear, flat, teeth-forming, cylindrical first leaves whereas the second leaves is growing gradually. The elongation of the primary lateral during summer-autumn is quite slow, no more than 1mm/day (**Yoshida 2005**). (2) Stem elongation period. The stem (primary lateral) elongates during the winter, > ~10 mm/day (**Yoshida 2005**), they forming complex lateral branches gradually. Vesicles transformed from mature leaves they gradually appear, beginning after mid-winter, and the number that is blasted abruptly in later winter. Few receptacles occur in this period. The planform surface area swinging perpendicular to fluid flow and volume of the frond are expanding. (3) Sex maturation period. A few receptacles occur in the apical section of the stipes otherwise appear among the leaves and vesicles in the spring. *S. horneri* individuals are dioecious. Male fronds produce sperms, opposite to the female generating eggs in the receptacles. Sperms in later spring are conjointly released from father fronds into the sea as waves pass through. They are brought and moved by tidal currents toward the female and then mate with female's eggs. Fertilized eggs are swaying together with the mother fronds and released into the sea with many times. Germlings are released over several days (3-4 d at a water temperature of 20°C) after fertilization of eggs in the female receptacles (**Koumoto and Tomiyama 1968**). **Koumoto and Tomiyama (1968)** found that 25–50 % of eggs were released onto the surface of the receptacles during a first liberation period and that the remaining eggs were released 2 wks later. **Tahara (1913)** reported that five egg release events occurred in *S. horneri* at intervals of 7–12 days during an approximately 1-mo study on the Misaki Peninsular near Tokyo. The elongation of thallus length is faster during this period. Larger weight and volume of the seaweed after sex maturation lead to larger drag force created by waves exerted on the frond. Moreover, mean thallus length of specimens in March 2014 in Nabeta Cove, Shimoda, Japan was shorter than in April 2014. It was due to effects of breakages created by waves to larger individuals. (4) Declining and detaching period. Roughly in later spring-early summer, most mature individuals are removed by drag force regardless of their sizes. However, <5% population's holdfast still has a possibility to stay on the substrata in a case of Nanji Islands in Zhejiang province of China (**Sun et al. 2008**) after this period. They can regrow again in future.

S. horneri populations in Nabeta Cove of Shimoda (**Figure 1.1 geographical location**)of Japan they mature in the mid-March (**Mikami 2007**), losing in the end of May.

Among seaweeds, annual species *Sargassum horneri* has larger sizes along the life history. Their weights generally are > 3 kg in the field, and in a case of raft cultivation, they grow to 13.2 kg (**Sun et al. 2009**). Their thallus lengths are generally growing to 2-3 m in the field and they can grow to a larger size of 7 m (**Sun et al. 2009**). Their vesicles and receptacles are important contributors to create larger force exerted on the thallus. Seaweed weight increases rapidly when many receptacles appear. Thus, mature thalli at a peak biomass are often damaged by strong waves, especially in wave exposure areas. In Obama Bay and Wakasa Bay, **Umezaki (1984)** found that mean primary lateral length was 99 cm (in May) in exposed area during August 1981-May 1982 whereas responding value is 161 cm (in May) in shelter area during October 1980-May 1981. Wave exposures and seaweed maturity might be most important environmental reasons to dislodge fronds and produce floating rafts. They control survival rates of *S. horneri* populations along life history in the field.

1.3 Floating rafts occurred in the East China Sea

Sargassum horneri is annual brown seaweed species that is common along northwestern Pacific coastlines. Germlings released from the receptacles settle on different substrata in the later spring–early summer period and develop into visible immature thalli in autumn-winter as rhizoids grow forming holdfast and the lengths and weights of the developing seaweed increase.

Thalli mature in spring. Dense *Sargassum* forests develop on hard substrata, such as bedrock or boulders, surviving from depths of 1-3 m (**Umezaki 1984; Komatsu et al. 2014**). Populations grow down from the surface layer into the subtidal zones, reaching depths >7 m. On the Sanriku coastlines of Japan, *S. horneri* populations form stands of thalli that are attached to the bottom to a depth of 7 m and float upward, forming canopies on the sea surface (**Komatsu et al. 2015**). When mature thalli reach maximum lengths in the later spring–early summer period, they are readily detached from substrata by waves action with or without their holdfasts. When the holdfasts are torn free from the substratum, the drag force created by waves has exceeded the attachment strength of the thalli (**Yoshida 1963**). The thalli have many gas-filled vesicles that support buoyancy for the floating canopies. Detached thalli float on the surface and often form rafts of tangled seaweeds (**Komatsu et al. 2006**).

China coastlines are supposed to be major origin to produce floating rafts in the East China Sea, and there *Sargassum horneri* is only species in the component of floating rafts

(**Komatsu et al. 2007; Komatsu et al. 2009; Komatsu et al. 2014; Mizuno et al. 2014**). Floating seaweed rafts provide important ocean habitats for a diversity of fish (**Senta 1986; Cho et al. 2001; Thiel and Gutow 2005**). These fishes include flying fish, Pacific saury (*Cololabis saira*), and Japanese halfbeak (*Hyporhamphus sajori*), which spawn eggs bearing filaments that attach to the floating seaweed rafts (**Komatsu et al. 2009**). Juveniles of yellowtail (*Seriola quinqueradiata*) and Japanese horse mackerel (*Trachurus japonicus*) search habitat in the floating seaweed rafts (**Senta 1986**). The Japanese yellowtail aquaculture industry captures these wild juveniles along with the floating rafts they inhabit and uses them to stock rearing facilities. This work is necessary because carnivorous juveniles of yellowtail prevent marine farmers from rearing the species from eggs. The farmers call yellowtail juveniles "*mojyako*"; "*mo*" and "*jyako*" mean seaweeds and juveniles in the Japanese language, respectively.

Thus, the details of the attachment/detachment mechanisms of *Sargassum horneri* are of commercial significance for the aquaculture industry. Information on the wave forces required to detach the thalli is relevant to this form of aquaculture.

1.4 Drag created by waves made seaweeds drifting

Under unidirectional stable flow condition, drag force created by stable flow is the bulk hydrodynamic force to determine their damage and dislodgement of the seaweeds (**Denny 1995; Gaylord 2000; Stewart 2006**). Drag force created by waves makes seaweeds drifting. Thus, studies of drag force varying among thallus sizes are crucial to understand supply mechanism of floating seaweed rafts.

Majority of drag force created by flows is pressure force that the flow pattern at the high Reynolds number (called *Re* in the below) around an organism results in a difference in pressures between the upstream and downstream sides of the organism, with the pressure higher upstream (**Koehl 1977**). Pressure drag is thus proportional to the formula of which "$0.5 \times Cd \times \rho_{seawater} \times (U^2) \times S$" (**Koehl 1977**). Here, Cd is an empirically determined drag coefficient that depends upon the shape of the organism and the *Re* of the flow situation. U is flow velocity. S is planform surface area of an organism. The drag force acting on a large organism (**e.g.** the seaweed *S. horneri*) when acceleration of flow current is low (**i.e.** flow is essentially steady) can be estimated using the above equation. *S. horneri* populations form large coastal forests at a bottom depth of 7 m along Sanriku coastlines of Japan (**Komatsu et al. 2015**).

Usually, drag force on alga is depending on their size, shape and flexibility (**Carrington 1990**). (1) Size. **Bettignies et al. (2013)** found that in high flow velocities, size ("total planform surface area") of the alga determined drag force. (2) Shape. Drag coefficient Cd is a constant for a given body shape generally, and however, this is not applicable to the case of alga due to their flexible shapes. **Utter and Denny (1996)** used shape coefficient to define drag coefficient of kelp *Macrocystis pyrifera* (L.) C.Ag. Some scientists (**Kris et al. 2011; Boller and Carrington 2006**) used Vogel E-value instead of velocity's square item in above drag formula to describe alga bending. (3) Flexible. "Going with the flow" (**Gaylord and Denny 1997**) is a usual strategy to protect several meters length seaweed from wave-strict field environment. If the plant is large enough and flexible enough, it can simply 'go with the flow' as waves pass by. Drag coefficient Cd is also decreasing with velocity increasing owing to this strategy (**Boller and Carrington 2007; Martone et al. 2012**).

The hydrodynamic force drag is important to annual species *S. horneri* ecologically during life history. In a case of spring-maturation (**Yoshida 2005**) such as populations in Nabeta Cove, Shimoda, visible immature sporophytes in later summer-autumn they grow very slow, exactly at 1 mm per day (**Yoshida 2005**), and then after 120 days' growth (roughly till December), even 3-5 cm length individuals at the age of ~4.5-mo produce receptacles as if good environment (**Pang et al. 2009**). After >30 days' growing (approximate in January), most individuals when they are <100 cm length form vesicles. During the winter (mean wet weight <100 g in December-January for the case of Nabeta populations), *Sargassum horneri* individuals they grow gradually till later winter-early spring of fast-growing period. When spring water temperature returns to 16 ℃ (optimal temperature beginning for sex reproduction; from **Sun et al. 2008**), thallus length of the plant elongates faster. Especially while receptacles appear, their weights are increasing huge. Mature male individuals of Nabeta populations usually in May begin to release gametes into the seawater, and they float and sway through wave actions into female's receptacles and then mate with oospores, resulting in next fertile offspring. These sex reproduction activities end soon, generally in 2 wks or very short time. Most individuals are detached by waves afterward.

Life history procedures described as the above according to **Yoshida (2005)**'s classifications are separated into early growth, elongation, maturing and declining 4 periods. *S. horneri* sporophytes are growing slow in the early growth stage. In the elongation period, their thallus length elongates rapidly, reaching >10 mm/day. **Komatsu et al. (2007)** suggested that *S. horneri* individuals were detached by waves from China coastlines in March or months earlier than March, so they might be transported to fringe areas of continental shelf and waters influenced by Kuroshio Currents from March. Stem breakages of Nabeta populations did not occur when their lengths were <50 cm prior to January 2014, but in January 2014 (lengths <100 cm) some cases of stem breakages happened on, especially for long thallus ones. There were more and more breakages after in February. Long thallus length individuals face higher risks against dislodgements and breakages due to larger size. In maturing period (roughly from mid-March to mid-May for Nabeta populations), wet weight, and thus planform surface area, rapidly increases, and that causes larger drag force exerted on them. Also, receptacles produce adhesive materials. Vesicles increase extra planform surface area. Large number of receptacles and vesicles growing they form added drag force acting on individuals. In April 2014, Nabeta populations their mean thallus length decreases unexpectedly compared with former month. Many larger individuals they have been broken or dislodged by strong waves. Floating *S. horneri* rafts were abundant in March-June off the water of Japan coastlines (**Segawa et al. 1957; Yatsuya et al. 2005**). In the declining period, except for a few holdfasts still attaching onto the substrata, other individuals disappear (**Sun et al. 2008**). Floating seaweed species most of them were detached with the holdfast

(**Kawamata 2012**).

Thus, variations of drag force varied among thallus sizes during *S. horneri* life history is crucial to understand supply time of floating rafts around Japan coastlines. We are the first to report the drag force on subtidal seaweed *Sargassum horneri* varying among thallus sizes and velocities.

1.5 The scope and aims of this study

The doctoral thesis focuses on studies of attachment *Sargassum horneri* C. Agardh to the substratum and its dislodgement forces released from the substratum, including external force-drag force causing its dislodgements. We conducted dislodgement forces required to detach or break the fronds from substrates during December 2013 through June 2014 and during December 2014 through May 2015, by an interval of 1 mo. We measured drag force acted on individuals under fluid flows, performing experiments of drag force on thalli including a boat method and a water tunnel method. We compared relationships between dislodgement force and morphological traits including holdfast basal area varying among seasons and water depths. We also measured morphological traits such as holdfast basal diameter area, planform surface area, and volume. The study promotes understandings relating with forming mechanisms of drifting seaweeds. The followings are the aims of this study.

(1) Changings of attachment strength/dislodgement force of *Sargassum horneri* individuals varying along its life history and among depths

Dislodgement forces for fronds among development stages and depths are measured with a force gauge of which the pointer indicates the maximum force in-situ of the field. Individuals of broken stem are discarded. We use the caliper to measure the cross area of a stem.

(2) Relationships between drag force and frond sizes along the life history

Drag force is the force in which detaches thalli from the substratum. Fronds are predicted to break when drag force and dislodgement force are equal, and maximum dislodgement force represents the maximum drag force that fronds could resist against. By using the method developed by Utter and Denny (1996), drag force on frond exerted by currents is measured by towing fronds from the boat. Small fronds suspended with spring gauge are set in a water tunnel for measuring drag force under steady flow. Based on these data, drag force coefficients of different sizes are organized with Reynolds number. Drag force is set equal to maximum dislodgement force to calculate the critical frond Reynolds number, the combination of frond size and water velocity that would dislodge all fronds. We get a relationship of allometric growths between thallus length and planform surface area.

(3) Modeling of forces on fronds by a long wave:

Strong wave is generally produced by a long wave in shallow coastal waters. We simulate drag force on thallus according to long wave theory, and compare it with field results.

Chapter 2 Attachment strength of the subtidal seaweed *Sargassum horneri* (Turner) C. Agardh varies among development stages and bottom depths

2.1 Introduction

Sargassum horneri (Turner) C. Agardh is an annual brown seaweed species commonly distributed around northwestern Pacific coastlines. Its germlings released from receptacles of *S. horneri* settle on different substrates in spring to summer and develop to visible immature sporophyte in autumn to winter accompanying with growth of rhizoid forming holdfast, thallus length and weight. They mature in spring. When its thallus lengths become longer in mature season from spring to early summer, waves detach them above or with holdfast on substrates since it has been considered that their drag force becomes greater than attachment force (Yoshida, 1963). *S. horneri* has many vesicles filled with gas to obtain buoyancy for keeping a thallus upward from the bottom. They can float due to buoyancy of vesicles after being detached or broken away from substrates by waves (Komatsu et al., 2006).

Floating rafts are most important habitats in the ocean as a forest for many fishes (Senta, 1986; Cho et al., 2001; Thiel and Gutow, 2005). Most of floating rafts include of detached *S. horneri* around Japanese coasts in spring to early summer (Yoshida, 1963; Ikehara, 2004). In East China Sea, floating rafts consist of only *S. horneri* (Komatsu et al., 2007; Komatsu et al., 2009; Mizuno et al., 2014, Komatsu et al., 2014). In East China Sea, commercially important pelagic fishes such as flying fish, Pacific saury (C*ololabis saira*), and Japanese halfbeak (*Hyporhamphus sajori*) spawn eggs with filaments on floating seaweed rafts (Komatsu et al., 2009). Juveniles of yellowtail (*Seriola quinqueradiata*) and Japanese horse mackerel

(*Trachurus japonicus*) accompany with floating seaweed rafts (Senta, 1986). Thus, it is important to know how much force of waves detaches *S. horneri* from substrates for understanding supply of floating *S. horneri* rafts.

Kawamata (2001) reported dislodgement of *Saccharina japonica* (J.E. Areschoug) C.E. Lane, C. Mayes, Druehl & G.W. Saunders ex *Laminaria japonica* surviving in wave-sheltered areas, respectively. He transplanted individuals from wave-sheltered areas to wave-exposed areas. Both of these studies showed that transplanted individuals were detached in a short time. The results suggest that wave intensity environment during early life history is an important physical factor to affect attachment force of seaweeds to substrates. However, they didn't examine attachment force of seaweeds along a bottom depth gradient. Sugawara et al. (1998) revealed that attachment force of *Eisenia bicyclis* (Kjellman) Setchell growing on shallow bottom was greater than that of *Ecklonia cava* Kjellman growing on deeper bottom at the same study site by dislodging them with a spring scale. They stated that the attachment forces of *Ei. bicyclis* and *Ec. Cava* reflected wave exposure of their habitats because decrease wave intensities depending on a bottom depth gradient may influence attachment force of seaweeds. Therefore, it is interesting to examine attachment force of *S. horneri* along a bottom depth gradient.

Environmental conditions such as storm, season and water temperature could relate to attachment force of algae on the substrates. Milligan and DeWreede (2000) investigated force to remove intertidal seaweed species *Hedophyllum sessile* (C. Agardh) Setchell from substrates, using a clamp and spring scale system. They distinguished a significant statistic difference in attachment force of *H. sessile* between a pre-storm group and a post-storm group, and concluded that there was a seasonal effect towards more resistant holdfasts against the storm. The attachment force of *S. horneri* may be changed with growth or season.

For understanding supply of floating *S. horneri* rafts from the coasts to offshore waters, this chapter aims to understand attachments of fixed *S. horneri* from the substrates depending on its growth, mature stage and a bottom depth gradient through in situ surveys.

2.2 Materials and Methods

Study site and sampling

Study site is located in Nabeta Cove, Shimoda, Izu Peninsular, Japan, which is a wave-sheltered rocky area near Shimoda Marine Research Center, Tsukuba University. Field surveys were conducted from December 15 2014 to May 28 2015 with intervals of about 1 month. In each survey, scuba divers randomly (random for thallus lengths and bottom depths) sampled about 20 to 40 individuals of *S. horneri* inhabiting at bottom depths between 0 and 4 m from mean low tide level (MTL) at Shimoda Port.

Dislodgement force of each individual was measured with a spring scale of 5 kg or 10 kg by twining rope around its stem just above its holdfast, connecting the scale at the end of rope and pulling the rope till its dislodgement from the substrate or breakage of holdfast as described in Sugawara et al. (1998). A piece of nylon webbing was placed around the stipe(s) and attached to the spring scale. The spring scale was hooked around the cable tie, and the thallus is pulled parallel to the substratum steadily, but quickly (1-2s), until the stipe broke. Thalli were placed in marked bags. Since the spring scale has a pointer remaining at the maximum weight, it is possible to measure the maximum force that dislodges holdfast with/without substrates or breaks holdfast or stem. We call the force as attachment force. Depth of the holdfast of each measured individual was immediately recorded during the survey. Through verifying its broken part, a dislodgement type was classified into: 1) main stem with an entire holdfast; 2) stem detached from the substrates without the holdfast (**Table 2.1**). Specimen where stipes broken before dislodgement were relatively few and were discarded.

Individuals sampled by the divers were individually numbered and transported to a laboratory for measuring sizes of organs and weight. When a part of holdfast or whole holdfast remained, it was also sampled from the substrate for measuring size of holdfast and weight. Samples were kept at -30˚C in a freezer till analysis. After thawed them, thallus length between the bottom of holdfast and the top of longest lateral branch was measured to an accuracy of one centimeter with a 30 cm-length ruler. Wet weight of plant was weighted to an accuracy of two grams with a balance (CR-5000WP, CUSTOM) after seawater on the seaweed was absorbed with paper towel. Basal edge of a holdfast was traced on tracing paper with a pencil and was digitized by scanning the paper with an image scanner (GT-X970, EPSON). The area of holdfast was measured with software, ImageJ 6.4 (NIH, USA, http://imagej.nih.gov/ij), processing the digitized image of the outer edge after one image of 5 cm × 5 cm area was determined firstly for calibration.

Statistical analyses

Differences in attachment force among groups of samples divided by intervals of a bottom depth of 1m along the bottom depth gradient by month was tested by one-way ANOVA (StatPlus:mac LE, AnalystSoft) after log transformed to fit to normal distribution and verification of homogeneity of variance in one-way ANOVA. Person correlation was applied in relationships among morphological traits and dislodgement force.

2.3 Results

2.3.1 Changes in dislodgement force by month

Mean dislodgement force of samples measured by month was increasing from December 2014 to April 2015 with increase in weight of *S. horneri* (**Figure 2.1**). However, mean dislodgement force was decreased abruptly in late May 2015. Mean wet weight of monthly samples was the heaviest in April 2015 during the whole study period and then dropped off rapidly in late May 2015 because most of large thalli disappeared. The maximum dislodgement force in each month was stable around 90 N. While only two dislodgement forces in April were more than 100 N, those of most samples in April were no more than or around 90 N and similar to those in other months. The minimum dislodgement force ranged from 4.5 to 21.1 N (**Figure 2.2**).

Dislodgement force variation across a bottom depth gradient

There was no statistically significant difference among dislodgement force of *S. horneri* samples along the bottom depth gradient ($p>0.05$, **Table 2.2**). A ratio of between-group variance to within-group variance was less than statistical *F* variable, F_{crit} ($p=0.05$) indicating no statistically significant difference among groups. However, there was a tendency that monthly mean dislodgement force of samples on shallower bottom was greater than those on deeper bottom from December to May (**Figure 2.2**). The maximum and minimum dislodgement forces along the bottom depth gradient were increased from

December to February.

Monthly changes in dislodgement force in relation to thallus growth

Thallus length and wet weight had no relations with dislodgement force for monthly surveyed groups (**Figure 2.3**). On the other hand, holdfast basal area had a linear relation with dislodgement force from December to February but not from March to May (**Figure 2.4**).

Monthly changes in holdfast basal area across a bottom depth gradient

From December to March, mean monthly holdfast basal area of samples on shallower bottom was greater than that on deeper bottom, while this tendency was not observed in April and May (**Figure 2.5**).

Compare with the results of December 2013-June 2014

Holdfast basal area during immature stage had a linear relationship with dislodgement force, but this did not occur in the case of mature stage (**Figure 2.6**). And there was a tendency that monthly mean dislodgement force of samples on shallower bottom was greater than those on deeper bottom from December to June in this year (**Figure 2.7**).

2.4 Discussion

Holdfast basal area is strongly related to attachment/dislodgement force from December to February while thallus length and wet weight don't relate with the force. Since holdfast basal area attaches directly to the substrate, dislodgement force of *S. horneri* fixed on the substrate is created by the holdfast basal area. Kawamata (2001) reported holdfast wet weight of *L. japonica* in exposed area was linearly related with dislodgement force (r^2=0.57, p<0.05). This report indicates that the holdfast is very important for seaweeds to attach the substrate. The wet weight of holdfast may correlate to its basal area. Thus, it is natural that dislodgement force of *S. horneri* is proportional to its area.

Mikami (2007) set three permanent quadrats in *S. horneri* dominant area in Nabeta Cove and numbered all the stipes of a quadrat. She reported that matured stipes had been found since mid-March and they disappeared in May. Based on this research, whole study period of monthly surveys was classified into an immature stage from December to February and a mature stage from later March to May. Holdfast basal area was proportional to dislodgement force during immature stage of *S. horneri* (r^2=0.53, p<0.001) (**Figure 2.6**). This result suggests that *S. horneri* maintains the holdfast to attach on the substrate during the immature stage. We name this maintenance of *S. horneri* to attach the substrate through the holdfast as a fixing strategy.

S. horneri is an annual and dioecious species. Germlings are released in several days (three to four days at a water temperature of 20˚C) after fertilization on female receptacles (Koumoto and Tomiyama, 1968). Koumoto and Tomiyama observed that half or one quarter of eggs were liberated on the surface of the same receptacle at the first liberation and remained eggs were liberated at the second times in two weeks after the first egg liberation. Tahara (1913) reported that egg liberations of *S. horneri* occurred five times at intervals of 7 to 12 days during about one month through his observations on *S. horneri* at the same location in Misaki Peninsular near Tokyo. Germlings are released from female receptacles at several times during the mature stage. Mean sedimentation velocity of *S. horneri* germlings was about 0.5 cm s^{-1} due to specific gravity of *S. horneri* heavier than seawater (Okuda, 1985). Assuming that receptacles are 3 m high above the bottom, it takes about 10 min to reach the bottom, which means that germlings settle on the bottom around their mother individuals. Settlement of germlings around mother individuals assures their colonization because the substrate and environments around the mother individuals are favorable for *S. horneri* to live there. The fixing strategy enables that *S. horneri* continues to survive in favorable environments with germlings released from the mother individuals fixing on the substrate. On the other hand, *S. horneri* can't expand their habitat with germlings released around the mother individuals fixing on the substrate.

The holdfast basal area was not proportional to the dislodgement force from April to May during the mature stage. Floating stage and heavier germlings are common for *Sargassum* species. *Sargassum muticum* (Yendo) Fensholt was accidentally introduced with seed oysters from Japan to France in 1960s. Rueness (1989) reported that floating *S. muticum* rafts were observed along Skagerrak coasts in Norway in 1984 before *S. muticum* fixed on the bottom was discovered in 1988. To expand their habitats to new other shallow waters, floating of matured individuals is a reproductive strategy for *Sargassum* species which have heavier germlings released at several times from the female individuals. Therefore, it is natural that *S. horneri* has no necessity to keep holdfast to attach on the substrate after maturation, especially the first release of germlings from female receptacles that assures safe reproduction around favorable environments where parents could survive and reproduce. This floating strategy is a common among seaweeds with floating stage. Hernández-Carmona et al. (2006) reported that sporophytes of drifting *Macrocystis pyrifera* (Linnaeus) C. Agardh remained fertile with high germination success as long as sori were present (125 days). Rafting bull kelp (*Durvillaea antarctica* (Chamisso) Hariot) near north central Chile was also found to be fertile (Tala et al., 2013). It is interesting to verify attachment strategy of seaweeds that form floating rafts with matured conditions.

In early maturation stage, *S. horneri* stops growing and invests energy for reproduction through production of receptacles and stay on the substrate for the first germlings on the substrate near parents. Thus, the holdfast must be necessary to maintain or improve dislodgement force of holdfast to the substrate in early stage of maturation. After early germlings, *S. horneri* stops maintaining holdfast to attach the substrate but continues production of eggs and sperms, receptacles and fertilization. Then, attachment force of holdfast may become weaker than in the immature stage. In Nabeta Bay, mature and immature *S. horneri* may be mixed in March (Mikami, 2007). The holdfasts of matured individuals are easily detached from the substrate after releasing germlings into the seawater. Since individuals in late March are composed of immature and mature ones, weak relation is found between the holdfast basal area and dislodgement force.

Most of individuals are matured in April and May. Holdfast basal area hasn't been correlated with dislodgement force in Nabeta Bay. Especially in May, dislodgement force in late was abruptly decreased compared with that in April. Dislodgement force distribution in March is composed of individuals with relation and no relation between holdfast basal area and dislodgement force corresponding to immature and mature ones.

Dislodgement forces of *S. horneri* along the bottom depth gradient also reflect the immature and mature stages. During the immature stage, the shallower the bottom substrate which *S. horneri* fixed on is, the stronger the attachment forces are. Stewart (2006) conducted experiments on transplantation of and *Turbinaria ornate* (Turner) J. Agardh exchanging between back reef area and fore reef to verify adaptation of *T. ornate* to a wave-exposed environment. The result showed that all of samples transplanted from back reef to fore reef disappeared but samples from fore reef to back reef were alive after the experiments, suggesting that attachment force of individuals in wave-protected site were weaker than dislodgement force produced by waves in wave-exposed site after settlement. Same results were found by Kawamata (2001) who transplanted *Laminaria japonica* attached on plastic plates between wave-protected and wave-exposed sites. Dislodgement forces of *Stephanocystis hakodatensis* in wave-exposure sites were greater than those in wave-protected sites (Kuwahara et al., 1999). Our results indicate that dislodgement force of *S. horneri* in shallower site in most cases were greater than in deeper site. Shallower the sea bottom is, the stronger waves forces are near the shallow coast. Thus, *S. horneri* adapts dislodgement force of holdfast to a wave-exposed environment as studies mentioned above.

Attachment forces of *S. hakodatensis* in wave-exposed sites ranged from 60 N to 140 N with a mean of 100 N (Kuwahara et al., 1999). Milligan and DeWreede (2000) measured attachment forces of intertidal seaweed species *Hedophyllum sessile* (C. Agardh) Setchell. They reported that those of adult individuals' were closed to 100 N. *S. horneri* generally grows in wave-protected waters. Mean dislodgement forces ranged from 67 to 85 N varying with month. Thus, dislodgement forces of *S. horneri* samples measured in this study are reasonable compared with other studies on seaweed species living in an intertidal zone or wave-exposed sites. Minimum monthly dislodgement forces of *S. horneri* were 4.5-21.1 N varying with month. Attachment forces of seaweed *L. japonica* in wave-protected sites were also less than 20 N (Kawamata, 2001). *S. horneri* isn't a robust species compared with its extra large biomass, which explains the reason *S. horneri* populations remain in wave-protected waters (Marui et al., 1981; Umezaki, 1984; Terawaki, 1986; Sun, et al., 2008).

2.5 Conclusions

This is the first study on dislodgement force of *Sargassum horneri* depending on month. The data presented here show that holdfast basal area produces attachment force against dislodgement force created by waves and proportional to the attachment force during the immature season while no relation between holdfast basal area and attachment force is found in mature season. Those are explained by two reproductive strategies of *Sargassum* species: one is a fixing strategy to assure reproduction on the substrates near their parents through settlement of germlings, and the other is a floating strategy to expand habitat through floating mature individuals with receptacles that release germlings on new substrates far away from the native home.

Chapter 3 Drag on small sizes of subtidal seaweed *Sargassum horneri* (Turner) C.Agardh varies with morphological traits

3.1 Introduction

Many *Sargassum* species (e.g. *Sargassum horneri*) have gas-containing vesicles transformed from leaves. After waves and currents have detached *Sargassum* species from the bottom, most of these plants become drifting (Komatsu, et al., 2009). Floating *S. horneri* rafts are abundant in spring (Ikehara, et al., 1986). Komatsu et al. (2007) suggested that *S. horneri* was detached by waves from Chinese coast in March or months earlier than March, so they can be transported to fringe area of continental shelf and waters influenced by Kuroshio Current from March. These floating plants were found in almost healthy condition with holdfast (Ohno, 1984).

Mikami (2007) set 3 quadrats at Nabeta cove, Shimoda and numbered all of stipes in a quadrat, and she found that *S. horneri* juveniles continued disappearing a lot especially for its early stage. Small thallus of *S. horneri* has tougher stem in contrast to its large ones. In winter, strong wind and storm exert larger drag force on the thallus. Along the Western Australian coastline, storms associated with mid-latitude depression are common in winter (Lemm et al. 1999), and generally, algal dislodgment occurs during such severe storms, which generate high water velocities (Seymour et al.1989; Dayton et al.1992; Blanchette 1997). Thus, it is important to understand drag force created by waves on small thallus sizes during early growing stage transferring from no vesicles to having vesicles for identifying floating supplies of *S. horneri* rafts.

Visible small fronds of *S. horneri* are ranging from 10 cm to 40 cm thallus length. Boat method to measure drag force on seaweeds developed by Utter and Denny (1996) is only suitable to larger giant kelp such as *Macrocystis pyrifera* (Neushul, et al. 1967), but not applicable to small sizes of seaweeds. It is difficult to read the records of spring scale in the boat even under peak flow of 4 m s^{-1}.

In this chapter, I report drag force on small sizes of *S. horneri* in a water tunnel of velocity speed adjusting from 20 cm s^{-1} to 130 cm s^{-1} in an interval of 10 cm s^{-1} in stages of no vesicles and vesicles.

3.2 Materials and Methods

Study site is located in Nabeta Cove, Shimoda, Izu Peninsular, Japan, which is a wave-sheltered rocky area near Shimoda Marine Research Center, Tsukuba University. Field surveys were performed in the morning of Jane 28 2015 and then immediately samples were sent to Tokyo Ocean University in that day to measure their drag force under velocities ranging from 20 cm s^{-1} to 130 cm s^{-1} in a 1.5m length recirculating flow tank (**Figure 4.1**). Samples of thallus length used in the experiment were no more than 40 cm.

Determining drag is similar to the method of Armstrong (1989). Drag force of without the seaweed under velocities was calibrated by known weights at first. Drag on the beam alone was subtracted in calculating afterward. Then, each thallus was attached to a beam, to which a strain gauge was mounted, that was projected about 10 cm below the water surface in the working section of the tank. Force was recorded on a chart recorder under a certain known velocity. And water temperature in this water tank was also recorded during the experiment.

After the experiment, thallus length between the bottom of holdfast and the top of longest lateral branch was measured to an accuracy of one centimeter with a 30 cm-length ruler. Wet weight of plant was weighted to an accuracy of two grams with a balance (CR-5000WP, CUSTOM) after seawater on the seaweed was absorbed with paper towel. The planform surface area of each individual to 0.01 cm^2 was calculated using ImageJ 6.4 (NIH, USA, http://imagej.nih.gov/ij) by measuring its area from a digital picture of the frond on its side after one image of 5cm*5cm square area was determined firstly for calibration. An estimate of planform surface area was made for five photographs of each samples and the mean of these estimates was used.

Also, a define for drag force (Fd) as:

$$Fd = \frac{1}{2} * \rho_{seawater} * U^2 * A * Cd$$

Where $\rho_{seawater}$ is the density of seawater (1.025 g cm^{-3} at 20°C), U is the fluid velocity relative to the seaweed, A is a characteristic area of the seaweed, and Cd is the drag coefficient, a dimensionless number that accounts for the interaction between the flow and the shape of the object.

And, a define for Reynolds number (Re) as:

$$Re = U * L/\upsilon$$

Where U is the velocity of the fluid relative to thallus, L is a characteristic length of thallus, and υ is the fluid's kinematic viscosity (0.01049 cm^2 s^{-1} at 20°C). In this section, characteristic length is equal to the division of planform surface area of a frond to thallus length.

3.3 Results

Wet weight of the seaweed had a significant linear relation against its planform surface area (r^2=0.98, p<0.001) as showing in **Figure 4.2**. Mean per gram wet weight of the thallus had 12.05 cm^2 of one side surface area in this experiment. In addition, thallus width acquired by the division of planform surface area against thallus length was compared with thallus width measured at 20 cm s^{-1} velocity, perpendicular to the direction of coming flow current in the water tunnel, finding that they were near diagonal line of measured width against calculated width. So calculated width can instead of measured width (**Figure 4.3**) in Reynolds number formula.

Plot of Reynolds number against Cd was separated into two groups, the case of thallus having no vesicles and the case of thallus having vesicles. When *S. horneri* attain and above a certain thallus length, they grow a lot of vesicles changing from its leaves to float on the sea. So the classification also was regarded as a comparison among thallus sizes. They have roughly same Cd with each other when Reynolds number increasing. Fronds of no vesicles had lesser half Reynolds number against fronds having vesicles described in **Figure 4.4**, rather than Cd values. Cd of no vesicles had attained around 0.6 compared with 1.0 for fronds of vesicles at low flow of 20 cm s^{-1}.

Drag force on fronds was classified into three types according to the standard of wet weight, and thus planform surface area (**Figure 4.2**), including 0-10 g, 10-20 g and 20-60 g. If wet weight of thallus was below 10 g, drag force was changed little under and around 1 N as showed in **Figure 4.5** If wet weight was in between 10-20 g, drag force become a little larger in about 1.5 N. And for the case of 20-60 g, drag force become larger compared with two other groups.

3.4 Discussion

At a low flow speed (~20 m s^{-1}), *Sargassum horneri* frond hang to a force beam fully expanded limply in the water tunnel. As water velocity increasing, the seaweed collapsed into a funnel shape, gradually turning to a position parallel to flow. As speed increased still further, the blades reconfigured into a fairly compact bundle, and began to flutter. At extremely high water velocities (>1 m s^{-1}), blades interleaf or clump together presented a compact body to the moving water. Dudgeon and Johnson (1992) found two similar anatomically, morphologically, ecologically and phylogenetically species *Chondrus crispus* Stackhouse and *Mastocarpus stellatus* Guiry had a similar drag on smaller fronds (<3g fresh weight) during the peak flow. This extreme reconfiguration in high flows can result in decoupling of hydrodynamic forces such that drag becomes relatively independent of the apparent differences of algae shape and flexibility (Denny and Gaylord 2002). Shape and flexibility might have a minor effect on drag exerted to small *S. horneri* fronds, about 1-1.5 N in this study.

Besides that, with the growth of the thallus, the number of vesicles and leaves increasing also contribute added drag force on the plant. Kris (2011) studied leaves to what extent contributed to the absolute drag for aquatic plant species with contrasting morphological strategies. Drag was determined before and after leaf removal. Removal of the leaves showed that for floating leaved and submerged species, the ratio of drag with leaves (N) to drag without leaves (N) was higher than 1. Along the different velocities, on average 60% of the absolute drag was contributed by the leaves. This higher drag was especially seen at lower angles (high velocities). This could be caused by differences in macrophyte movements (flapping of leaves) owing to leaf shapes.

Drag coefficient Cd decreased across water velocities, corresponding to the change in the angle of the crown (Hoerner, 1965). The decrease in the drag coefficient with increasing velocity is consistent with the findings for drag force and corresponds to a reconfiguration of the algae to a more streamlined and compact shape (Koehl 1984; Koehl and Alberte 1988; Gaylord et al. 1994; Denny and Gaylord 2002). Cd tended to reach an asymptote at higher velocities, and the effects of shape change were greatly reduced (Carrington 1990; Gaylord et al.1994; Bell 1999; Boller and Carrington 2006). In this study, fronds of no vesicles and fronds of vesicles have roughly same Cd with each other when Reynolds number increasing. However, Fronds of no vesicles had lesser half Reynolds number against fronds having vesicles described in **Figure 4.4.** Differences among morphological traits affecting their Cd values also were described in the case of a same algal species but living in different wave-exposure environments. Stewart (2006) compared macroalga *Turbinaria ornate* across a barrier reef in a disparity for morphological variation. The drag coefficient Cd was higher for forereef (wave-exposure) fronds than backreef (wave-shelter) fronds at low flow speeds (0.32 and 0.5 m s^{-1}), but the difference decreased at higher flow speeds. The more upright posture of forereef fronds at low flow speeds correlated with higher values of Cd. At high flow speeds, the forereef fronds were bent over in the flow to a similar extent as backreef fronds and the Cd approached that of the backreef fronds.

3.5 Conclusions

This chapter is the first to study drag force exerted on small sizes of the frond especially for low flow speeds. This study verified the conclusion that small fronds had similar drag force.

Chapter 4 Drag on subtidal seaweed *Sargassum horneri* (Turner) C.Agardh varies among thallus sizes and velocities

4.1 Introduction

Sessile organism dislodgement, which is fatal to seaweeds, such as *Sargassum horneri*, usually results in the mortality of the individual and causes floating for Sargassum species. Pressure drag force is a bulk force to make the thallus broken or dislodged from the substratum. Charters et al. (1969) calculated the relative effects of skin friction drag and pressure drag on seaweed species *Eisenia arborea* (Aresch 1876), and found that the overwhelming proportion of drag on the alga was due to pressure drag.

Pressure drag is built the relationships among the size of an object, the viscosity and velocity of the fluid as described by the drag-equation (e.g. Vegel 1984). The force exerted on the seaweed considered only the hydrodynamic forces due to steady, nonaccelerating water flow (Jones and Demetropoulos 1968; Branch and Marsh 1978). However, owing to *Sargassum horneri* surviving in wave-strict tidal zones, "subjected to a given current velocity, macrophytes experience a drag force 25 times higher than terrestrial plants exposed to a similar wind speed (Denny & Gaylord 2002)", this species face high risks from the detachment or breakage especially in wave-exposure areas.

Annual *Sargassum* species *Sargassum horneri* is detached by waves after sex reproduction and then becomes floating, generally in May or June depending on its living latitude (Mikami 2007; Xu 2008). Mikami (2007) set 3 permanent quadrats at Nabeta Cove in observing detachment information of local *Sargassum horneri* populations. She found that the number of individuals in these quadrats was decreasing through month variation and thallus growth. Detachment of this species through variations of life history is vital to understand forming mechanisms of floating rafts in the East China Sea.

Sargassum species are the finely branched plants with narrow, cylindrical axes and many thin, lanceolate blades. Among them, *S. horneri* has very soft stipes, so it might be expected to reconfigure during the peak flow to a drag-reducing shape for avoiding the detachment and breakage. In December, the thallus size remains very small, generally no beyond 30 cm thallus length, so they might reorient or reshape to a clump in flow to limit the pressure drag force, which results in a more compact profile and effectively a reduced surface area. However, when they grow to a huge size standing via many vesicles in the sea in March when the population is in between maturity and no maturity, they are going with the flow as waves pass along the thallus like many other giant kelps such as *Nereocystis luetkeana* (Koehl and Wainwright 1977) and *Macrocystis pyrifera* (Utter and Denny 1996). These bull seaweeds employ a survival strategy "going with the flow" facilitated by the flexibility and great length to avoid the brunt of hydrodynamic forces. It is effective primarily only for plants longer than approximately 1 m. Since shorter plants cannot reorient during a fraction of the wave cycle, this strategy is less effective for them.

Drag coefficient Cd vary with algal size, the physical characteristics of fluid, and the fluid velocity. To compare coefficients between objects of different sizes, Cd are plotted as a function of Reynolds number, Re, a dimensionless measure of the relative contribution of inertial and viscous processes in determining flow patterns around objects. Cd values used in this study were obtained at Re~10^5. As Vogel (1981) observed, the variation of Cd with Re decreased as the irregularity of the object increased. Thus, for a flexible organism, e.g. *Sargassum horneri*, Cd were varying with Re number significantly. As the Reynolds number rises through the range 10^4-10^6 in steady state flow, Cd generally decreases gradually (Hoerner 1965, Vogel 1981).

It is necessary to understand changings of drag force on different sizes of the seaweed at velocities. In this chapter, I examined changings of pressure drag with increasing thallus size through fixed life history period and identified corresponding values in different size ranks.

4.2 Materials and Methods

4.2.1 Study site and in-situ experiment

Study site was located at Nabeta Cove, Shimoda, a wave-sheltered rocky area near Shimoda Marine Research Center (SMRC), Tsukuba University, Japan. Scube divers collected healthy individuals of *Sargassum horneri* randomly for sizes on April 17 2014 and Feb 25 2015, independently. After field sampling ended, fronds temporarily were kept in an outdoor tank, covered by a blackout cloth, and continuously, under moving seawater fluid for <24 h before in-situ experiment.

The method measuring drag force on the seaweed was following Utter and Denny (1996)'s study. The seaweed was connected to the spring scale via a non-flexible cord running on low-friction wheels along a vertical pole attached to the side of the boat. The pole, extending approximately 0.5 m into the seawater, and kept thallus submerged, out of the wake of the boat. Thallus was towed behind a boat at known speeds while forces were recorded with Pesola spring scales. Spring scales used in in-situ experiment had been calibrated by known weights firstly. Each frond was subjected to velocities roughly in 0.5-4 m s^{-1}. Four readings were recorded at each velocity, and 3-4 different velocities were arranged for each frond.

Plants used after in-situ experiment were immediately transported to a laboratory and frozen them till furthermore analyze morphological traits. After thawing, thallus length was measured from the bottom of holdfast to the top of longest lateral branch. They were determined to an accuracy of $\pm$1cm using a 30 cm-length ruler. Wet weight of the plant was weighted to an accuracy of $\pm$ 2g with a balance (CR-5000WP, CUSTOM) after absorbing seawater on the plant with paper towel. The planform surface area of each individual been precise to 0.01 cm^2 was calculated using ImageJ 6.4 (NIH, USA, http://imagej.nih.gov/ij) by measuring its area from a digital picture of the frond photographing on its side, following calibration with a square of known area (5 cm×5cm). We prepared 5 photographs for each specimen and used the mean of these estimates.

Also, a define for drag force (Fd) as:

$$Fd = \frac{1}{2} \times \rho_{seawater} \times U^2 \times A \times Cd$$

Where $\rho_{seawater}$ is seawater density (1.025 g cm^{-3} at 20°C), U is the fluid velocity relative to the seaweed, A is a characteristic area of the seaweed, and Cd is the drag coefficient, a dimensionless number that accounts for the interaction between the flow and the shape of the seaweed.

And, a define for Reynolds number (Re) as:

$$Re = U \times L / \upsilon$$

Where U is fluid velocity relative to thallus, L is a characteristic length of thallus, and υ is the fluid's kinematic viscosity (0.01049 cm^2 s^{-1} at 20°C). In this paper, characteristic length is equal to the division of planform surface area of thallus to thallus length.

Other implementations

In field, main stem elongating from rhizoid forming holdfast base of the seaweed was often tensed by waves. Thus, thallus length was used replacing it to fit the relationship of length against planform surface area.

Fractional error for an individual was following the formula "Fractional error=(predicted item-measured item)/(predicted item)" (Utter and Denny, 1996). Wet weight was separated and divided into 3 groups: dividing point by 60 g, dividing point by 100 g and all the data combined. Absolute fraction error applying in relationships between wet weight and planform surface area, results showing that dividing point of 100 g was the best way among them, the error only remaining 20.2%±21.2% (**Table 3.1**).

Two reasons for 0.5 m s^{-1} velocity taken as a start point to estimate drag force were that 1) a value with division of project area toward vertically to flow to planform surface area of *Sargassum* species was approaching to stable after 0.5 m s^{-1} velocity (Hasegawa, 1999) and that 2) most of field data in our experiment were recorded beginning from 0.5 m s^{-1}. Planform surface area was used instead of characteristic area of *S. horneri* as they changed less after 0.5 m s^{-1} velocity and were easily measured. In addition, seaweeds experienced velocities of which were monitored to over 2 m s^{-1} (up to 3-4m s^{-1}) during the storm at Marmion Lagoon, 20 km north of Perth, Western Australia (32°latitude) (Bettignies, et al. 2013). Thus, maximum velocity of 4 m s^{-1} had met ecological requirements for the plant experienced by storm velocities. In this paper, flow was classified into 3 types: low flow in 0.5-1 m s^{-1}, median flow in 1-3 m s^{-1} and peak flow in 3-4 m s^{-1}.

Dimenionless drag coefficient Cd was estimated by Reynolds number as described in Sugawara (1998). We obtained them according to Reynolds number of which included factors of both velocity and thallus size. Specimens were grouped depending on their size by an interval of thallus length 50 cm. Especially ＞200 cm thalli were grouped into a size group owing to few number.

Similar thallus length of two fronds had large weight disparity between each other. Thus, their Cd values were calibrated by a division of Cd value predicted by model weight against Cd value predicted by real weight according to relationships between wet weight and mean individual Cd (**Figure 3.4**). Model weight was predicted by thallus length as showing in **Figure 3.1**.

4.3 Results

4.3.1 Fit planform surface area by thallus length and weight

Planform surface area and wet weight independently were fitted by thallus length of *S. horneri*, as showing in **Figure 3.1**. There had exponent relations among them. Planform surface area was fitted by wet weight (**Figure 3.2**), when they had a linear relationship against each other.

4.3.2 Estimate Cd by Reynolds number

As Re increasing, Cd was decreasing, and limit threshold of Cd decreasing from 0.02 to 0.002 varying among thallus sizes (**Figure 3.3**). Small size thalli had less Re number compared with larger ones (**Figure 3.3** and **Figure 3.4**). Cd varying with thalli length calibrated by weight factor was better than the case of no calibration (**Figure 3.5**).

4.3.3 Drag force on the seaweed depending on its size

Drag force was increasing with thallus length especially in peak flow (**Figure 3.6**). Most of forces in the group of 0-50 cm lengths were <5 N at velocities except for few data being a little >5 N during peak flow. Forces on individuals of the group of 50-100 cm lengths were <15 N whereas most in the group of 100-300 cm length were <20 N. During low flow, drag forces on all the groups were in 0-5 N. During median flow, specimens of all the groups were

<15 N. Few drag forces were >30 N during peak flow. Final, most of fraction errors comparing force measured by field with force estimated by drag model were in ±50% and drag force in ±5 N (**Figure 3.7**).

4.4 Discussion

Thallus size is crucial to determine how much drag force on algae (Dudgeon and Johnson, 1992; Jensen, 2008; Bettignies et al., 2013; Demes, 2013). For example, reproductive sporophylls of *Alaria marginata* (Postels and Ruprecht, 1840) experienced greater drag than non-reproductive sporophylls. However, after correcting for differences in size among sporophylls, there were no differences in (size-specific) drag between reproductive states. It was suggested that increased size was an important component of the added drag force (Kyle W.Demes, 2013). Larger thallus sizes of which include factors of length and weight of *S. horneri* usually had much more drag force than small individuals. (**Figure 3.6**). Dudgeon and Johnson (1992) found that drag on *Chondrus crispus* (Stackhouse) thalli of >3g fresh weight was greater than on *Mastocarpus stellatus* (Stackhouse) Guiry of the same biomass, whereas drag on smaller fronds(<3g fresh weight) of the two species was similar. Drag forces on small individuals of *S. horneri* had few differences among each other, since they might hold on similar morphological traits (**Figure 3.6**).

Compared with other species such as maximum 90 N drag force created by water flow on giant kelp *Macrocystis pyrifera* (Neushul et al., 1967), *S. horneri* belongs to a median type in drag force contrast to its large size, only attaining 30 N during peak flow in the size >250 cm length (**Figure 3.6**). *Sargassum horneri* has very soft stipes so it can change its shape suiting to different flow intensities, thus, avoiding the risk from wave-strict field been breakages and dislodgements. Same situations of seaweeds' morphological traits and their flexible shape adapting to flow intensities (e.g. Carrington 1990; Koehl et al. 2008) had been discussed a lot.

Morphological factors tightly linked with variations in hydrodynamic performances of seaweeds (Koehl, 1986; Koehl and Alberte, 1988; Gaylord & Denny, 1997; Koehl, et al., 2008). For example, Koehl and Alberte (1988) found that differences in blade morphology of *Nereocystis luetkeana* caused greater flapping of wide, undulate blades in flow, as opposed to narrow, flat blades, that increased the wake size, and therefore, drag.

In addition, traditionally for Cd value, the drag coefficient, this is a gross oversimplification. Cd, initially that is used to estimate drag on fixed shape of organisms (Koehl, 1977), however, now it is applied in cases of flexible algae, generally having very small value in the limit threshold during peak flow. This Cd was changing among velocities and thallus sizes of the seaweed. In a case of *Sargassum horneri*, when thallus length varying from the group of 0-50 cm to the group of 200-300 cm, Cd limit threshold also was decreasing 10 times from 0.02 to 0.002. Past studies demonstrated similar results of drag coefficients Cd for several kelps under high flow regime: 0.01-0.05 (Koehl 2000; Kawamata 2001; Thomsen 2004). When Cd converged at water velocities characteristic of storms (4 m s^{-1} in this study), morphological traits had a minor effect on drag due to the algae being equally streamlined at these higher velocities. Final, drag coefficient Cd was estimated by Reynolds number. When Reynolds number is a level of 10^{5-6} such as the case of *S.horneri*, visocity force of the flow becomes very small and pressure drag is main force to determine the thallus (Koehl, 1977; Denny, 1985).

4.5 Conclusions

This chapter is the first to determine influences of drag force on subtidal seaweed *Sargassum horneri* (Turner) C.Agardh in between thallus length 21 cm to 263 cm among velocities. We estimated its drag force depending on different size ranks, and developed a weight factor to calibrate initial Cd in Koehl(1977)'s drag formula.

Chapter 5 Conclusions

5.1 Evaluate risks of dislodgement from the field

Mikami (2007) found the number of *Sargassum horneri* individuals in the quadrats was decreasing with the growth of the thallus. *S. horneri* need to balance the force between attachment strength and dislodgement along the life history to resist against wave force with the growing in the field. Thus, it is necessary to assess risks exerted on the *Sargassum horneri* fronds in the field especially for the dislodgement.

We conducted in-situ surveys in the Nabeta Cove that was close to Shimoda port. We used waves data (December 2014-May 2015) collected in Shimoda port provided by social organization "nowphas" of which offer free open waves data covering Japan main port. Orbit velocity calculated by waves was changing with the time. We applied significant waves data into small amplitude waves model to calculate x-axis velocities among the depths. Then, we used drag model developed as the above chapter showing to predict drag force for each dislodgement frond at a certain depth. These predicted drag forces were compared with dislodgement force to identify dislodgement information from December 2014 to May 2015 along its life history.

Small-amplitude waves formula:

$$u = \frac{H}{2}\sigma \frac{\cosh k(h+z)}{\sinh kh} \cos\,(kx - \sigma t)$$

Where H is wave height; σ is radian frequency; k is radian wave number; t is time the fluid point moving; h is water depth; u is x-direction velocity.

Among them, the model's assumptions are included:

(1) When t=0, x-axis horizontal water velocity is the maximum value. The orientation of water velocity propagation is perpendicular to planform surface area, and at that time, drag force is the maximum exerted onto the frond.

(2) All the fronds are set to the location (0, depth). Where x-location is 0, y-location is the depth the seaweed attached onto.

(3) Disparity of dislodgement forces between specimens collected date before 5 days and that day after 5 days is none. During immaturity season, attachment strength of the frond is improved with the growth of holdfast basal area.

(4) All the fronds are simplified to a point. We use their attaching depth as the point y-location to calculate the water speed at that point. *S. horneri* grows to 2-3 m in the field, and upper planform surface area is far more than the bottom. In addition, the water depth is shallower, the velocity is higher. Thus, actual drag force exerted on the frond is more than predicted drag force.

During in-situ measurements to acquire data of drag force about *S. horneri* fronds on the boat, the case of stem breakage was happened in high boat speed in around 4 m s^{-1}. In this case, planform surface area perpendicular to the speeds is smaller than real frond surface area. In the field, water velocity created by waves attains to 15-20 m s^{-1}. Thus, survival environment and development strategies of *S. horneri* are important to the population. In addition, the size of the frond (biomass and thallus length) determines the drag force on them. In peak flow of 4 m s^{-1}, most of the fronds kept attachment on the substratum, and however, the thallus has a more possible to be attached during very high water speeds (e.g. extreme weather typhoon). In sea surface, water speed is much more large than the bottom, so fronds close to sea surface are easy to be detached. Drag force is increasing with velocity in exponent way, so when velocity becomes higher with time passing, possibilities of seaweed detachments are higher.

In **Figure 5.1**, winter storm is strong, especially in December. Many juveniles had a high possibility to be detached in December as showing in **Figure 5.19**. However, after December, 78-96% specimens were expected to be kept in the substratum, especially in February to April. At that time, *S. horneri* population experience two rapid growths. In a first time, with water temperature rising, *S. horneri* population is the first fast growing. In a second time, after receptacles appear, weight of *S. horneri* becomes weigh. During immaturity stage, dislodgement force is increasing with holdfast basal area, and in a converse, during maturity stage, dislodgement force has no relations with morphological traits such as frond weight, thallus length and holdfast basal area. In December, dislodgement force of most juveniles are weak, and under storm weather or strong wind weather, these individuals are easily to be detached.

In December 2014, for water depth 2.2-2.4 m (see **Figure 5.2**), whatever for each group of biomass, they have a possibility to be detached in a bad weather such as in 16^{th}, or 21^{th}. In this group drag force is roughly proportional to the biomass. In **Figure 5.3**, comparing same biomass of fronds but with different depth (shallower against deeper), we find that they have a same possibility to be removed away in bad weathers such as in 16^{th},21^{st} or 29^{th}. In **Figure 5.4**, comparing different biomass but with similar depth of bottom depth 2-3 m, they are detached in most time. In **Figure 5.5**, comparing different biomass but with similar depth of bottom depth 1-2m, they are detached in most time. **Figure 5.2** - **Figure 5.5** show that seaweed detachment has no relation with bottom depth in December but relating with wave information of this month.

In January 2015, for **Figure 5.6**, comparing a same biomass but with similar depth of bottom depth 1-2m, they are safe in all dates of the month. In **Figure 5.7**, comparing different biomass but with similar depth of bottom depth 2-3 m, they are safe. In **Figure 5.8**, comparing different biomass but with similar depth of bottom depth 3-4 m, they are safe. **Figure 5.6-Figure 5.8** show that for each bottom depth and biomass, most of specimens are safe. However, in **Figure 5.9**, when for specimen of among different biomass and depths, they have a possibility to be detached in the peak flow (e.g. in 7^{th}, 15^{th}, 23th, 28^{th}, 31^{st}). In **Figure 5.10**, for different biomass of fronds but with similar depth of bottom depth 1-2m, they have a possibility to be detached in the peak flow (e.g. in 7^{th}, 15^{th}, 23th, 28^{th}, 31^{st}). **Figure 5.9** and **Figure 5.10** show that in January, part of specimens whatever for depth or biomass, they has a possibility to be detached in a peak flow.

In February 2015, for **Figure 5.11**, comparing different biomass but with similar depth of bottom depth 1-2 m, they are safe in all dates of the month. In **Figure 5.12**, comparing different biomass but with similar depth of bottom depth 2-3 m, they are safe in all dates of the month. **Figure 5.11**-**Figure 5.12** show that for each bottom depth and biomass, most of specimens are safe. In **Figure 5.13**, for a specimen with a larger biomass and a shallower depth, it is expected to be detached in most dates of the month.

In March 2015, for **Figure 5.14**, comparing different biomass but with similar depth of bottom depth 1-2 m, they are safe in all dates of the month. In **Figure 5.15**, for a similar biomass but with a same 1.5 m depth, they have different dislodgement forces, so one frond is expected to be detached in most dates of this month whereas another frond is expected to be safe in most dates of this month. Meanwhile, for a specimen of which live in a shallower depth with a heavier weight, it is expected to be detached in most of dates of this month although its dislodgement force is tending to the attachment strength limit of this species.

In April 2015, for **Figure 5.16**, comparing different biomass but with similar depth of bottom depth 1-2 m, they are safe in all dates of the month. In **Figure 5.17**, comparing different biomass but with similar depth of bottom depth 2-3 m, they are safe in all dates of the month. Even for larger biomass but with deeper depth (biomass=~2038 g and depth=3.0 m), it is also safe in this month. In **Figure 5.18**, comparing similar biomass but with different depth of bottom depth (1.6 m against 2.6/2.8 m), these fronds have a possibility to be detached in peak flow (common in 21st,this month).

For these fronds that are larger size and earlier maturation, they are easier detached by waves. This is also the reason floating seaweeds occurring in March or earlier than this month. During February 2015 to April 2015, the kelp is fast growing and being mature, and at that time, fluid flow they experienced by is very small. These physical conditions are beneficial to

S. horneri populations' growth and reproduction. In these months, dislodgement force is far more than drag force for most of specimens. Thus, dislodgement force relating with the seaweed has a relation with the season.

5.2 Balance strategies between attachment strength and drag risk along life history

The survival of *S. horneri* will depend of the balance between the drag force (as the main hydrodynamic force) and the force required to break or dislodge the algae (break force). Results in this study verified the assumption that some *S. horneri* individuals were detached in March or earlier than this month and being floating rafts in the East China Sea. Results also were agreed with Mikami's founds that the number of individuals were decreasing across temporal variation. In juvenile stage of this species, *S. horneri* individuals had a high possibility to be moved from substrata during the winter of storm weather. In rapid growth stage and sex reproduction stage, although *S. horneri* individuals biomass were several ten times than the before, detaching possibilities were lower owing to peaceful wave conditions in most of the time. Because annual seaweed species *S. horneri* adults had no opportunities to pass the examination of strict winter storm, they finished the stage of biomass extremely expanding in only 2-3 mo and generate next generations around mother fronds after maturity in only 2 wks. Then, they are decayed away and being detached by waves in later spring-early summer. Typhoon weather in each year will attack on Japanese and Chinese coastlines in the summer. Very small spores of this species enter into a dormant period and thus avoid the physical risk created by typhoon weather. Meanwhile, floating rafts produced by fixed ones bring benefits to expand survival habitats through the floating. Annual seaweed species *Sargassum horneri* through these life history development strategies makes itself to become a common species along northwestern Pacific coastlines and successfully survives till now.

5.3 Summary

The main conclusions of the thesis are described as follows.

In Chapter Ⅱ, mean dislodgement forces of the shallow specimens tended to exceed those in deeper water through life history. Thallus length and wet weight had no relationships with dislodgement force across varieties of the month. Holdfast basal area was linearly related to dislodgement force during immature season, though not in mature season. The maximum attachment strength was keeping stable around 90 N. The minimum attachment strengths were in the range of 4.5 to 21.1 N.

In Chapter Ⅲ, as dimensionless index Reynolds number Re increasing, drag coefficient Cd was decreasing, and limit threshold of Cd decreasing from 0.02 to 0.002 varying from small sizes to larger ones. Drag force was increasing with thallus length especially in peak flow. Most of forces in the group of 0-50 cm thallus lengths were <5 N at velocities of no beyond 4 m s^{-1}. Forces on individuals of the group of 50-100 cm lengths were <15 N whereas most in the group of 100-300 cm length were <20 N. During low flow of 0-1 m s^{-1}, drag forces on all the groups were in 0-5 N. During median flow of 1-3 m s^{-1}, specimens of all the groups were <15 N. Few drag forces were >30 N during peak flow of 3-4 m s^{-1}. In addition to the model, most of fraction errors comparing force measured by field with force estimated by drag model were in $\pm 50\%$ and drag force in ± 5 N.

In Chapter Ⅳ, fronds of no vesicles had half lesser Reynolds number against fronds keeping vesicles. They had similar Cd as Re increasing. Cd of individuals of no vesicles had been about 0.6 in a contrast to 1.0 for fronds of vesicles at low flow of 20 cm s^{-1}. Drag force was varied less under and around 1 N in a case of wet weight no beyond 10 g. Drag force became a little ≥ 1.5 N in a case of wet weight in between 10-20 g.

In Chapter Ⅴ, extreme fluid flows created by waves in a whole month of December 2014 for Shimoda Port where was near to Nabeta Cove were often happened, the peak arriving at ~20 m s^{-1}. In this month, most of specimens had risks to be detached or broken by waves. Especially among them, usually specimens in shallower depth were easier detached than in deeper depth; heavier biomass of individuals was easier detached than lesser biomass of ones. In January 2015, most of specimens were stayed in a safety range, but several specimens had a possible to be detached in 7^{th}, 15^{th}, 23^{rd}, 28^{th}, 31^{st}. In winter season, thalli kept smaller size. During February 2015 to March 2015, almost all of specimens among depths were remained safe at that time, however, large biomass specimens in shallower depth of < 1m would be loss in most time. In April 2015, all the specimens among depths were safety but in 21^{st} of this month, some individuals had low possibilities to be detached. In May 2015, our specimens were collected in 28^{th},May, and at that time, *S. horneri* individuals only remained holdfasts on the substratum. Holdfasts among depths were exerted by low drag force, so all the specimens in almost time remained safety.

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Figures and Tables

Table 2.1 Dislodgement force category assignments for *Sargassum horneri* thalli from December 2014 to May 2015.

Month	Stem breakage	Dislodgement with holdfast *	Other	Total
December	0	29	1 (data lost)	30
January	1	28	1 (aggregated heptera)	30
February	4	25	1 (substrate: crustacea and coralline algae)	30
March	2	17	1 (attached to *E. bicyclis* holdfast)	20
April	2	17	1 (data lost)	20
May	1	38	1 (data lost)	40

* Thallus dislodgement force data were used in the statistical tests.

Table 2.2 One-way ANOVA testing for statistical differences in dislodgement force along the bottom depth gradient from December 2014 to May 2015.

		Analysis of Variance (One-Way)				
Stage	Month	Source of Variation	MS	F	*p*-value	F crit
Growth	15 Dec.	between groups	0.20	2.72	0.11	4.24
		within groups	0.07			
	28 Jan.	between groups	0.31	2.70	0.09	3.44
		within groups	0.11			
	25 Feb.	between groups	0.02	0.33	0.57	4.32
		within groups	0.07			
Mature	31 Mar.	between groups	0.01	0.14	0.71	4.67
		within groups	0.05			
	28 Apr.*	between groups	0.03	0.72	0.42	4.84
		within groups	0.04			
	28 May.	between groups	0.36	0.51	0.60	3.29
		within groups	0.71			

* Only measurements collected for depths of 1–2 m and 2–3 m were included in the analyses of thalli sampled on April 28 because the sample size for the 0–1 m depth range on that date was too small for inferential testing (two specimens only).

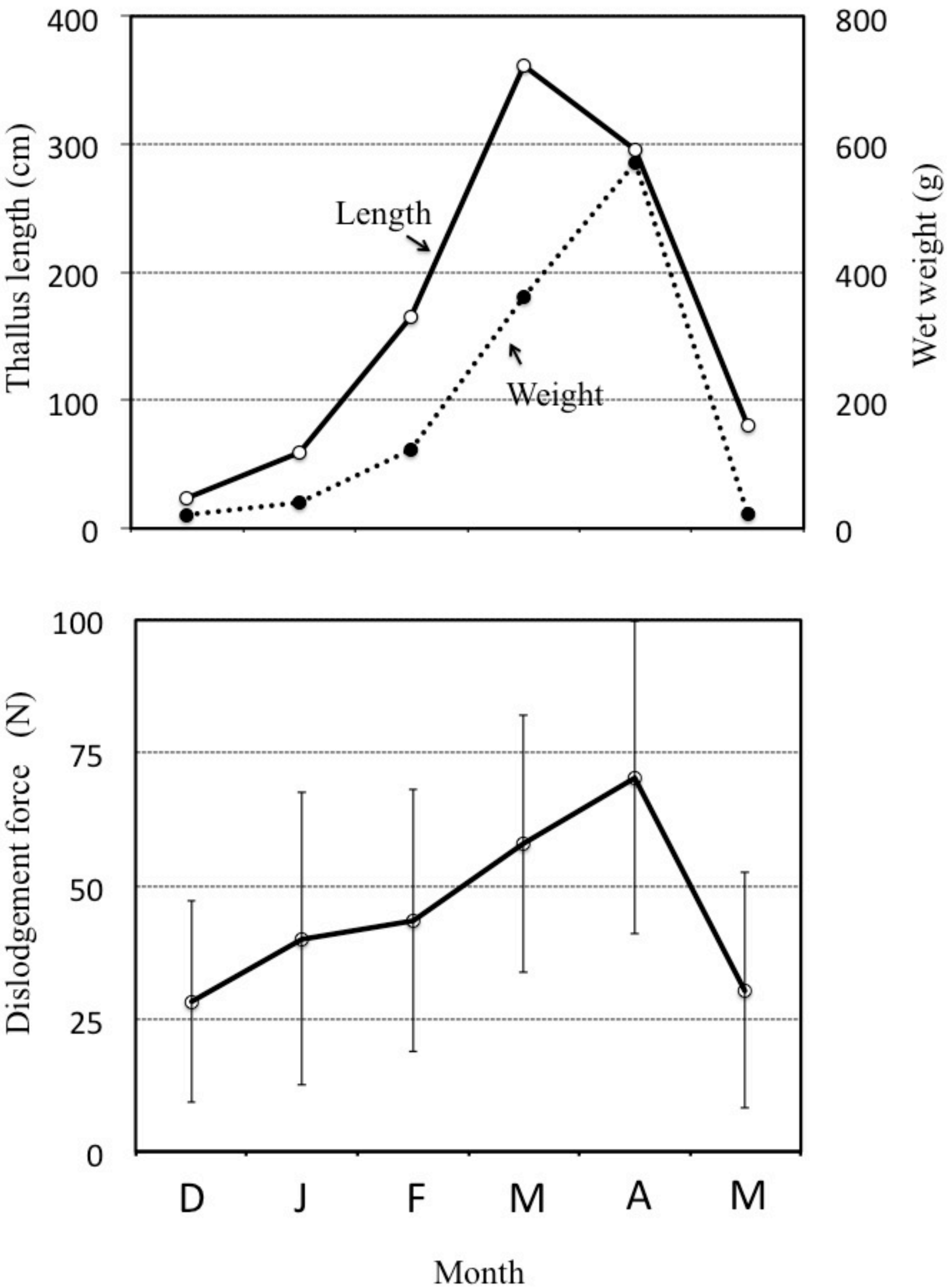


Figure 2.1 Thallus lengths (cm) and wet weights (g), and dislodgement forces (N) measured monthly from December 2014 through May 2015. Values are means ± SD.

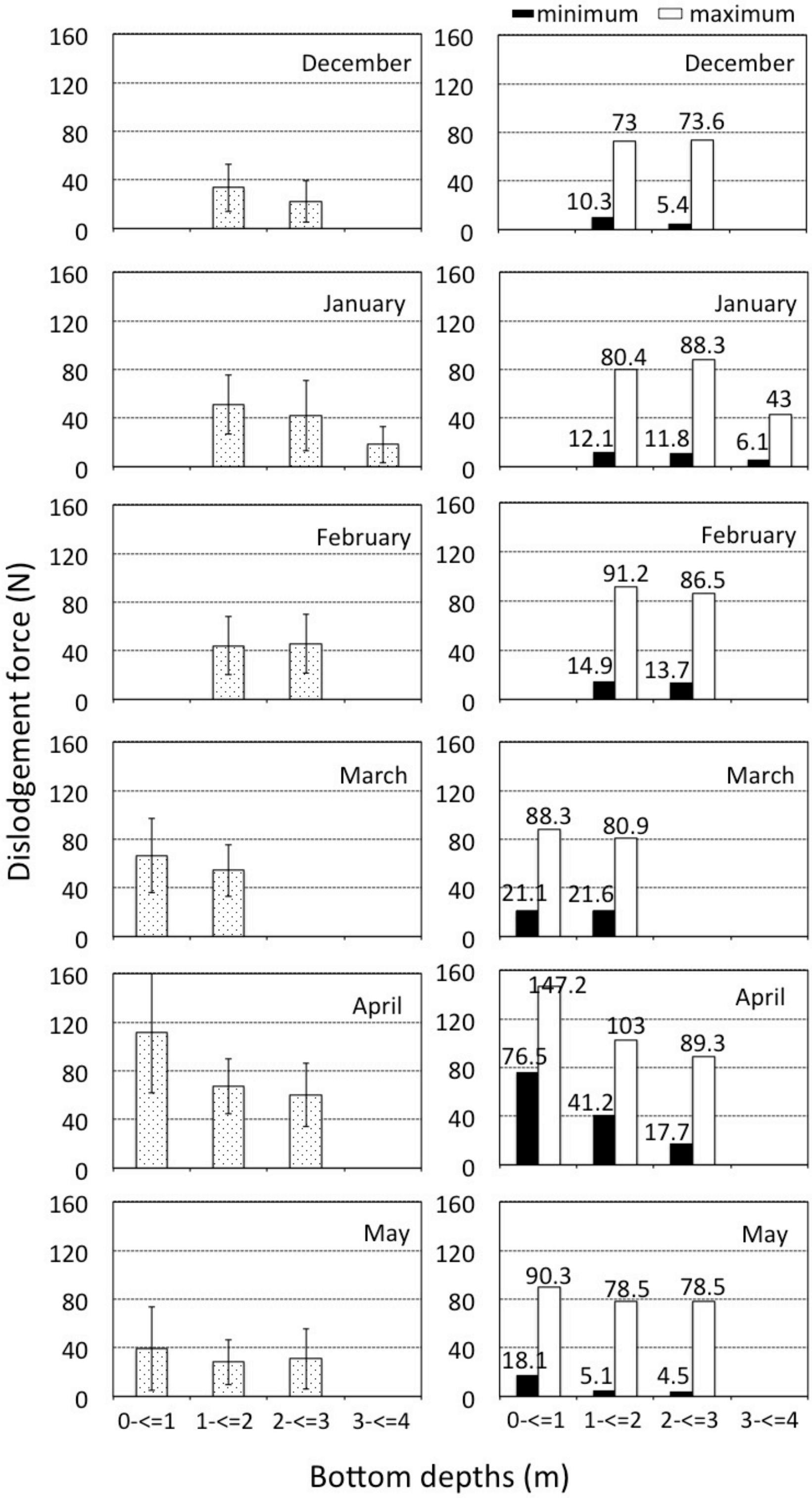


Figure 2.2 Mean (± SD; narrow vertical error bars) (left column), minimum (filled bar) and maximum (open bar) dislodgement forces (N) (right column) measured along the bottom depth gradient from 0 to 4 m below the mean tide level for each month from December 2014 through May 2015.

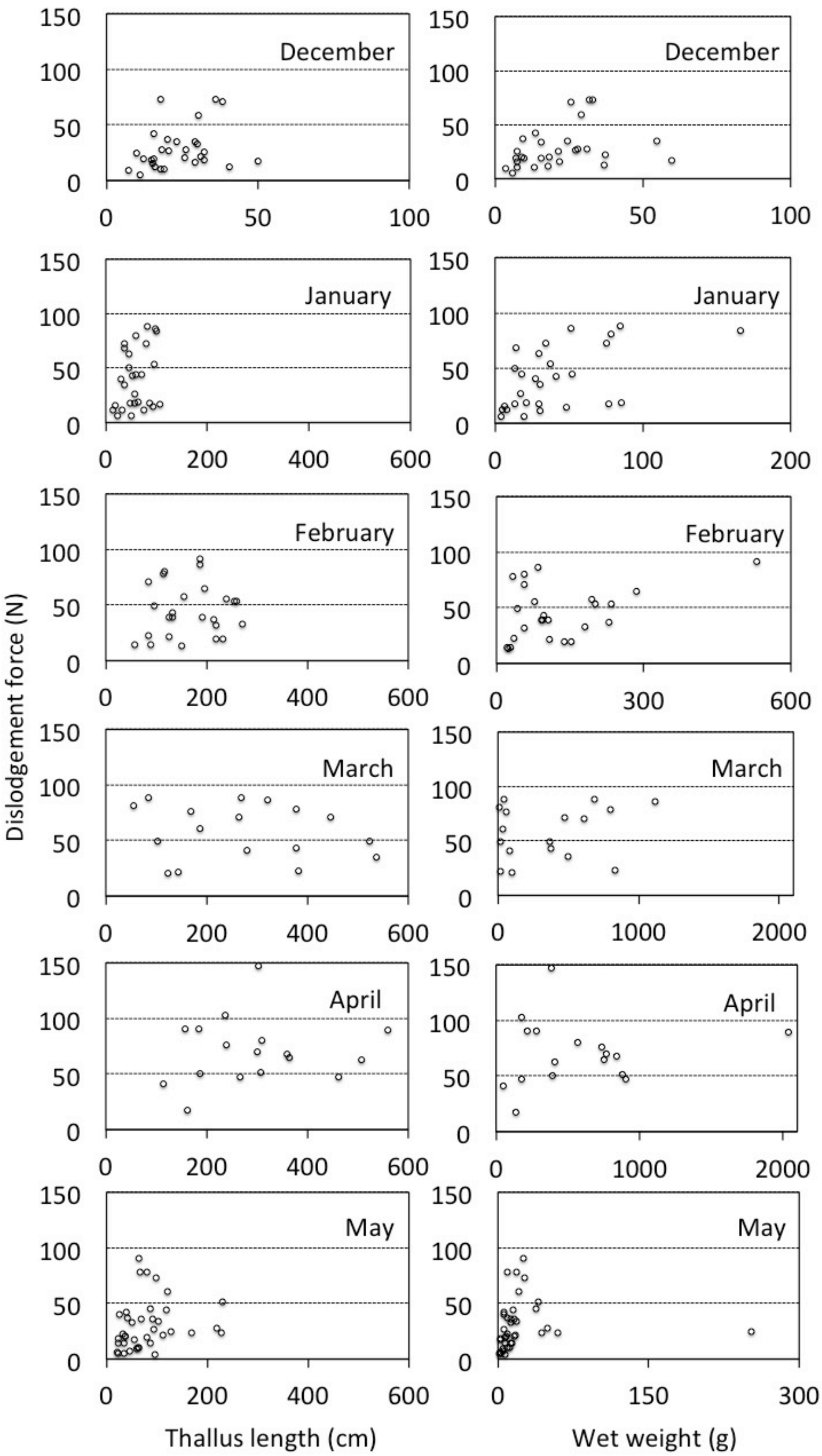


Figure 2.3 Relationships between dislodgement force (N) and (i) thallus length (cm) (left column) and (ii) wet weight (g) (right column) for each month from December 2014 through May 2015.

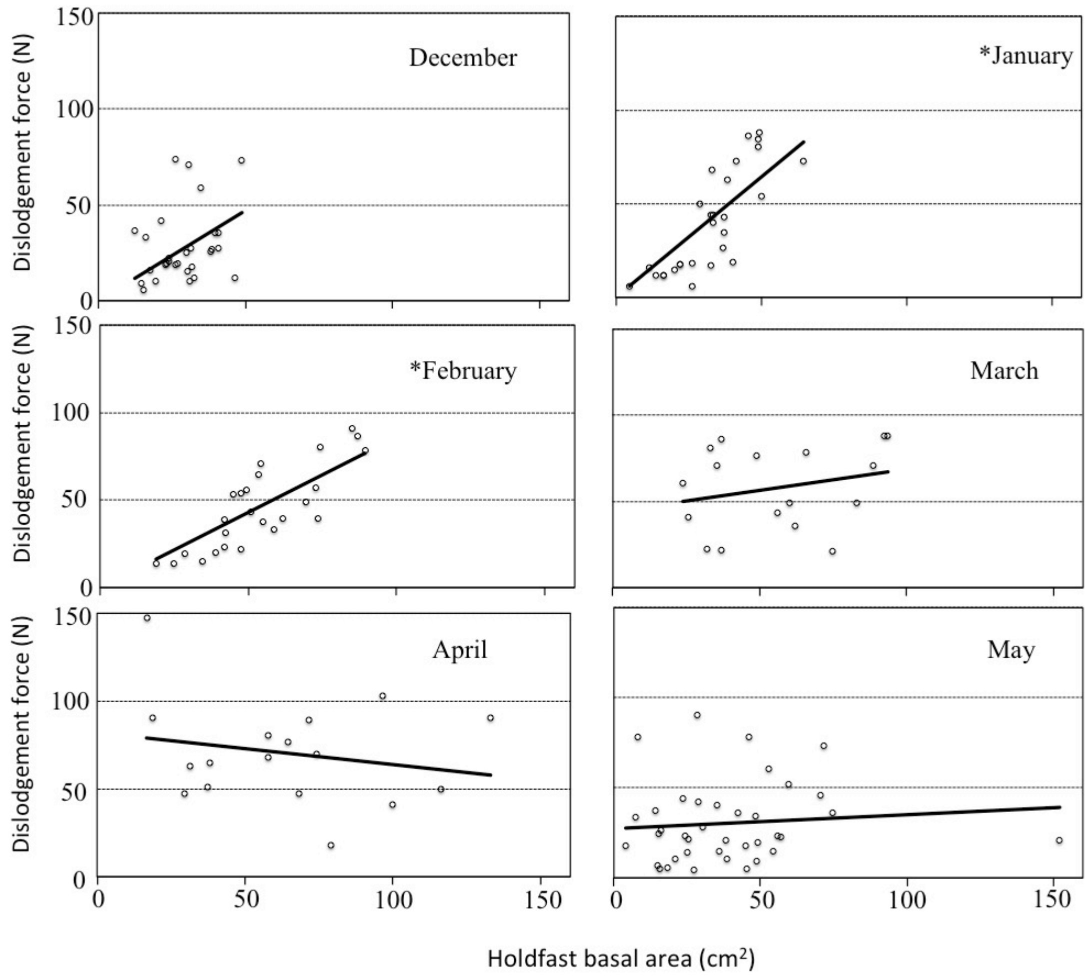


Figure 2.4 Relationship between dislodgement force (N) and holdfast basal area (cm^2) for each month from December 2014 through May 2015. Solid lines are regression fits. *, significant linear correlations in January 2015 ($r^2 = 0.86$, $p < 0.001$) and February 2015 ($r^2 = 0.81$, $p < 0.001$).

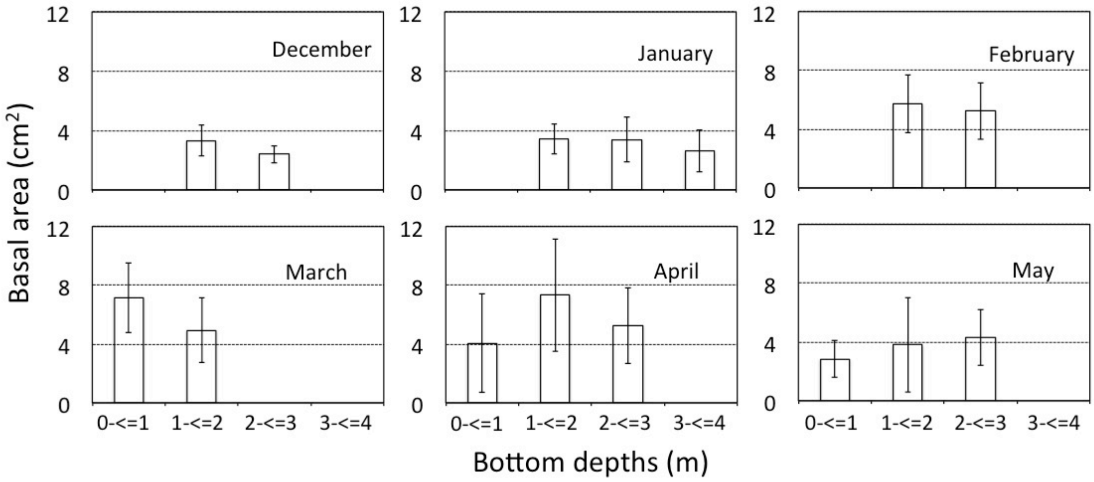


Figure 2.5 Mean holdfast basal areas (cm^2) (open bar) (±SD; narrow vertical error bars) measured along the bottom depth gradient from 0 to 4 m below the mean tide level for each month from December 2014 through May 2015.

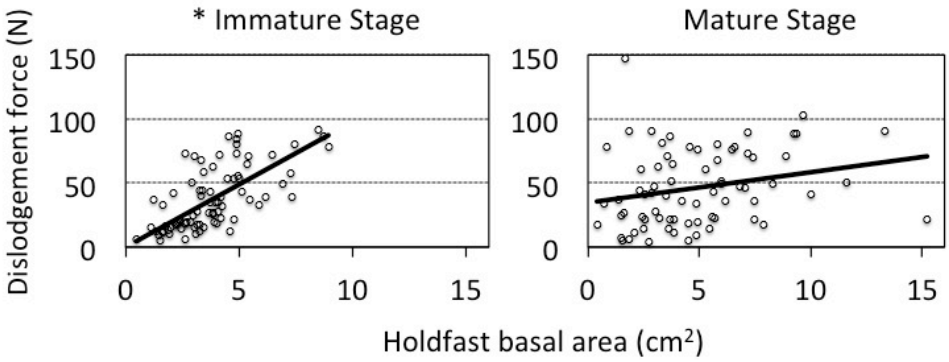
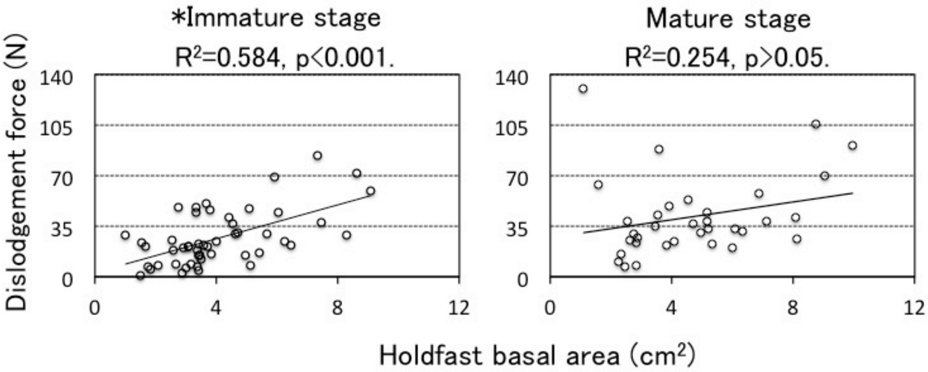


Figure 2.6 Relationship between dislodgement force (N) and holdfast basal area (cm^2) for immature and mature thalli (December 2014–February 2015 and March 2015–May 2015, respectively). Solid lines are regression fits. *, significant linear correlation in the immature stage ($r^2 = 0.67$, $p < 0.001$).



(Data were tested by Pearson correlations in R software.)

Figure 2.7 A plot of holdfast basal area (cm^2) against dislodgement force (N) during December 2013 through June 2014.

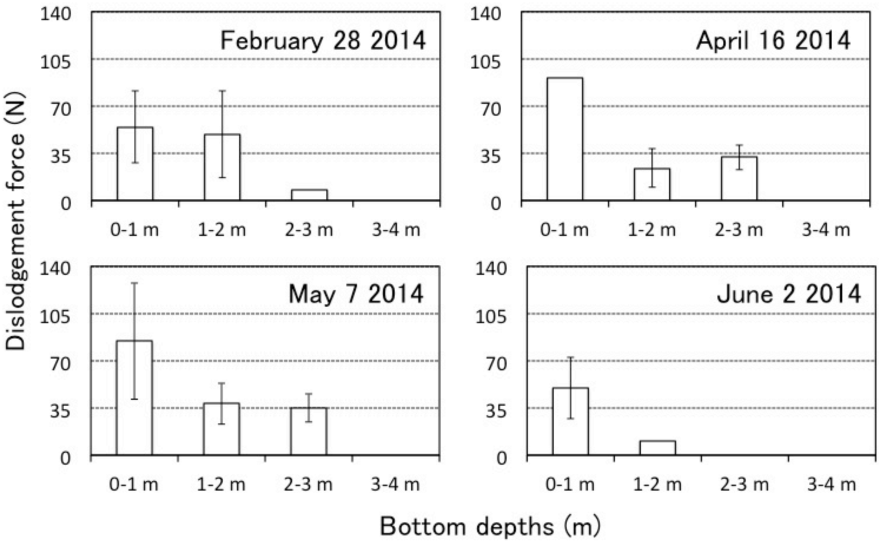


Figure 2.8 Changings of dislodgement force (N) among bottom depths during February 2014 to June 2014.

Table 3.1 Comparisons of absolute fraction error in estimating planform surface area separated by 3 groups: dividing point of 60 g, dividing point of 100 g, and all the data combined.

	Point of 60 g	Point of 100 g	Data combined
*Ab. F. Err	29.2%±38.0%	20.2%±21.2%	112.3%±60.0%

*Absolute fraction error=|predicted item-measured item|/(predicted item)

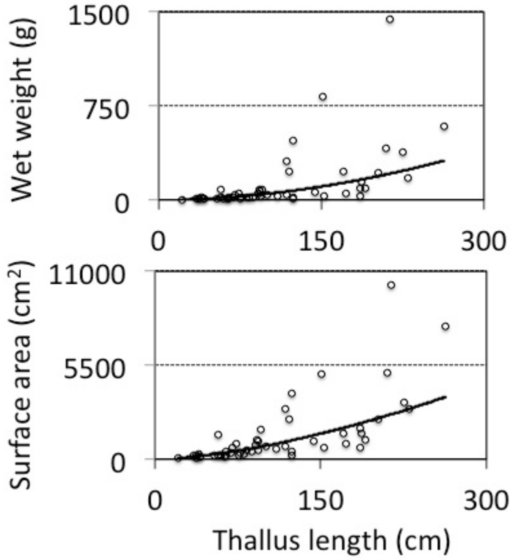


Figure 3.1 Planform surface area (cm^2) and wet weight (g) against thallus length (cm), independently.

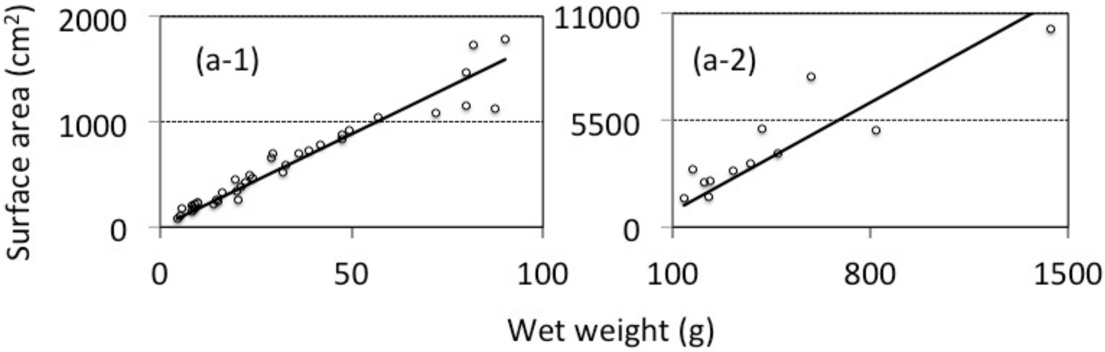


Figure 3.2 Planform surface area (cm^2) against wet weight (g) separated by the point of 100 g, including two parts of < 100 g and > 100 g.

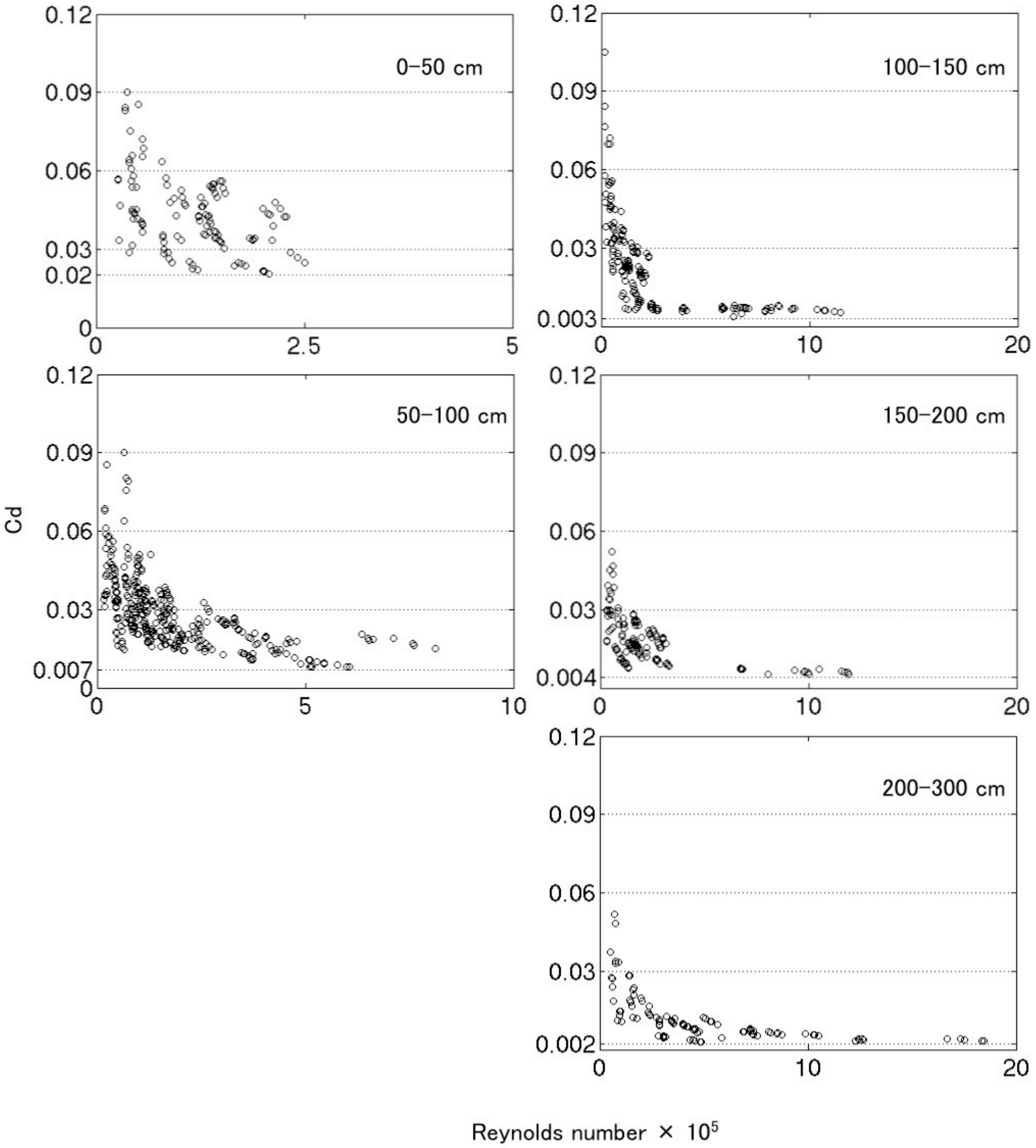


Figure 3.3 Drag coefficient Cd against Reynolds number in a function of thallus sizes among the group of 0-50, 50-100, 100-150, 150-200 and 200-300 (cm).

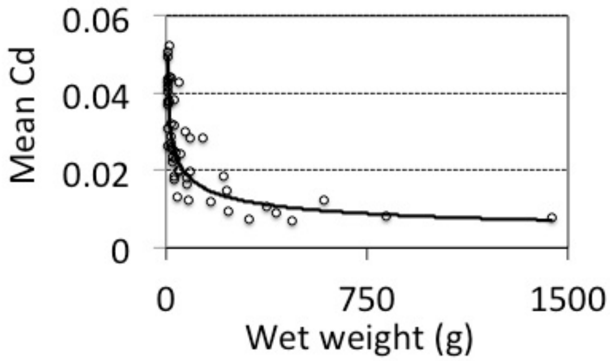


Figure 3.4 Relationships in mean Cd of a single frond against its wet weight.

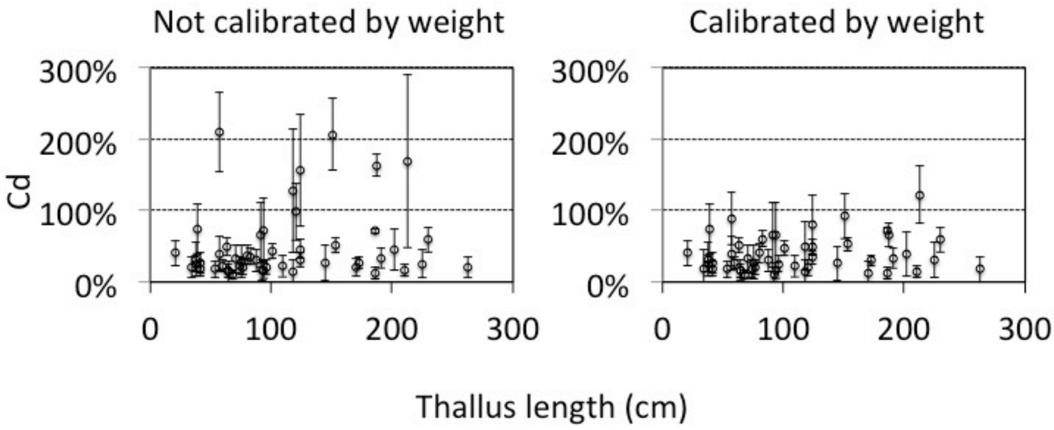


Figure 3.5 A comparison for Cd (±SD) against thallus length (cm) between the group of not calibrated by weight and the group of calibrated by weight. Values were calculated by absolute fraction error.

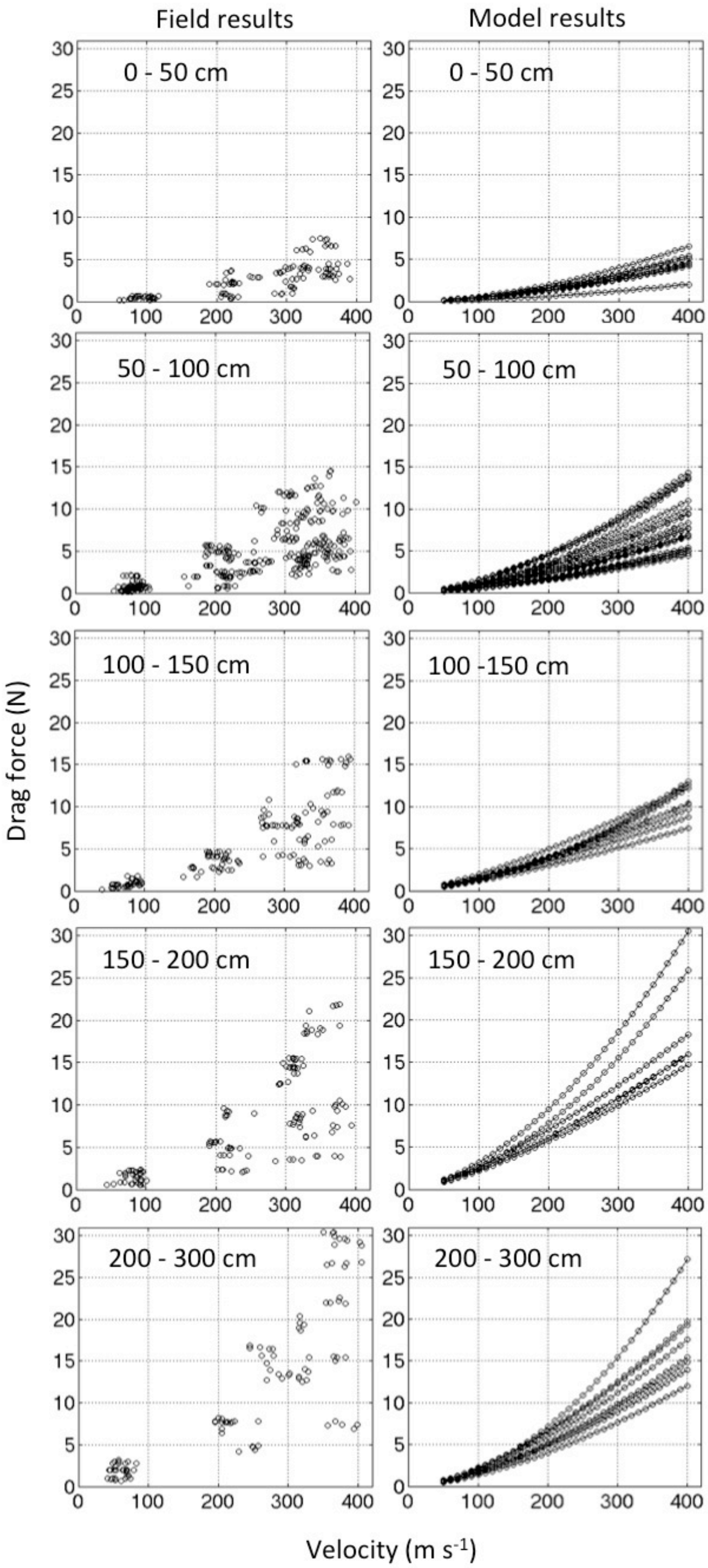


Figure 3.6 Plots of drag force (N) in a function of the group of thallus length of 0-50 cm,

50-100, 100-150, 150-200 and 200-300 cm against velocities (cm s^{-1}).

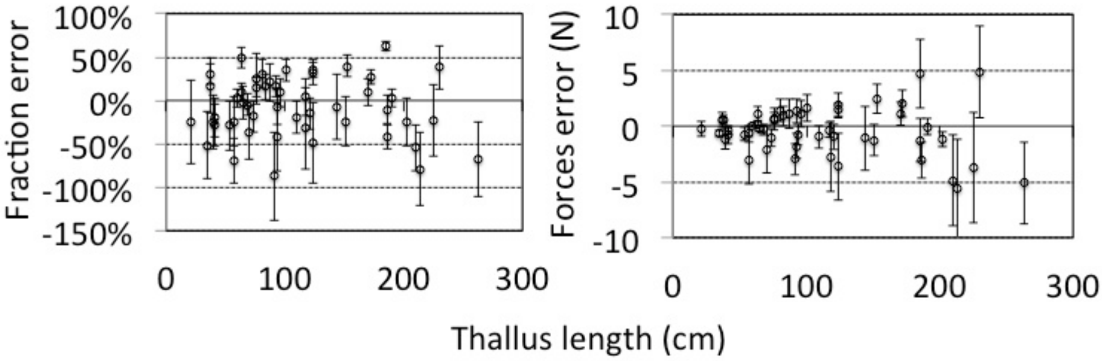


Figure 3.7 Fraction error±SD (%) and *force error±SD (N) against thallus length (cm), independently.

* Force error=|predicted force-measured force|.

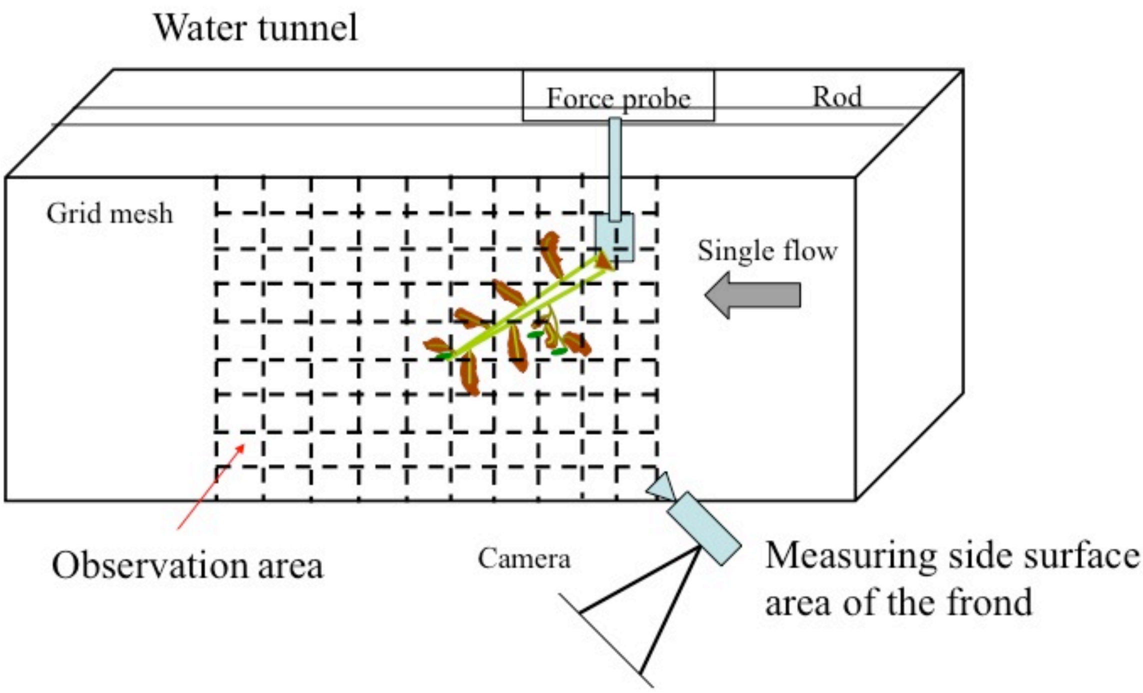


Figure 4.1 Experimental design for measuring surface area of the frond with wave perturbation. The seaweed is hanged upside down from the top of the tank. This posture ensures projected area to be influenced in low fluid speed (<0.1 m s^{-1}).

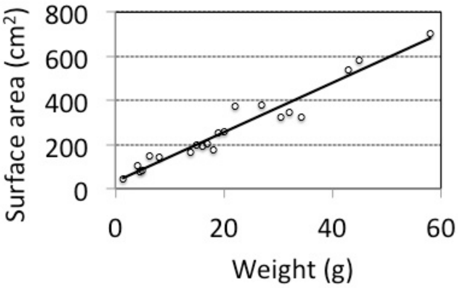


Figure 4.2 A plot of planform surface area (cm^2) against weight (g)

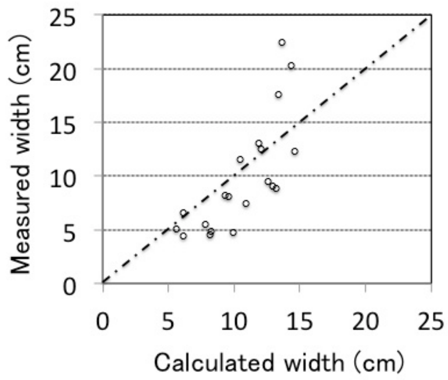


Figure 4.3 Calculated width (cm) against measured width (cm) at 20 cm s^{-1}

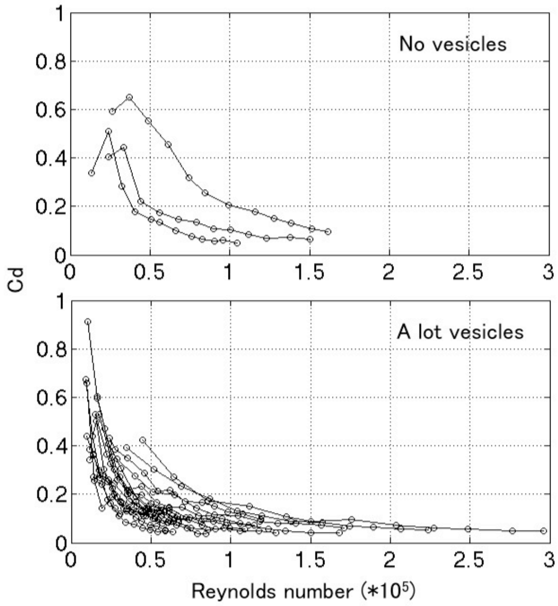


Figure 4.4 A plot of Reynolds number (10^5) against Cd value

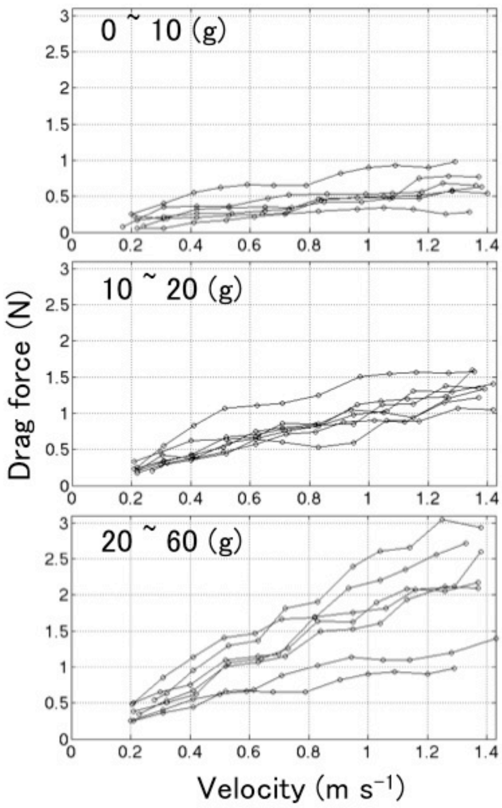


Figure 4.5 Plots of drag force (N) against velocity (m s^{-1})

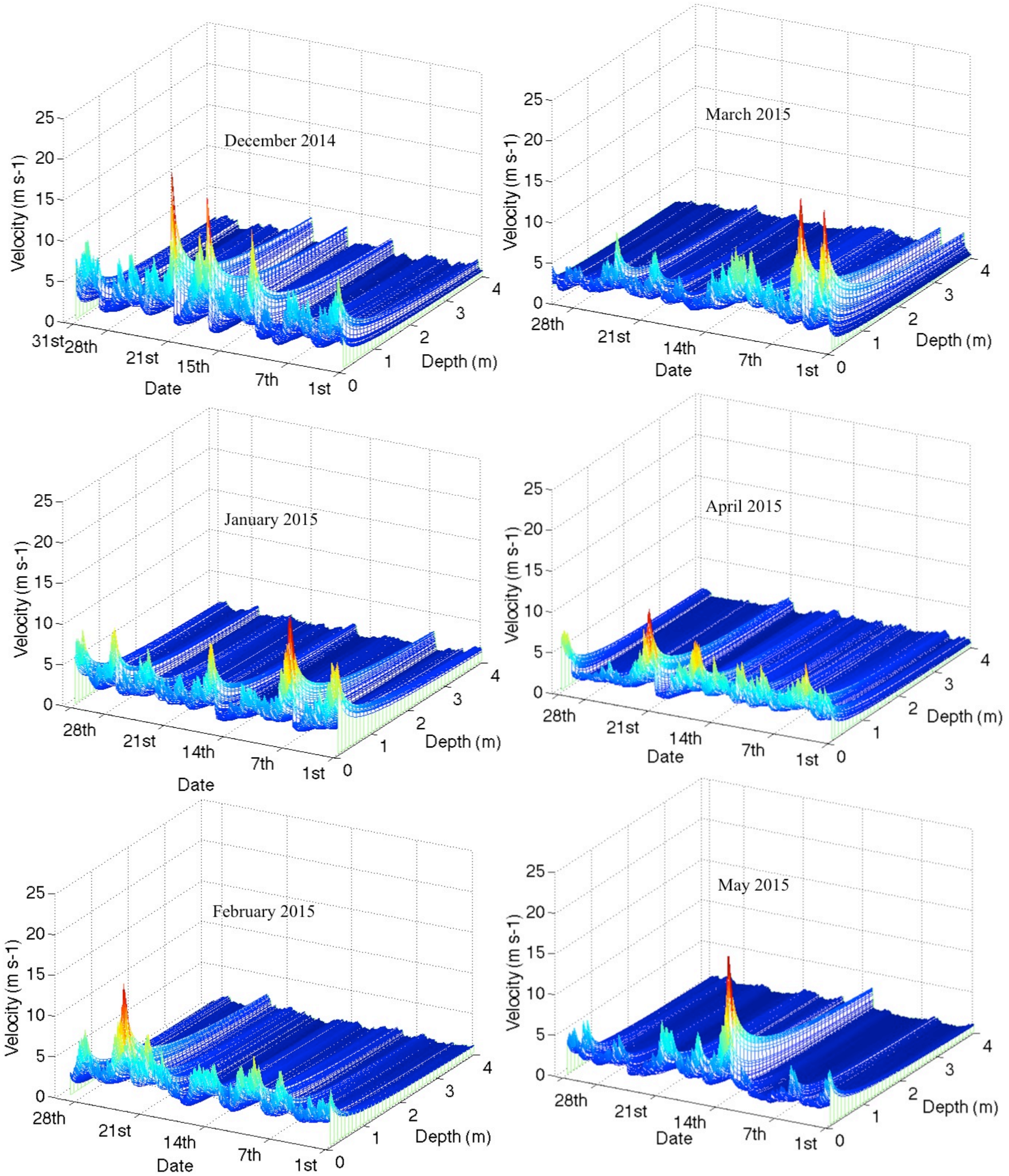


Figure 5.1 Temporal variations of velocities among depths during December 2014 to May 2015 in Shimoda port. Significant waves data is applied in this purpose.

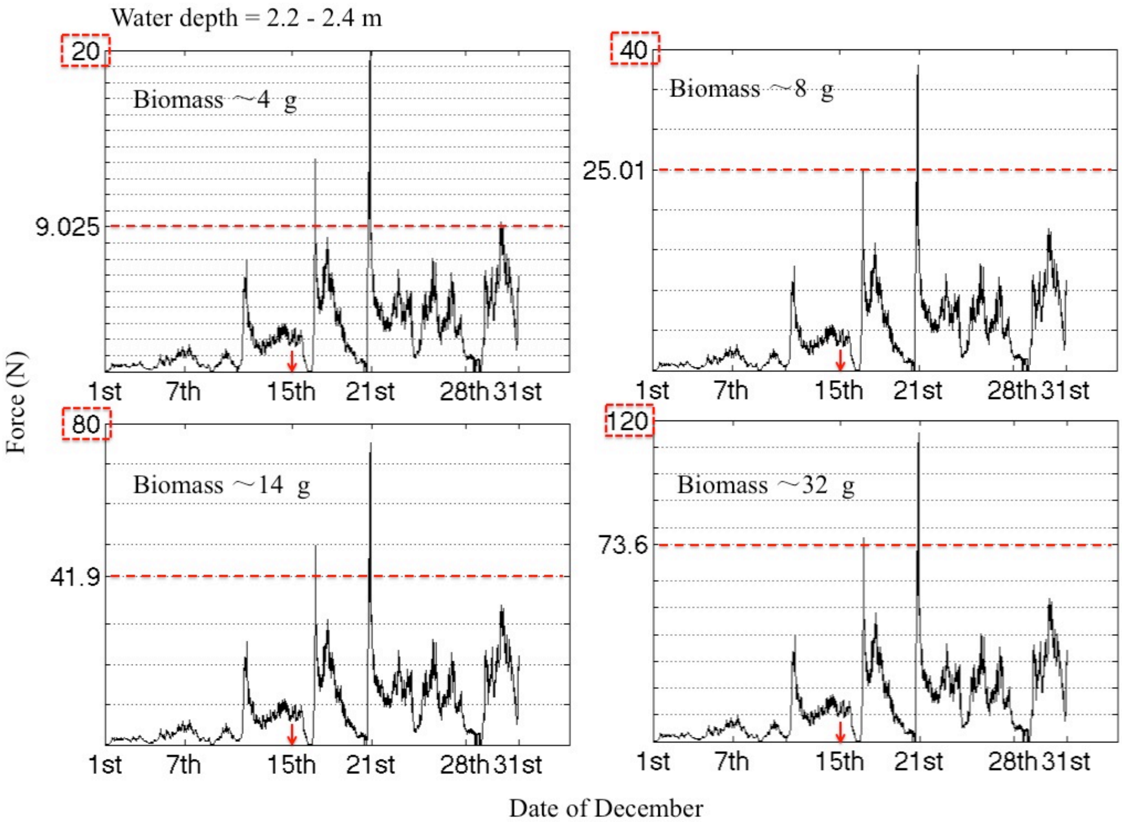


Figure 5.2 Compare drag force with dislodgement force in similar water depths 2.2-2.4 m with different biomass varying with temporal changings in December. Data were collected in December 15 2014. Red arrow is specimens collecting date.

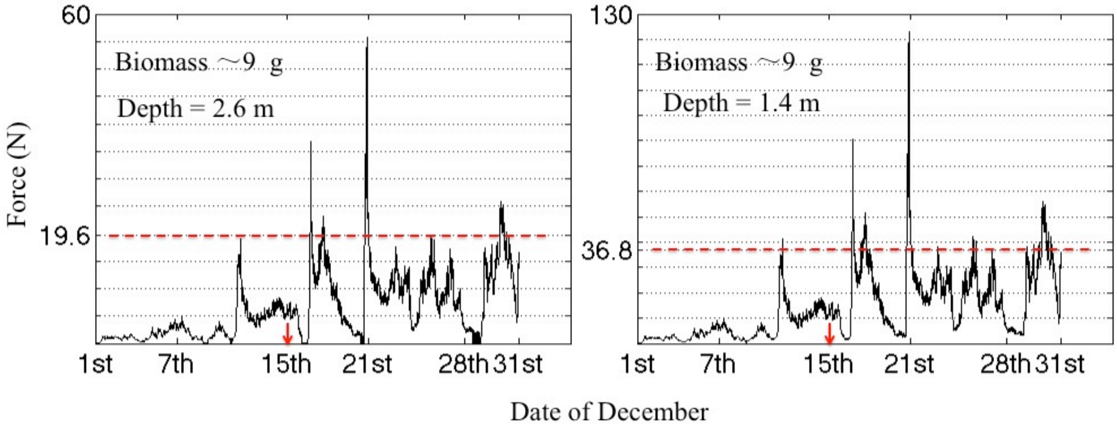


Figure 5.3 Compare drag force with dislodgement force in different water depths with same biomass varying with temporal changings in December. Data were collected in December 15 2014.

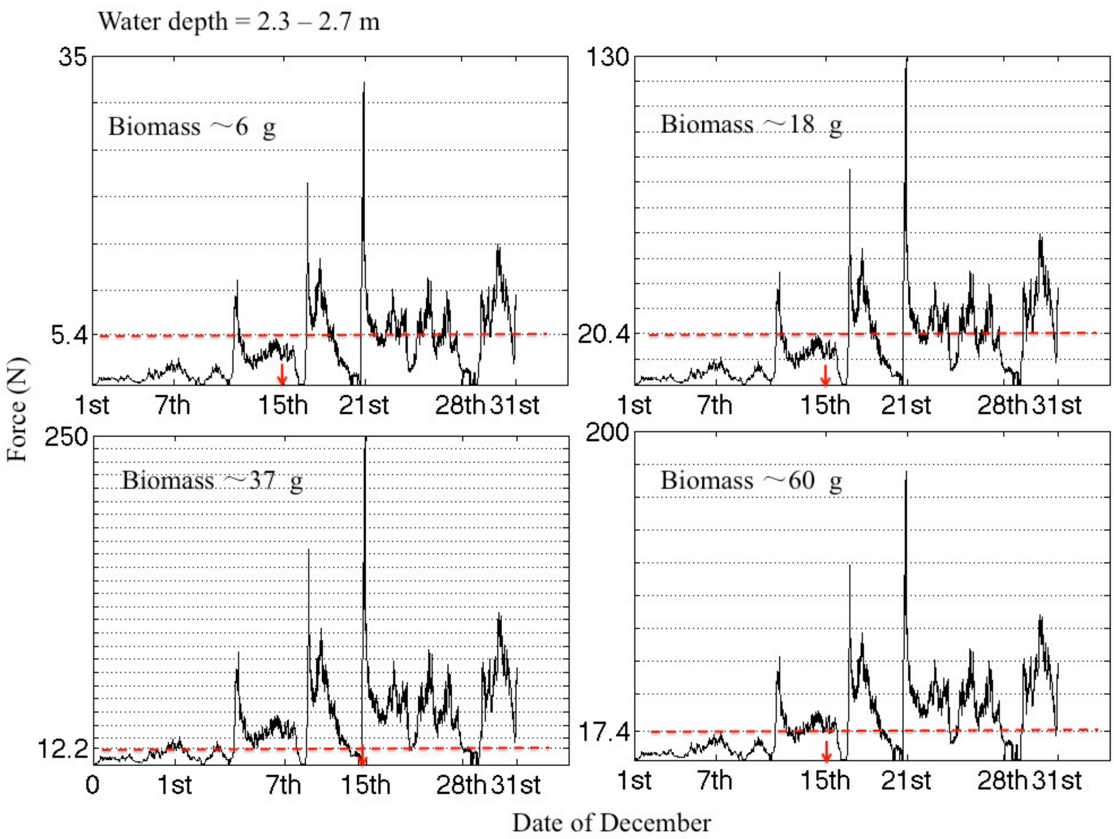


Figure 5.4 Compare drag force with dislodgement force in similar water depths of bottom depth 2-3 m with different biomass varying with temporal changings in December. Data were collected in December 15 2014.

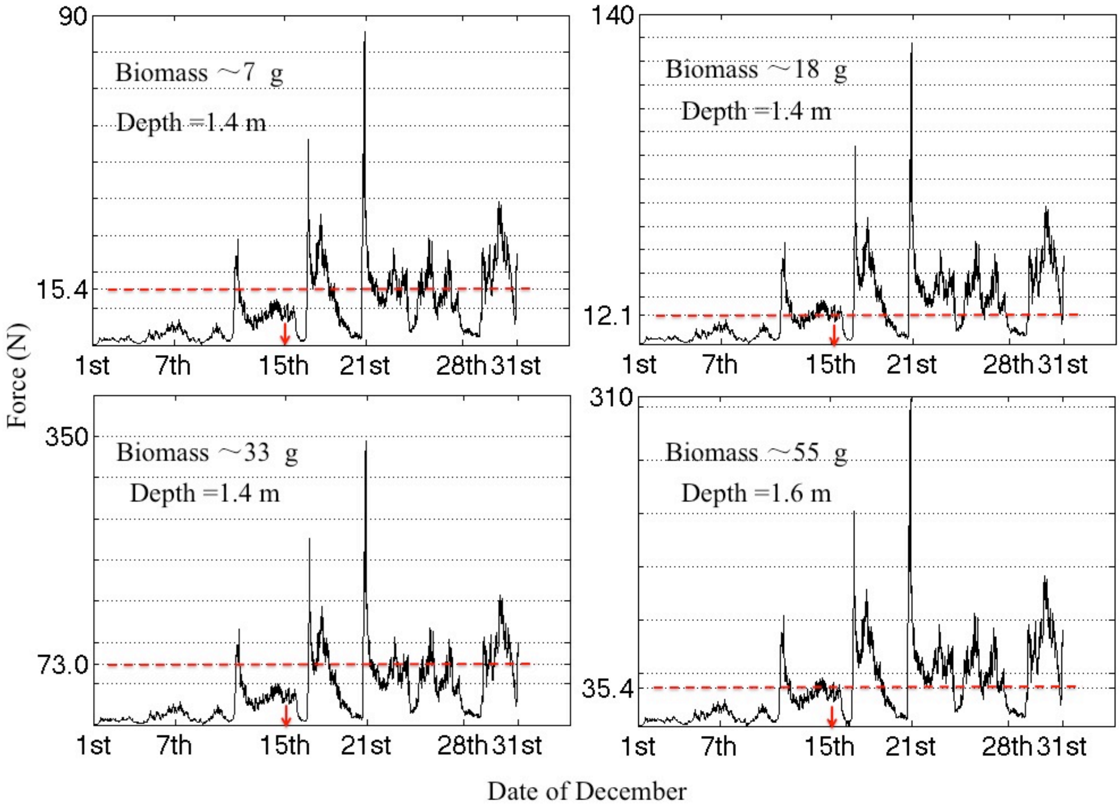


Figure 5.5 Compare drag force with dislodgement force in similar water depths with different biomass varying with temporal changings in December. Data were collected in December 15 2014.

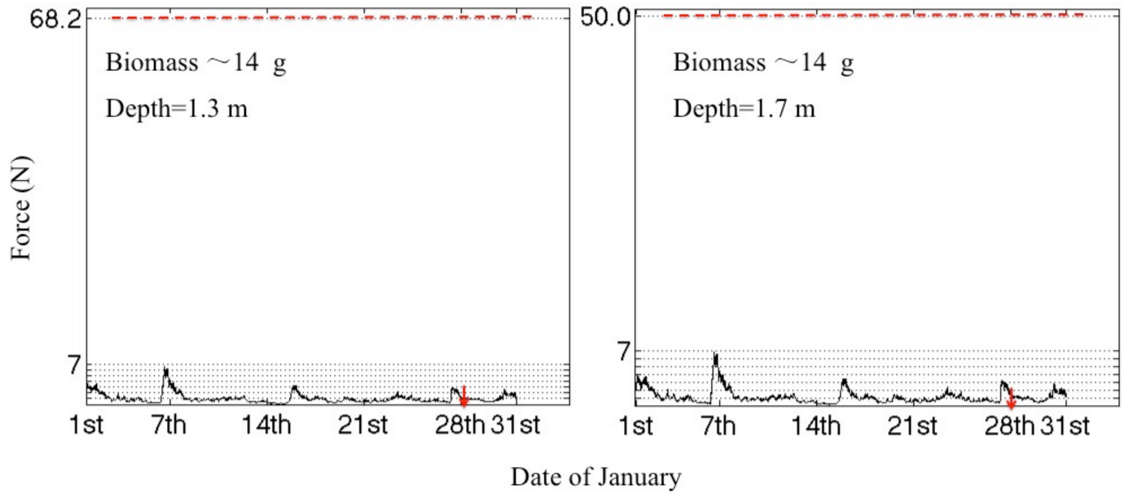


Figure 5.6 Compare drag force with dislodgement force in different water depths of bottom depth 1-2 m with same biomass varying with temporal changings in January. Data were collected in January 28 2015.

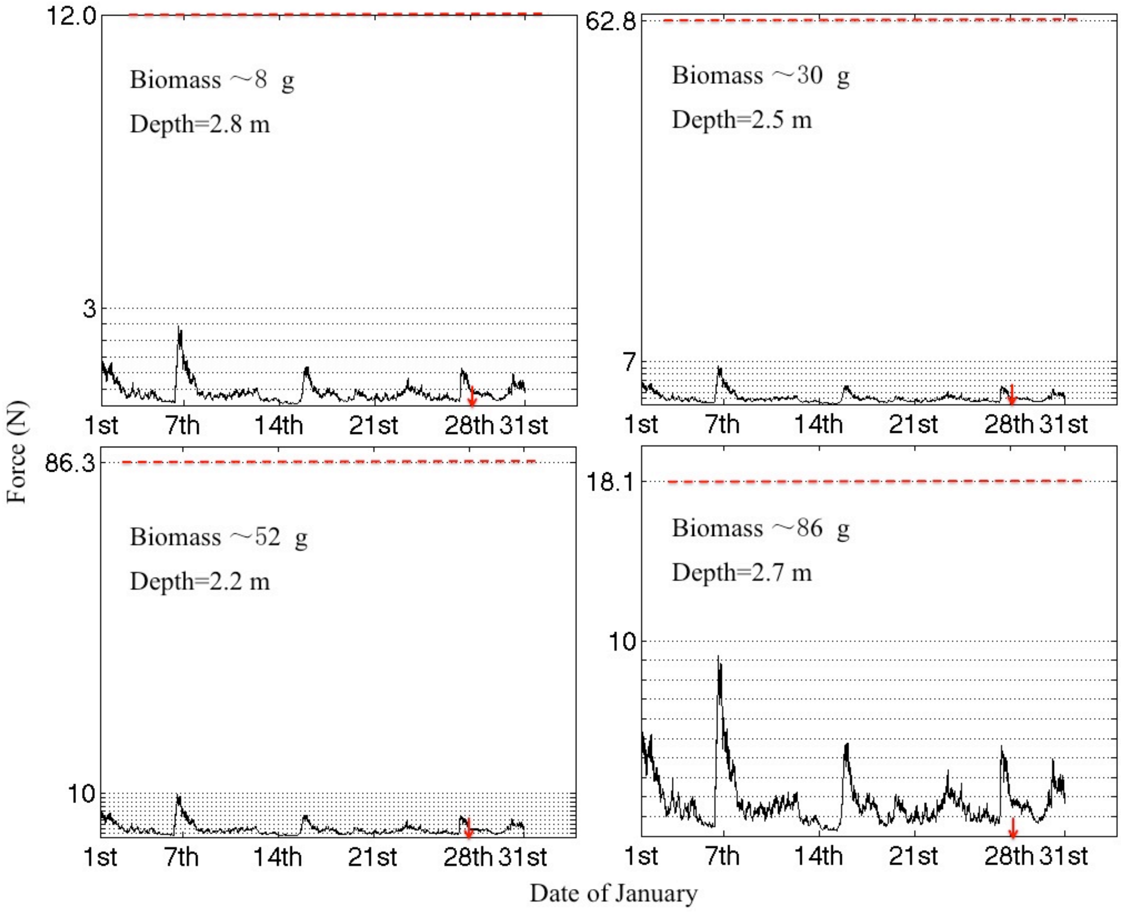


Figure 5.7 Compare drag force with dislodgement force in similar water depths of bottom depth 2-3 m with different biomass varying with temporal changings in January. Data were collected in January 28 2015.

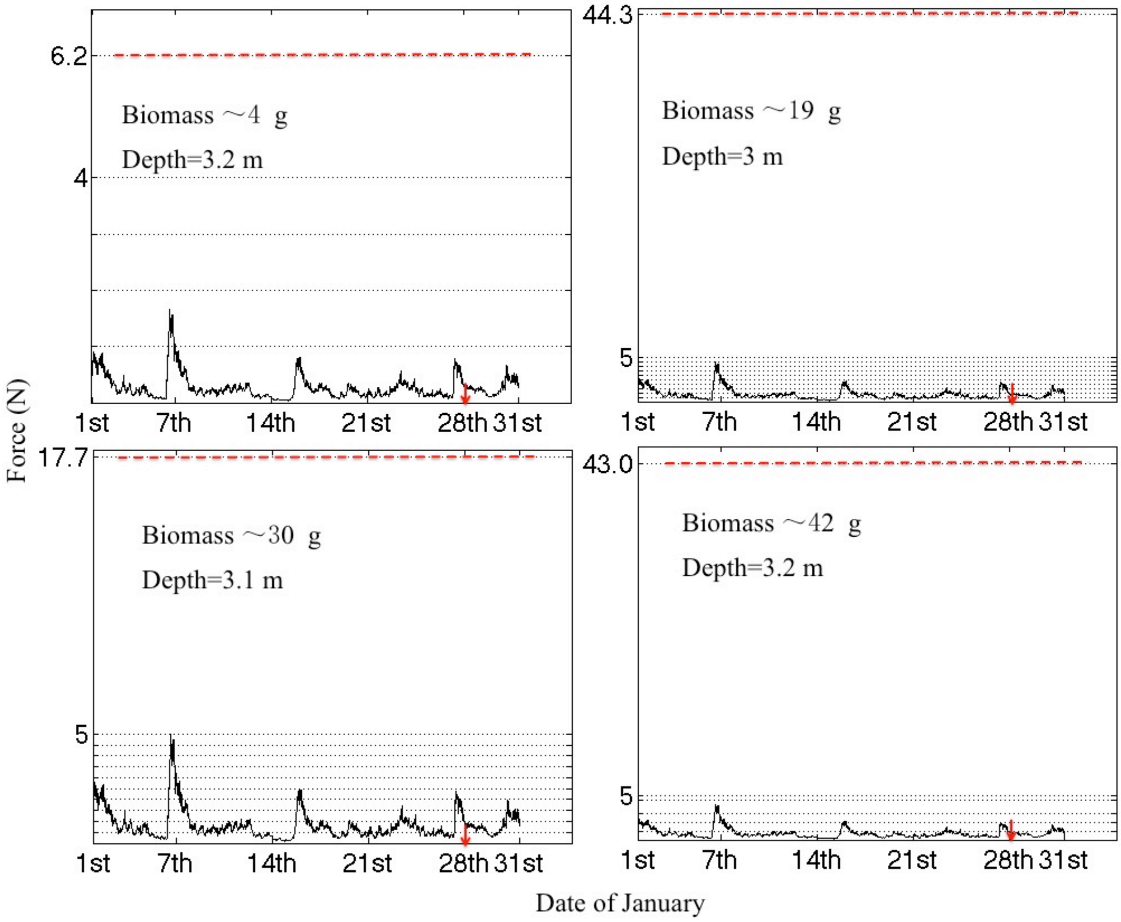


Figure 5.8 Compare drag force with dislodgement force in similar water depths of bottom depth 3-4 m with different biomass varying with temporal changings in January. Data were collected in January 28 2015.

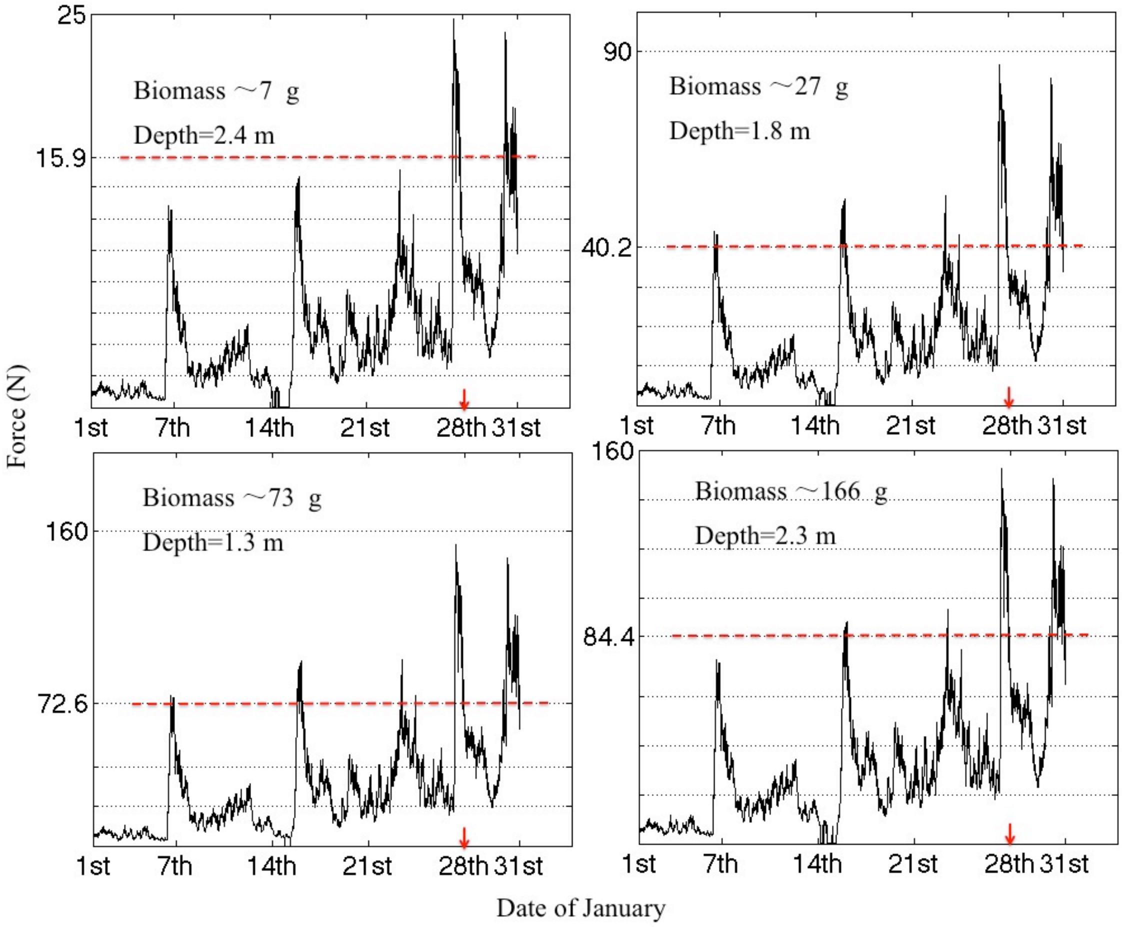


Figure 5.9 Compare drag force with dislodgement force in different water depths with different biomass varying with temporal changings in January. Data were collected in January 28 2015.

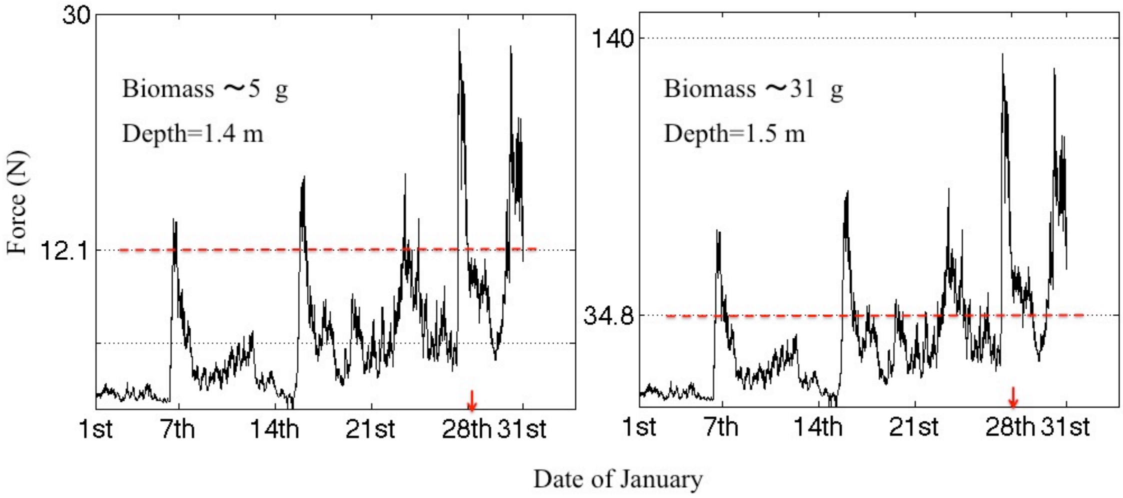


Figure 5.10 Compare drag force with dislodgement force in similar water depths of bottom depth 1-2 m with different biomass varying with temporal changings in January. Data were collected in January 28 2015.

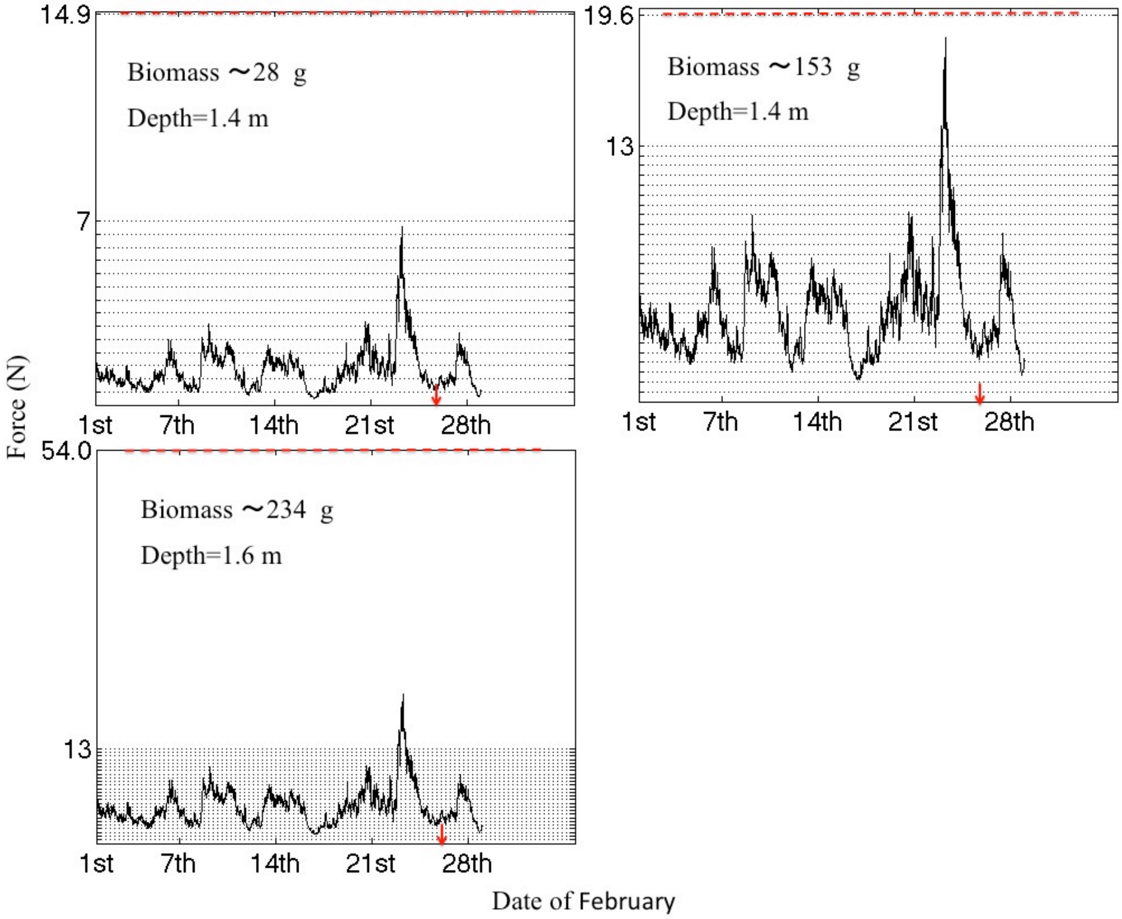


Figure 5.11 Compare drag force with dislodgement force in similar water depths of 1-2 m bottom depth with different biomass varying with temporal changings in February. Data were collected in February 25 2015.

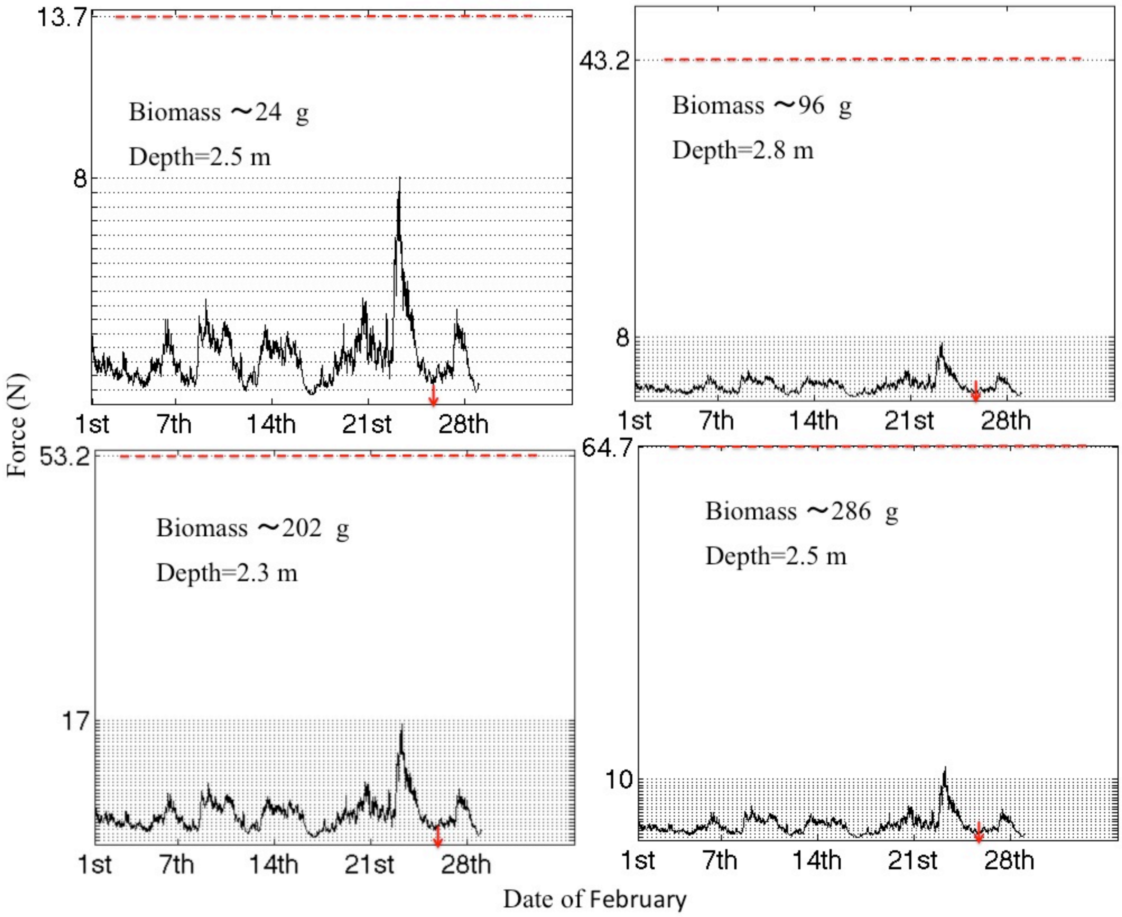


Figure 5.12 Compare drag force with dislodgement force in similar water depths of 2-3 m bottom depth with different biomass varying with temporal changings in February. Data were collected in February 25 2015.

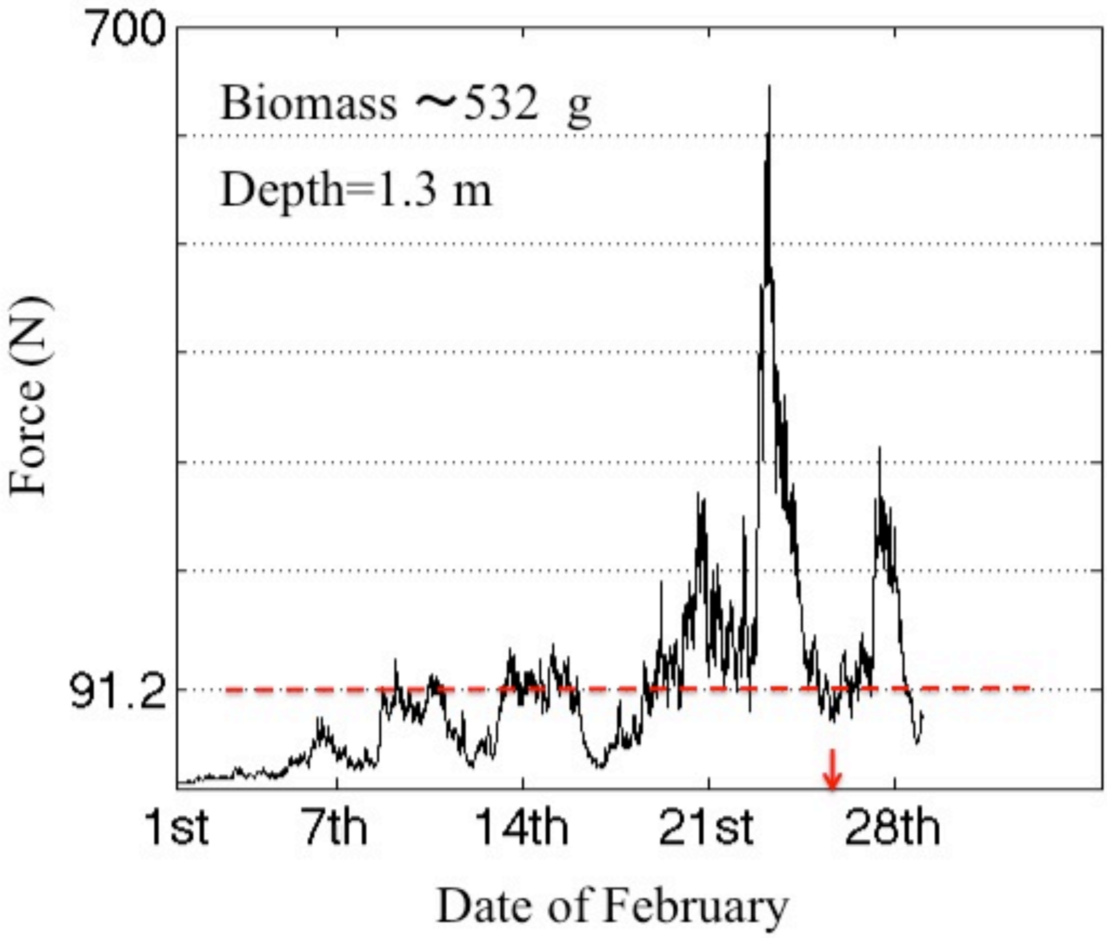


Figure 5.13 Compare drag force with dislodgement force in shallower place with larger biomass varying with temporal changings in February. Data were collected in February 25 2015.

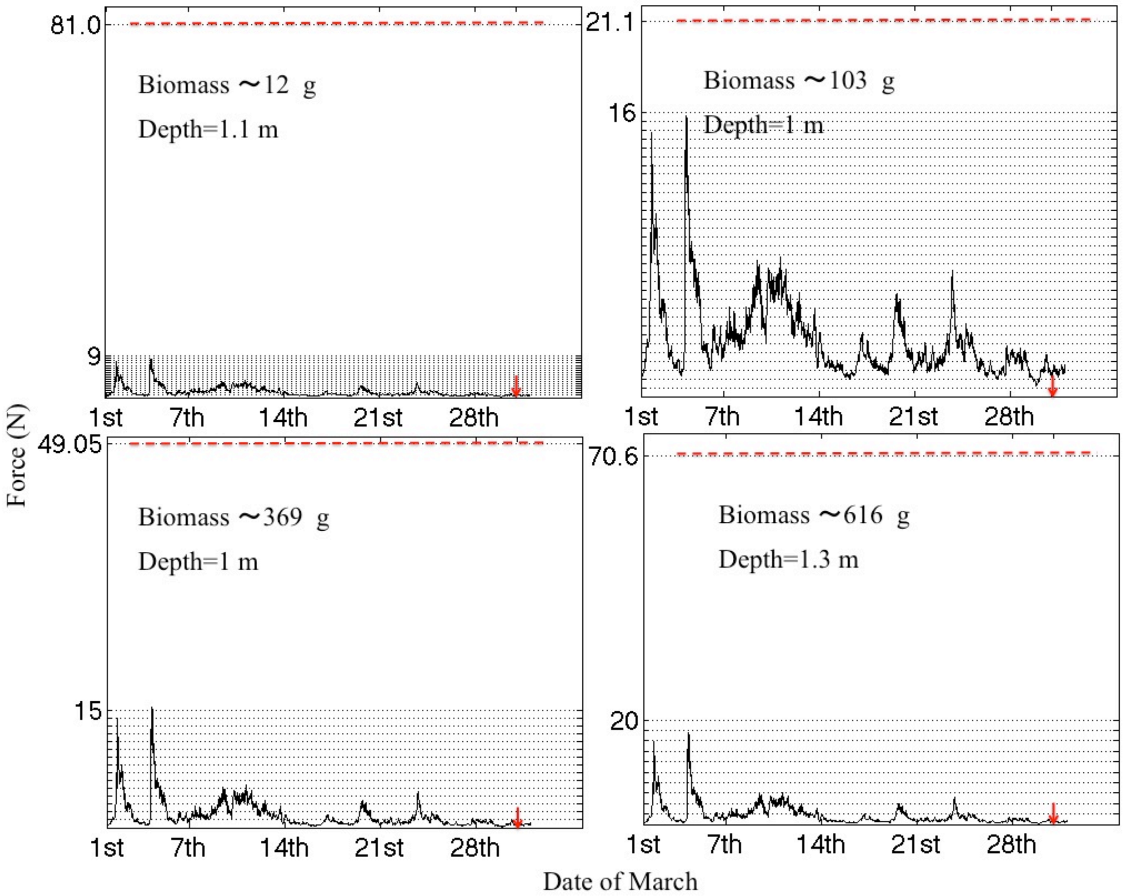


Figure 5.14 Compare drag force with dislodgement force in similar water depths with different biomass varying with temporal changings in March. Data were collected in March 31 2015.

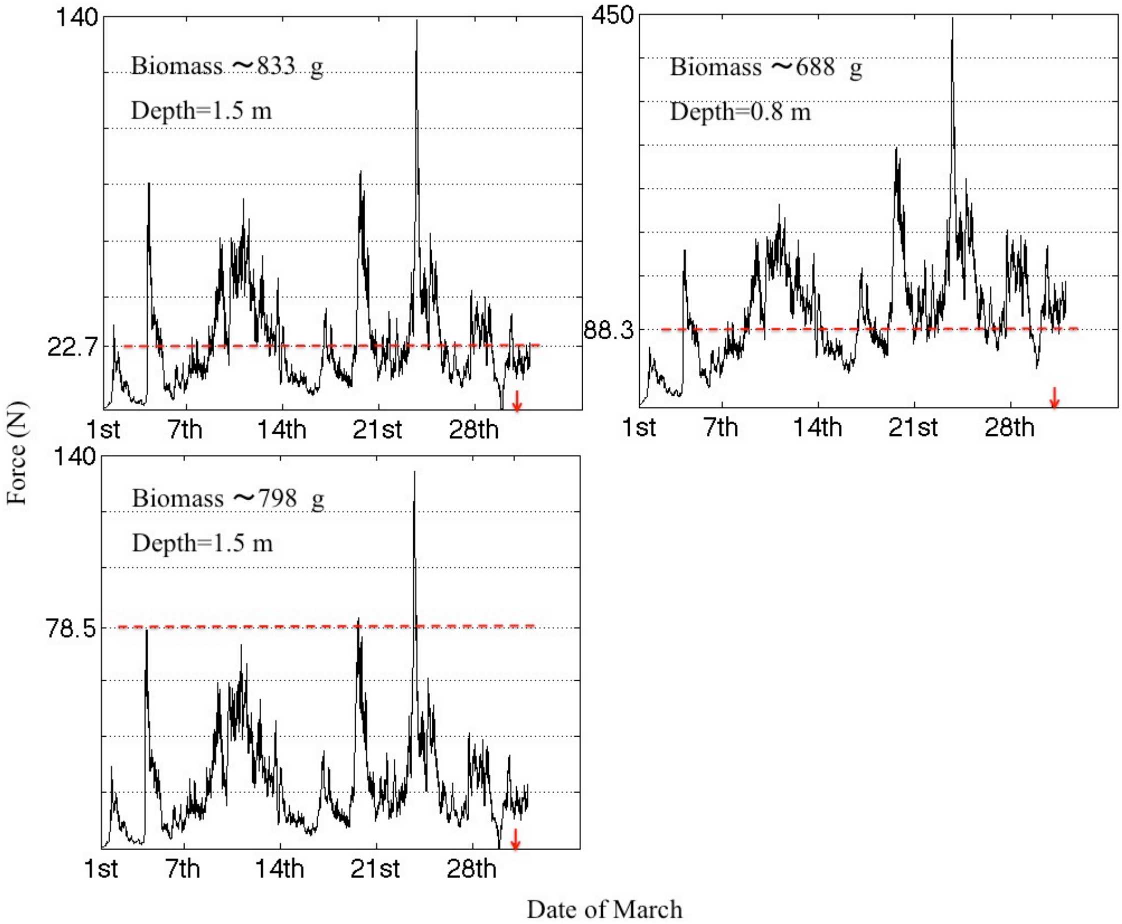


Figure 5.15 Compare drag force with dislodgement force among water depths with similar biomass varying with temporal changings in March. Data were collected in March 31 2015.

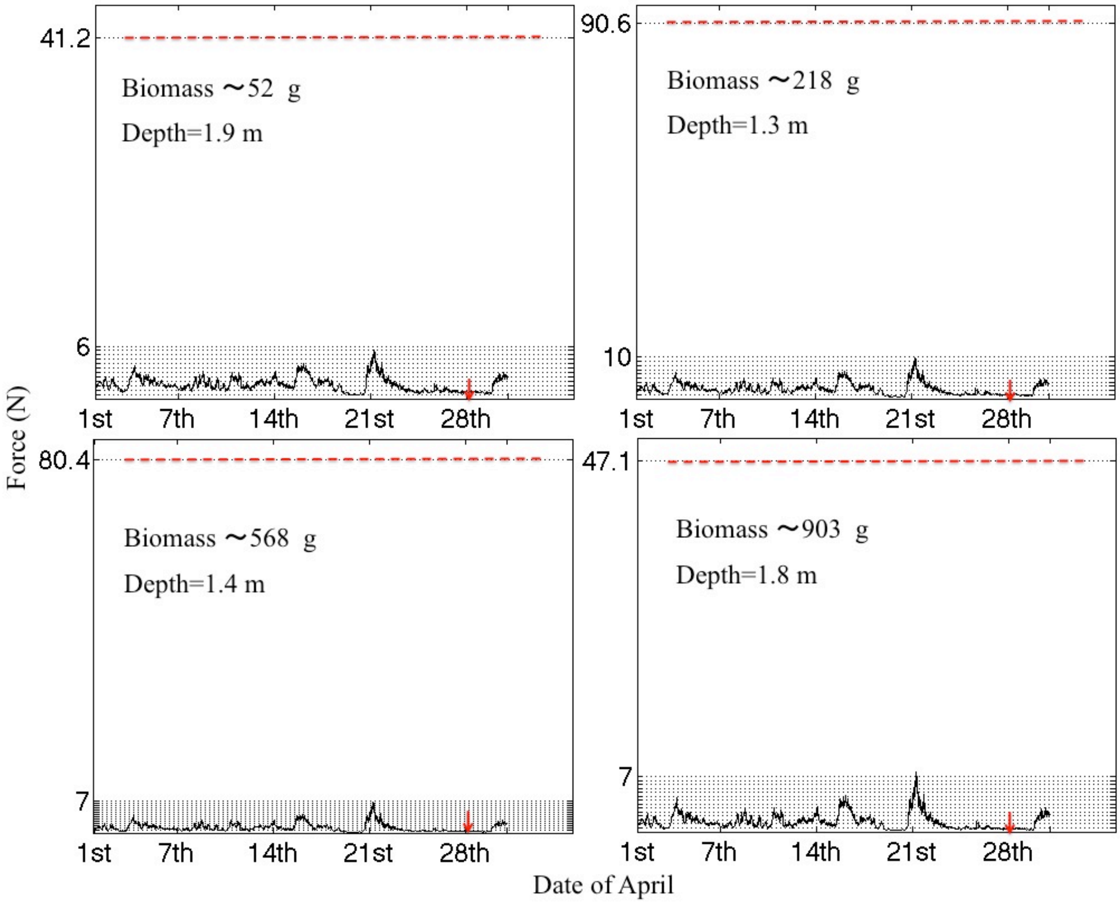


Figure 5.16 Compare drag force with dislodgement force among water depths of bottom depth 1-2m with similar biomass varying with temporal changings in April. Data were collected in April 28 2015.

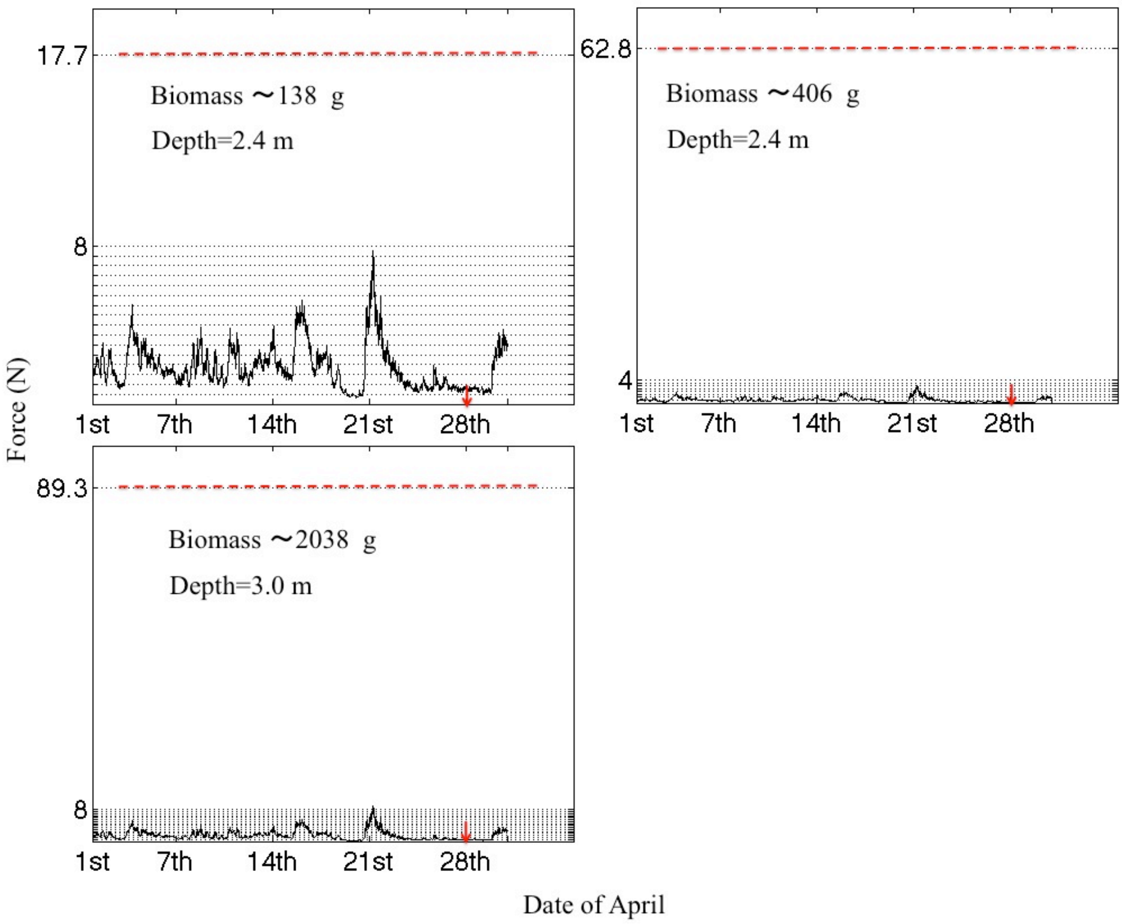


Figure 5.17 Compare drag force with dislodgement force among water depths of bottom depth 2-3m with different biomass varying with temporal changings in April. Data were collected in April 28 2015.

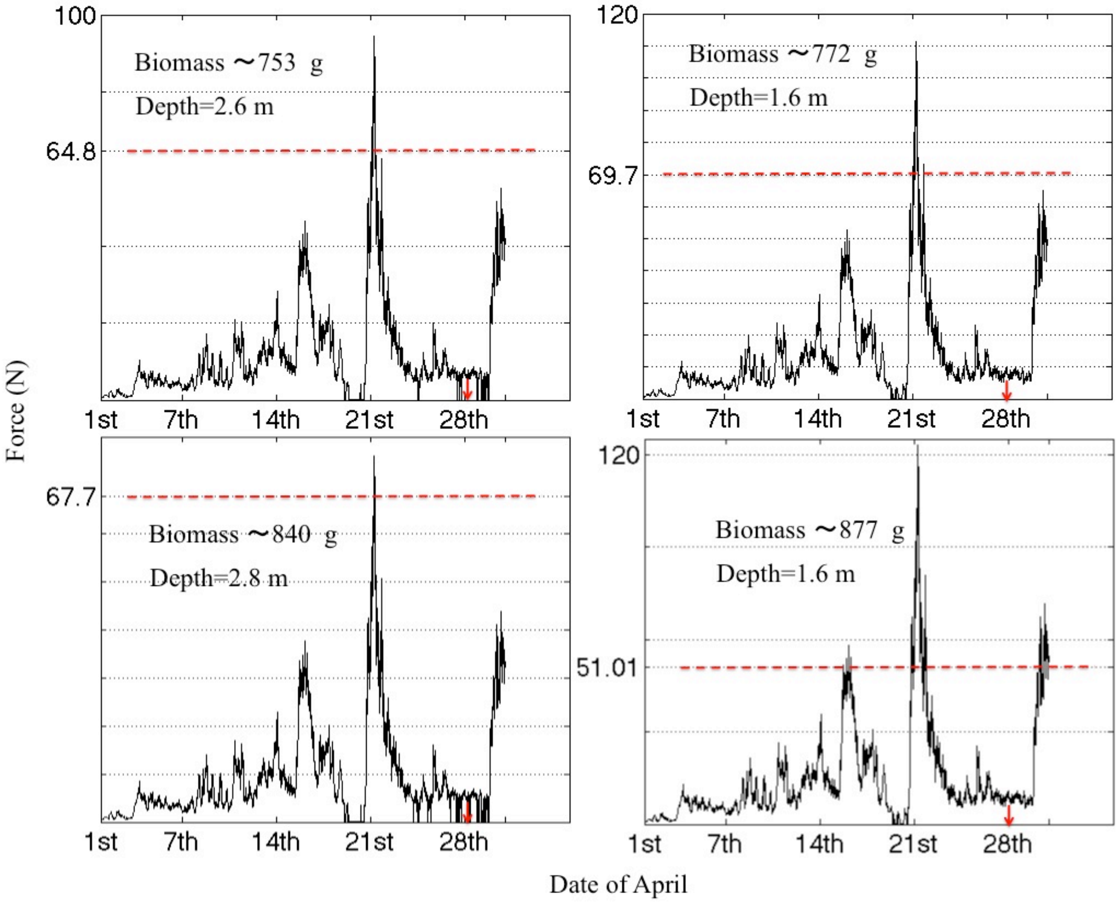


Figure 5.18 Compare drag force with dislodgement force among different water depths separately with similar biomass varying with temporal changings in April. Data were collected in April 28 2015.

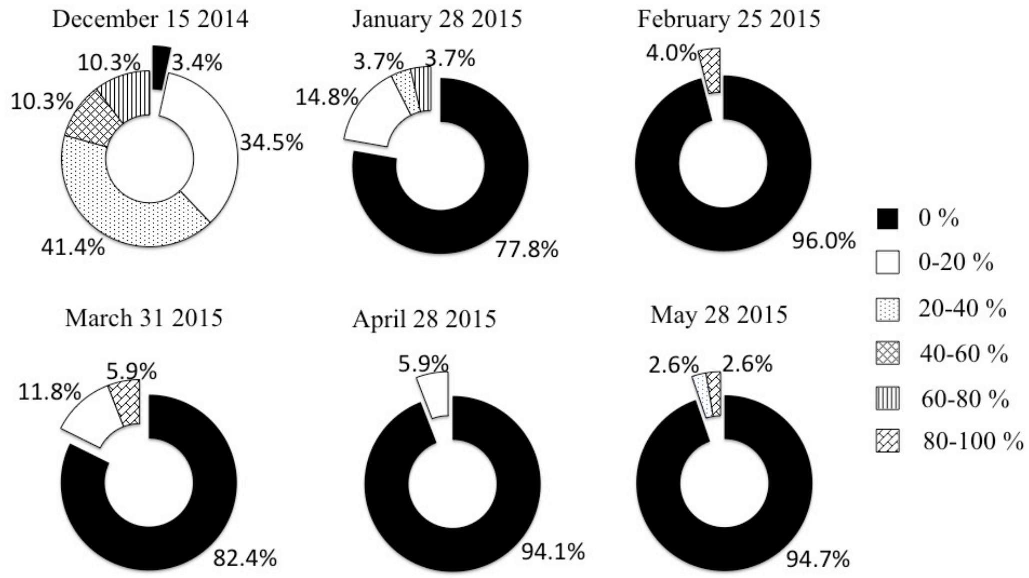


Figure 5.19 Dislodgement force possible percentage in all of specimens varying among the month. Calculating method: the chance number of drag force being above dislodgement force in collecting date 5 days ahead and 5 days afterward, and then the number is divided by the total time number. For example, Black bar 0% is meaning no chance being detached by waves.

Programs

Matlab codes

The codes to calculate the drag force on thalli of different sizes showing as bellows.

```
function [F,cd,A,Rey]=sardrag(LL,L,ww,u)
% LL/unit: primary lateral length (cm)
% L/unit: thallus length (cm);
% ww/unit: wet weight (g);
% u/unit: velocity(cm/s); an interval of 10 cm/s
% u should more than 50 cm/s; u=50-400 cm/s;
% F/unit: drag force(N);
% cd,dimensionless index;
% Copyright by Komatsu Lab
%
m=length(L);
mm=1;
% calculate A and Cd
n=length(u);
A=zeros(n,m);
cd=zeros(n,m);
w=0.0074*L.^1.9211;%model's weight,R^2=0.62147
beta=ww./w;
```

```
cd1=0.082*w.^-0.336;%model's cd,R^2=0.73887

cd2=0.082*ww.^-0.336;%real weight's cd

lamda=cd1./cd2;

visco=0.01049;% kinematic viscosity (cm^2/s)

ru=1.025;% ru is seawater density,unit:g/cm^3

for i=1:m

if ww(i)>1.38*w(i)|ww(i)<0.62*w(i) % allow 76% error range

% based on wet weight (g) to get surface area;

    if ww(i)<=100

        A(:,i)=17.676*ww(i);% R^2=0.92875;

    else

        A(:,i)=8.0246*ww(i);% R^2=0.73307;

    end

else

% based on maximum frond length (cm) to get surface area;

% A/unit: projected surface area,cm^2;

    A(:,i)=0.5218*L(i).^1.5884; %R^2=0.66274;

    A(:,i)=beta(i).*A(:,i);

end

end

for i=1:m

    Rey(:,i)=(u(1:n).*(A(:,i)'/L(i))/visco);

    if L(i)>=0&L(i)<50

        cd(:,i)=0.9024*Rey(:,i).^-0.268; % R^2=0.24023;
```

```
        elseif L(i)>=50&L(i)<100
            cd(:,i)=2.6041*Rey(:,i).^-0.391; % R^2=0.51784;
        elseif L(i)>=100&L(i)<150
            cd(:,i)=49.885*Rey(:,i).^-0.664; % R^2=0.76006;
        elseif L(i)>=150&L(i)<200
            cd(:,i)=2.8125*Rey(:,i).^-0.43; % R^2=0.52983;
        elseif L(i)>=200&L(i)<300
            cd(:,i)=27.999*Rey(:,i).^-0.626; % R^2=0.71218;
        elseif L(i)>=300
            cd(:,i)=0.002;
        end
end
istest=1;
% Modify Cd according to s. horneri's morphologies
if istest==1
for i=1:m
j=0;
cd(:,i)=cd(:,i)./lamda(i);
if ww(i)/w(i)<=3
        if ww(i)/L(i)>=2&ww(i)/L(i)<3&ww(i)>500
        for k=1:n
        if u(k)>=50
              j=j+1;
              cd(k,i)=(1+j*0.015/mm)*cd(k,i);
```

```
        end
        end
        end

else
        for k=1:n
        if u(k)>=50
              j=j+1;
              cd(k,i)=(1+j*0.018/mm)*cd(k,i);
        end
        end
end
end

if cd<0.002, %make Cd to 0.002
  for k=1:n
        if cd(k,i)<0.002
                cd(k,i)=0.002;
        end
  end
end
end

for i=1:m
```

```
F(:,i)=0.5*ru*A(:,i).*cd(:,i).*u'.^2;%here,unit is dyn

F=F./(10^5);%here, unit is N

end
```

The codes to calculate the velocities among the depths from 0.0-4.0 m created by waves.

```
function [ux]=waves(tau,H)

% ux is x-axis direction velocity

% tau is wave period;

% H is wave height;

d=0:0.1:4.0; % d is depth;

g=9.81; % g is gravity acceleration;

for i=1:length(d)

lamda=((g*tau.^2)./(2*pi)).*sqrt(tanh((4.*d(i)*pi^2)./(g*tau.^2)));

kap=2*pi./lamda;

omega=2*pi./tau;

ux(:,i)=pi*H./tau.*cosh(kap*d(i))./sinh(kap*d(i));

end
```