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Ecology of immature octopus *Enteroctopus dofleini*
Growth, Movement and Behaviour

Submitted in partial fulfillment of the requirements for the degree of Doctor of
Philosophy (Fisheries Science)

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by

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Abstract

This thesis considers the physiological and behavioral phenomena associated with the biannual migrations of immature *Enteroctopus dofleini* (Wülker, 1910) commonly known as the North Pacific Giant Octopus on and off the south coast of Hokkaido. Experiments were done in Usujiri, Minamikaityo where coastal temperatures range from 0°C in January, February, and March to 21°C in July, August, and September. Immature (3g-9 kg) *E. dofleini* are found in the coastal waters above 30 m when water temperatures are between 5 and 12°C. When water temperatures are outside this range, octopuses move offshore into deeper water, so there is a biannual inshore-offshore migration pattern.

Chapter 1. Juvenile and immature *E. dofleini* found inshore in southern Hokkaido are often confused with *O. conispadiceus* and *O. honkongensis*. Identification of immature octopus is difficult because they lack the three important features used to identify adults: a very long ligula; dark longitudinal grooves along the orange/red mantle; and small non-protruding eyes. Here *E. dofleini* is distinguished by features unique to immature animals, such as light skin colour and characteristic white patches, and other features that are common to both mature and immature animals.

Chapter 2 Part A considers the effect of temperature on energy input, somatic storage and expenditure by monitoring the food ingestion, growth and respiration of captive immature octopuses. Three different experiments were conducted. The first tested the oxygen use of starved inactive acclimatized individuals in a dark flow through chamber. The second tested the effectiveness of different diet regimes by measuring intake, growth and faecal production of captive octopuses fed different amounts and types of food. The third experiment was a calculation of the energy budgets of animals living at 7, 9.5 and 12°C. Consumption and growth were measured and the results of the previous

two experiments were used to calculate basic metabolic rates and devise appropriate diets for optimal growth. The results suggest that a mixed low lipid diet of approximately 3% body weight provided every 3 days and temperatures of 7-9.5°C lead to more energy being available to fuel growth and activity.

Chapter 2 Part B is a brief description of the growth rates of juvenile and immature individuals from long-term maintenance of 5 g - 5 kg *E. dofleini*. Time from hypothetical settlement to potential maturity (1g -15 kg) differed depending on temperature however in general; *E. dofleini* growth is best described by an exponential curve in the juvenile phase and a linear rate in the immature phase. With these results it is confirmed that *E. dofleini* in southern Hokkaido take approximately 30 months to reach maturity from the time they settle.

Chapter 3 Part A is a description of behaviours seen in captivity. The behaviours are organized into 16 activity levels (from resting to continued fighting) that are scaled according to awareness, responsiveness, movement and (apparent) energy consumption.

Chapter 3 Part B investigates behavioral response in terms of space use, interactions and activity of immature *E. dofleini* at 2, 5 and 7°C. Locations and interactions between octopuses kept in captivity were recorded on the hour for ten days. Recording was done by hand and video. Temperature was shown to influence space use, which in turn influenced interactions between octopuses. As temperature increased territorial behaviour (physical defence of home areas) increased and larger octopus became more dominate (has greater access to preferred areas).

Chapter 4 describes the daily movement patterns of immature *E. dofleini* at the entrance to Funka Bay. Three radio acoustic buoys (Vemco Ltd) set in a 300 m equilateral triangle, less than 500 m, offshore recorded locations of eight tagged

immature *E. dofleini* from mid April to late June 2003. Tagged octopuses showed movement patterns that suggest foraging occurred within a 250 m² area (home range), and that they spent 42% of their time within a mean area of 46m² (core area).

The **Discussion** deduces that the seasonality of *E. dofleini* around southern Hokkaido as noted in local seasonal culinary specialties, occurrence in fishermen's nets and the scientific literature: Kanemaru 1964; Kanemaru and Yamashita 1969; Hartwick *et al* 1978; Yamashita 1975, is a behavioral response to changes in water temperature. Only juvenile and immature octopuses migrate inshore, taking advantage of the seasonal temperature fluctuation while inhabiting small temporary home ranges. The rocky shore line provides immature octopuses with many potential den opportunities while heavy vegetation lowers visibility and ample but small prey items contribute to a large percentage of time foraging within small home ranges.

Interpretation of the results of the present studies suggests that the biannual migration of immature *E. dofleini* in southern Hokkaido can be explained with reference to energy balance and behaviour. During summer, a combination of decreased feeding rates and increased metabolic activity created by high coastal water temperatures keep the octopuses offshore. In late fall when coastal water temperatures decrease to 12°C, octopuses are able to migrate back inshore. The octopuses stay inshore until increased metabolic activity can no longer be balanced by increased feeding. From January to April, octopuses are found only offshore in deep waters where temperatures (around 5°C) are higher than they are along the coast. In spring when coastal temperatures increase above 5°C, octopuses migrate inshore staying on the coast until temperatures become less adverse.

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Introduction

The North Pacific Giant Octopus (Turgeon *et al.* 1998) is a coleoid cephalopod in the Family Octopodidae. It was first described by Wülker, 1910 as *Polypus dofleini*. It is now identified as a species in the genus *Enteroctopus* Rochebrune & Mabile, 1889 which has been recently redescribed by O'Shea (1999; see also Hochberg 1998). The current identification of the species is *Enteroctopus dofleini* (Wülker, 1910) (Fig i.1 - Taxonomic tree). Synonyms include the following: *Polypus dofleini* Wülker, 1910; *Polypus gilbertianus* Berry, 1912; *Polypus apollyon* Berry, 1912; *Octopus dofleini* (Wülker, 1910); *Octopus dofleini dofleini* (Wülker, 1910); *Octopus dofleini apollyon* (Berry, 1912) *Octopus gilbertianus* (Berry, 1912); *Octopus punctatus?* Gabb, 1862; *Paroctopus asper?* Akimushkin, 1963; *Octopus dofleini martini* Pickford, 1964; *Paroctopus dofleini* Wülker; *Paroctopus dofleini dofleini* Wülker and *Paroctopus hongkongensis* Kanemaru 1964; Kanemaru and Yamashita 1968; Okutani *et al.* 1987; not Hoyle 1885; not Adam 1939.

Enteroctopus dofleini (Wülker, 1910) is distinguished from other large long-ligula octopuses (*Octopus conispadiceus* and *O. honkongensis*) by differences in size, skin sculpture, reproductive systems, gill filament counts, funnel shape, head size, eye size, relative arm length and to web depth. *E. dofleini* adults can reach up to 3 m total body length. Dark longitudinal grooves run lengthwise along an orange/red mantle. The male ligula is long and thin. The pseudophallus is slim and grows to be as long as the mantle and is distinguished by the attachment of the spermatophoric duct close to its anterior end (Glendall 1993; 2002). Spermatopore length in mature males may be more than 1 m (Sato 1996). There are 12-15 gill filaments per demibranch or 24-29 in total. The funnel is shaped like a W with short lateral limbs. The head is short and narrow. The eyes are relatively small (compared to other species of octopus) and do not protrude (although

protrusion depends on posture). The arms are subequal but arms 1 and 2 are usually longest, and the hetocotylus (third right arm of males) is shorter than the third left arm. The inter brachial web is shorter between the dorsal arms (arms 1) and ventral arms (arms 4) (referred to as web sectors A and E) than it is between the other arms (Sasaki 1929; Pickford 1964; Okutani *et al*; 1987Gleadall 1993)

Enteroctopus dofleini (Wülker, 1910) is found in the temperate North Pacific from Northern California through the Sub Arctic to the Tsugaru Straits between the Japanese Islands of Hokkaido and Honshu (Fig.i.2- *E. dofleini* distribution). The North Pacific across the Aleutian Islands has not been sampled for mature animals. However, Kubodera (1991) found paralarvae measuring 3-13 mm in mantle length during surface tows along transects in the area (north of 45° N between 167° E and 155° W in the North Pacific and south of 57° N between 180° and 165° W in the Bering Sea), and suggested that paralarvae are transported by the Alaskan stream, thus connecting the otherwise geographically separated Eastern and Western Sub Arctic regions of the distribution.

In the southwestern part of the *E. dofleini* distribution lies Usujiri at the entrance to Funka Bay, the southeastern coast of the peninsular projection of Southern Hokkaido. Throughout most of the year this part of Southern Hokkaido (known as the Dounan area) is under the influence of the warm Tsugaru current, a branch of the northeasterly flowing Tsushima current. In the early part of the year, this changes when winter monsoons are thought to cool the water in the south East China Sea. The cooling of the water results in a depression of the Tsushima current volume and lessens the influence of the Tsushima current on the Dounan area (Conlon 1982; Otani and Murakami 1986). The unchecked cold, low salinity water from the North Pacific brought in to the area by the Oyashio current thus encroaches on the southern Hokkaido shoreline. The strength,

depth and southern most point of the Oyashio current varies interannually but regardless of yearly fluctuations the temperatures in Funka Bay reach their lowest some time between February and April (Fig i. 3- Influential currents around Hokkaido) (Murakami 1994). In Usujiri, temperatures range from an average summer high of 21°C to around 0°C (Fig i.4- Graph of Temperature and Salinity at the entrance of Funka Bay).

The Dounan area shoreline from 0 – 30 m is dominated by large rock formations and concrete fortifications against wave erosion. In the rugged inter-tidal zone, where small *E. doffleini* are seen so frequently, poets have commended them for seeming to be perched and waiting for the fishermen's baskets.

There is a long history of octopus fishing in the Dounan area as attested by native stories. Including those of the spirits of the gods lining the shore in the form of eight legged monsters (Sudou 1985); and illustrations of Ainu fishermen standing spear-in-hand as bright red heads surrounded by eight flailing arms dance over the cresting waves (Fig i.5- Illustrations of hunting by Dounan Ainu). The area continues to supply almost 30% of the annual haul from Hokkaido (Fig i.6- *E. doffleini* hauls in Hokkaido) with fishing methods that have not changed greatly over the years. Spear fishing as pictured below is still practiced, although usually off a low-hulled boat rather than directly from the shore. Box traps and shrimp baskets are used offshore while in shore fishermen still specialize in buoy jigging.

Today more revered for its taste than being a spirit of the gods, *E. doffleini* remains a Dounan area specialty. Locally called Mizudako (water octopus) or Shiodako (salt octopus), in reference to the clear mucus that has to be washed off well with salt before it is edible, these octopuses have long been a subject of local lore. Stories date back to the Meiji Era. One tale in particular tells of coastal fisherman being filled with relief

over the disappearance of the small octopuses, which plagued their coastal fishing grounds (Soudou 1985). This can be interpreted as the earliest recorded mention of an awareness of octopus seasonality in the Dounan area. Seasonal availability is reflected in the local octopus recipes. Dounan area winter nights are warmed by stews that typically have a few red arms curling above the rim. Instructions for how to make Esan soup (Esan in a town on the Dounan coast) come with the stipulation that a small winter octopus be used, innards and all. Roadside stalls sell `tako yaki`, which is cubed octopus baked in dough. It is now popular throughout the year in many parts of Japan, and is said to taste best in May, when it is eaten outside under cherry tree blossoms.

The seasonality of *E. dofleini* has been noted beyond local kitchens. It is a fact of life for fishermen around Hokkaido who follow the migrations and whose catch reflects availability in the areas accessible to them (Fig i.8- Seasonal *E. dofleini* hauls in Usujiri). The occurrence (Kanemaru and Yamashita 1969, Hartwick *et al* 1978), timing (Yamashita 1975) and mode (Kanemaru 1964) of biannual migration of immature *E. dofleini* have been also been documented in the scientific literature. But the reasons behind this seasonal movement and its relationship to behaviour and growth have not been elucidated and so are the focus of the present thesis. First, physical attributes of the immature stages found in the area are outlined from dissections and observations. Second, individual ranges and social structure are shown by acoustic tagging experiments. Third, the changes of physiology and behaviour that occur with temperature are ascertained through laboratory-based experiments focused on energy balance and behaviour at different temperatures and with different food sources.

Fig i.1 - Taxonomic tree

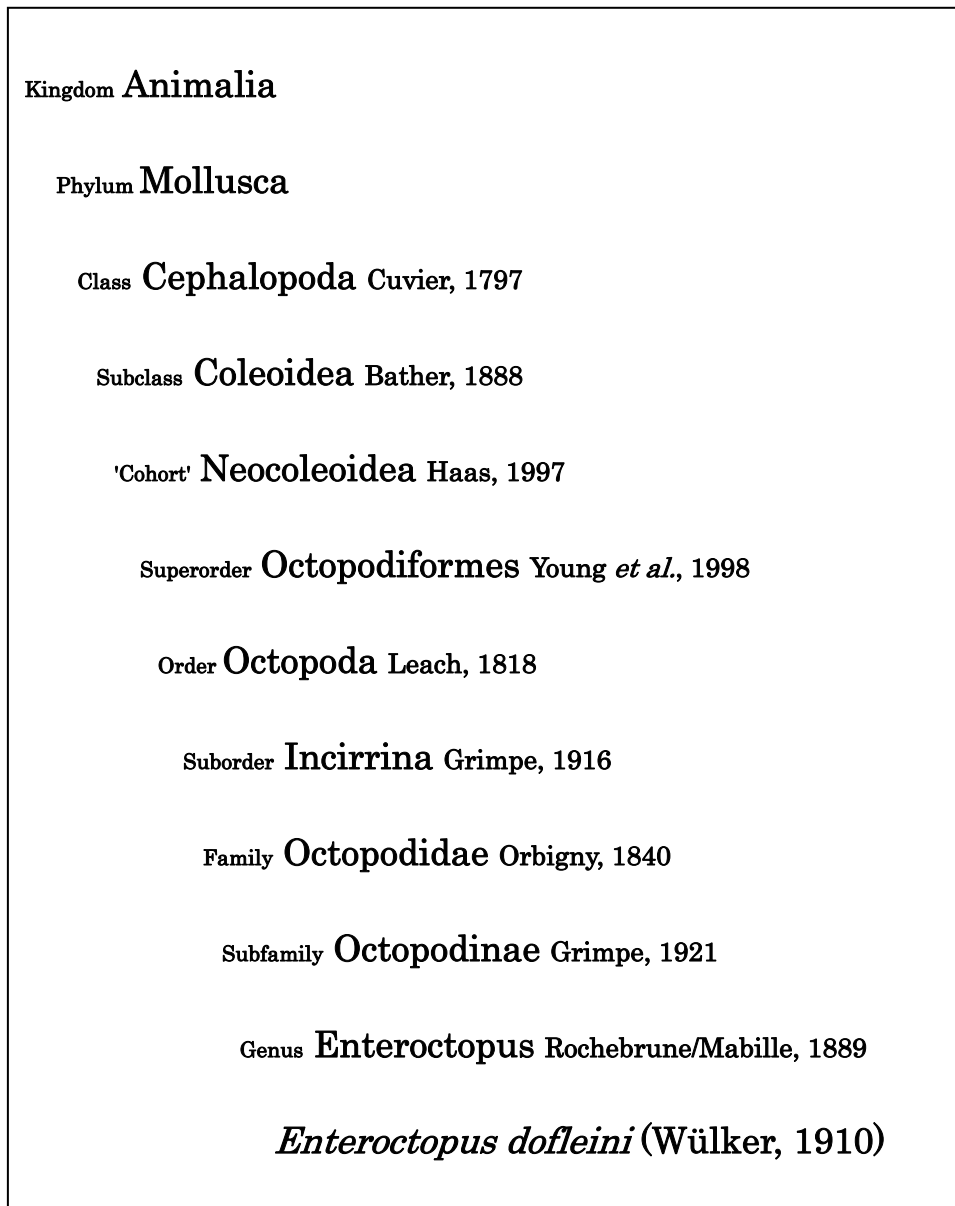


Fig.i.2- *E. dofleini* distribution

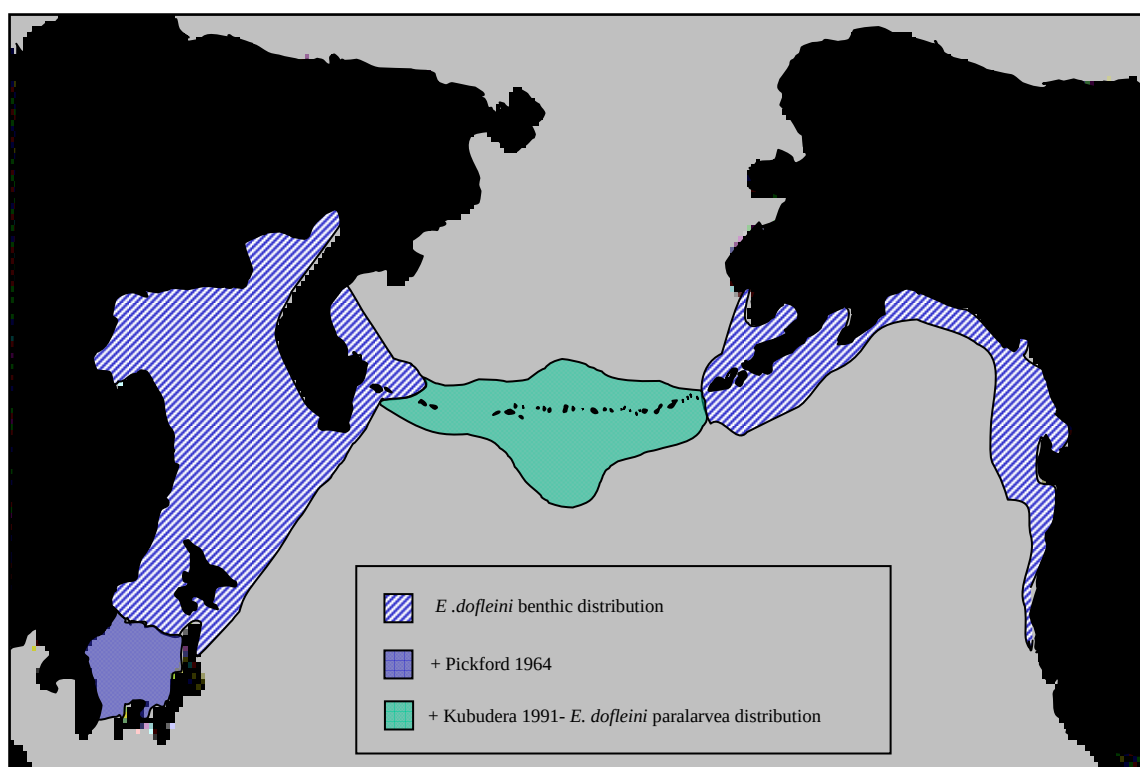
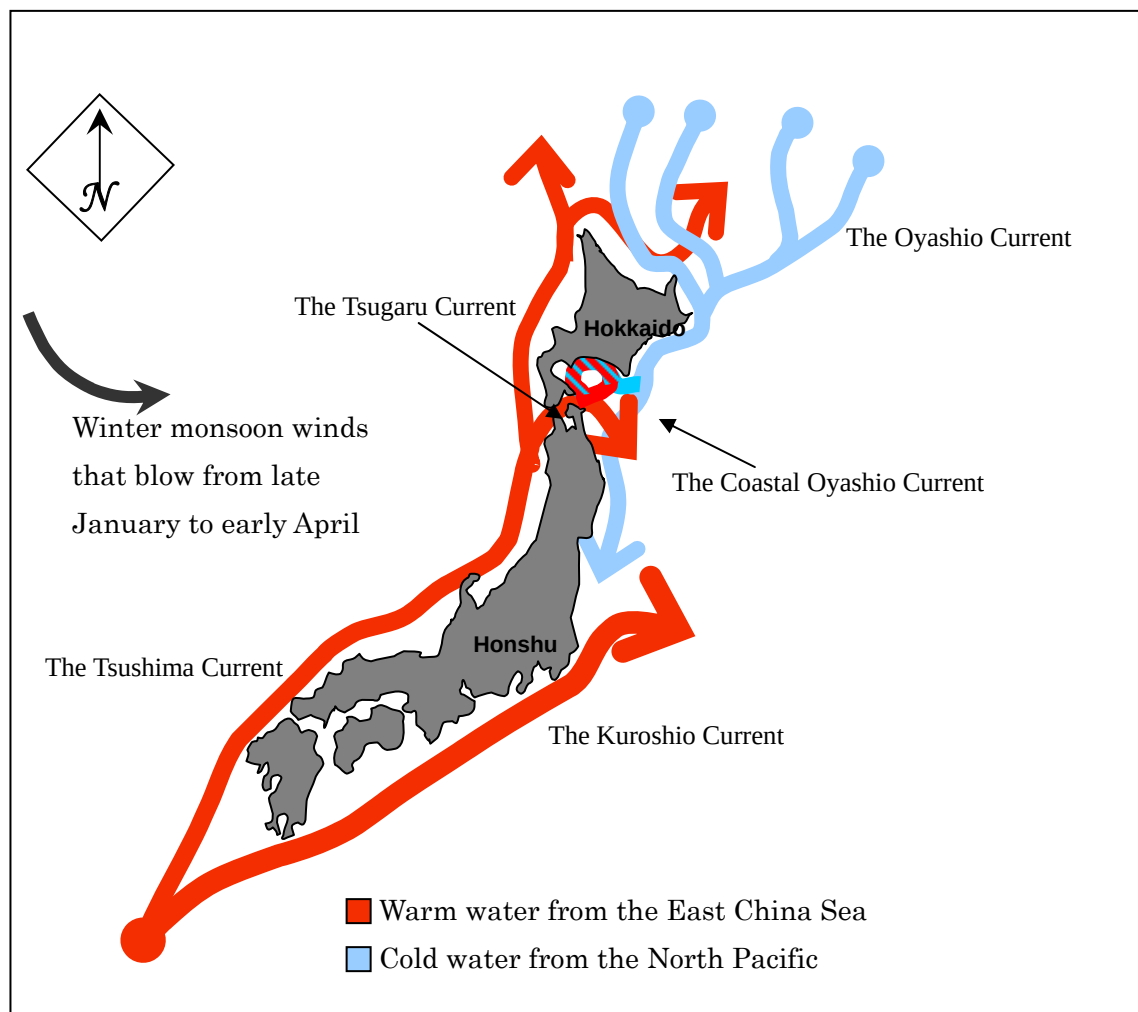
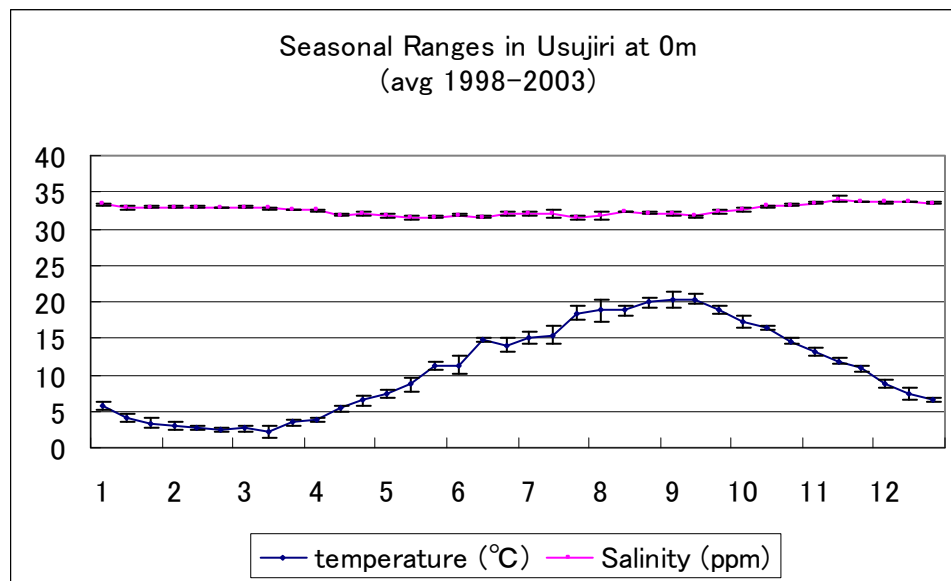


Fig i. 3- Influential currents around Hokkaido



Adapted from Takano and Kawai (1972)

Fig i.4- Monthly fluctuations of temperature and salinity in Usujiri



Surface salinity and temperatures recorded in Usujiri. Temperatures are lowest in February, March, and April and highest in August, September, and October. Salinity appears fairly stable in this graph, due to the influence of the warm regime that lowers the impact of the low saline water of the Oyashio current and has been in place since 1988. Measurements provided by Hokkaido University Northern Biosphere Research Station.

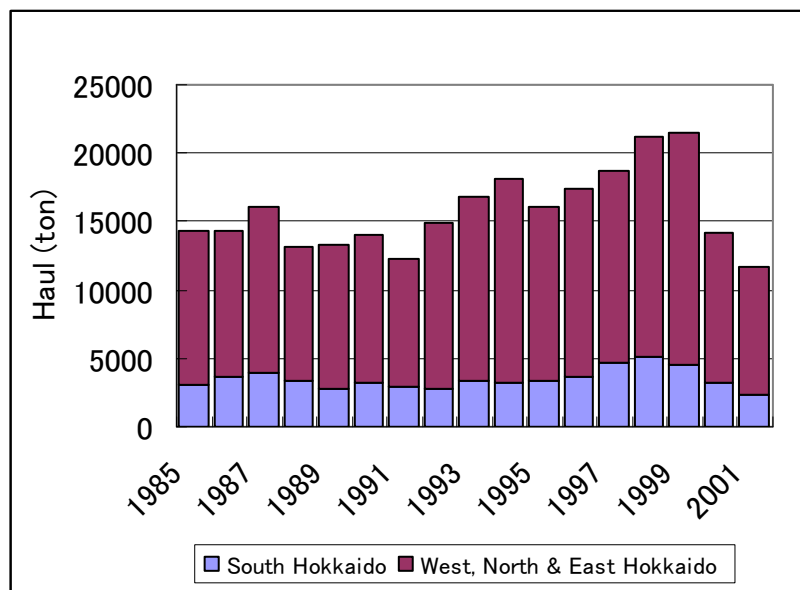
Fig i.5- Illustration of octopus hunting by Dounan area Ainu



Octopus spear fishing; as practiced by the Ainu of the Donaun Area, Hokkaido.

Wall hanging provided by the Hakodate City Library, Motomachi Hakodate.

Fig i.6- *E. dofleini* hauls in Hokkaido



Southern Hokkaido fishermen haul in app. 30% of the total island annual *E. dofleini* catch

Chapter One

Physical attributes of immature *Enteroctopus dofleini*

Abstract

The original *E. dofleini* holotype was an immature male from Todohokke in southern Hokkaido (Sasaki 1929; Gleadall 1993). Although immature octopuses have been used to describe species traits there is a lack of specific description of the juvenile and immature life stages. Here findings from 0.5-13 kg *E. dofleini* are reported with notes on distinguishing features and general appearance.

Introduction

Immature *E. dofleini* octopuses have been a cause of confusion. They have been used to describe the species and variations of it. The holotype of *E. dofleini* (Wulker 1910) from Todohokke, in southern Hokkaido, was an immature male (Sasaki 1929; Gleadall 1993). The confusion of immature *E. dofleini* and other species in the waters of southern Hokkaido has lead to descriptions of variations between the species in different areas. An example of this is in Pickford (1964) where *Octopus honkongensis* Hoyle is described as the subspecies *O. dofleini dofleini*. Some of the confusion that has resulted in the numerous synonyms for *E. dofleini* (see introduction) can be explained by the use of various measurements on unspecified life stages at different ranges of preservation.

E. dofleini are characterized by: shorter back (3rd and 4th) arms than front (1st and 2nd) arms, shortened hetocotylus (third right arm of males); W-shaped funnel with short lateral limbs; rounded mantle; a web that is shorter in front and back (sections a and e)

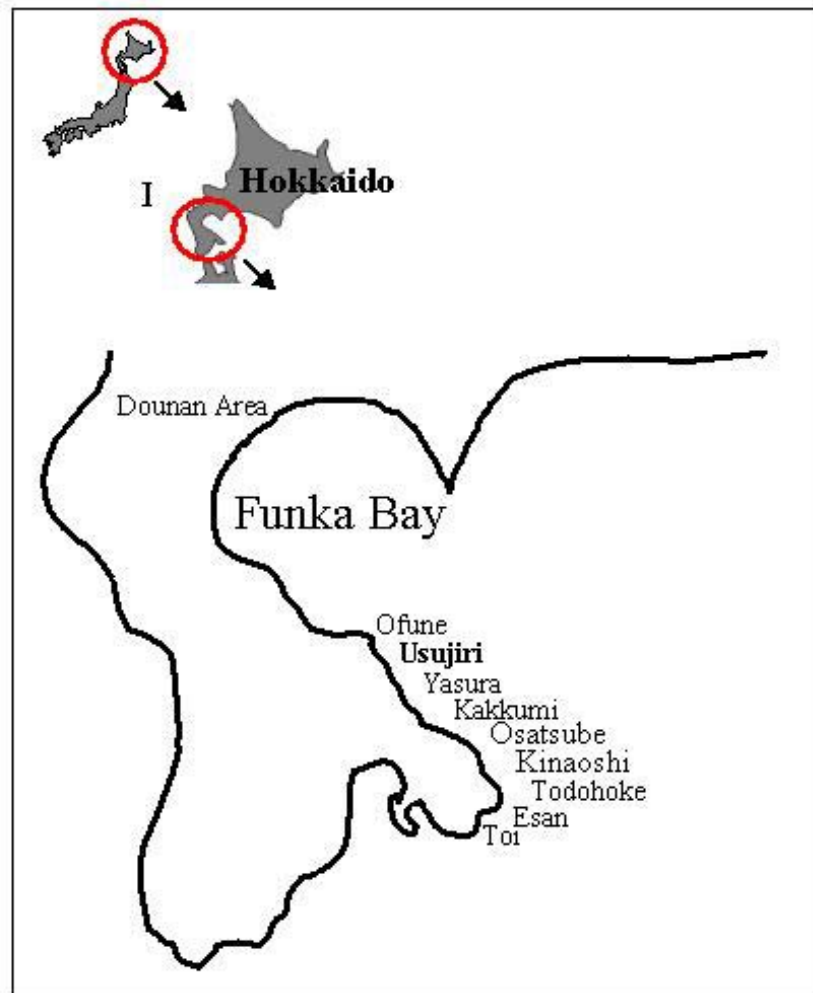
than it is between the legs (sections b, c, and d), with an average depth that is equal to 25% of the arm length; 250-300 biserial suckers along each arm; and longitudinal grooves running lengthwise down the mantle (Gleadall 1993; Okitani *et al* 1987; Pickford 1964; Sasaki 1929; Wulker 1910)

The following are findings were from dissections that were performed to confirm that the specimens used in the following chapters were *E. dofleini*.

Methods

Measurements were made on 20 fresh specimens (frozen and unthawed: not preserved in alcohol or formalin) between 0.1-15 kg, collected from the Dounan area between the years 2001- 2003. Work was done at the Research Station for Northern Biosphere, Usujiri, Hokkaido. Specimens of less than 1 kg were collected by hand from the shore of Usujiri at depths of 1 to 20 m. Specimens over 1 kg were caught in the same area by hand and box trap at depths of 1 to 30 m. Specimens over 10 kg were caught off the coast of Esan and Toi, refer to the map in Fig1.1-Funka Bay Area. Measurements of length were performed with sting so as to follow the body outline as closely as possible. Attempts were made to measure lengths without stretching the part being measured, however some variation was unavoidable due to the nature of octopus bodies (having neither an internal nor external skeleton they tend to shrink and are easily stretched or distorted).

Fig. 1.1 - Funka Bay area



Measurements:

TL - Total Length: Top of mantle to tip of longest arm (Figure 1.2a)

TW - Total Weight

VML- Ventral Mantle Length: Top of mantel to opening at the base of the mantel.

DML- Dorsal Mantle Length: Top of mantel to the mid eyes (Figure 1.2a)

M cir- Mantle circumference: Circumference of the widest point of the mantle.

MW- Mantle weight (after the viscera were removed)

EWb - The width between the eyes (Figure 1.2a).

EWi - The width including the eyes (Figure 1.2a).

FL- Funnel length (Figure 1.2b).

FLf- Length of the free part of the funnel (Figure 1.2b).

FD –Diameter of the tip of the funnel (Figure 1.2b).

AL – Arm Length (Figure 1.2c).

AW- Arm weight.

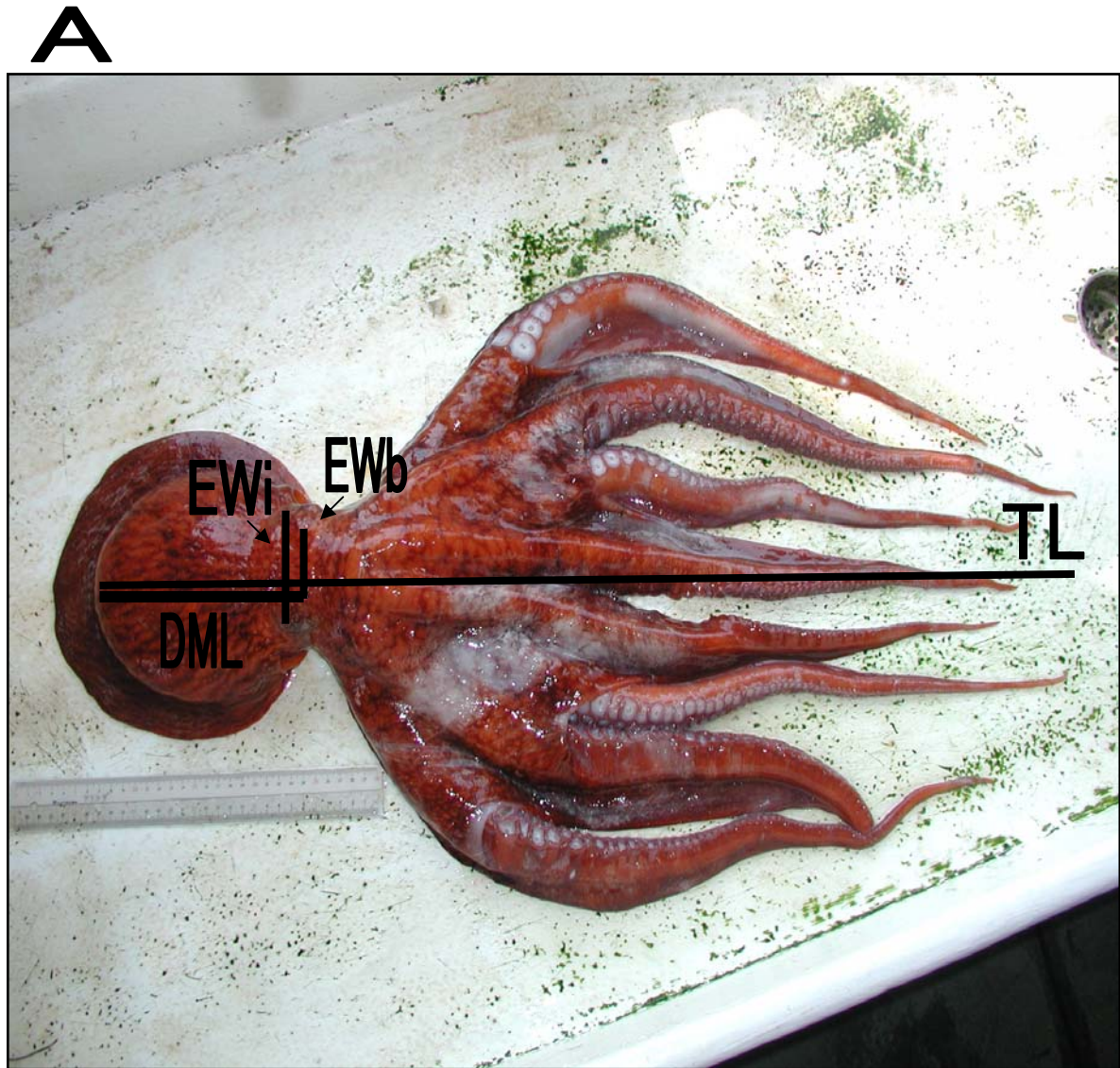
LL- Male Ligula Length (Figure 1.2d).

H cir- Head circumference: taken under the eyes

WD- Web depth: distance from body to the edge of the (relaxed) web (Figure 1.2c).

SN— Number of suckers on undamaged arms (Figure 1.2c)

Fig 1.2 a- Dissection measurements



TL: total body length

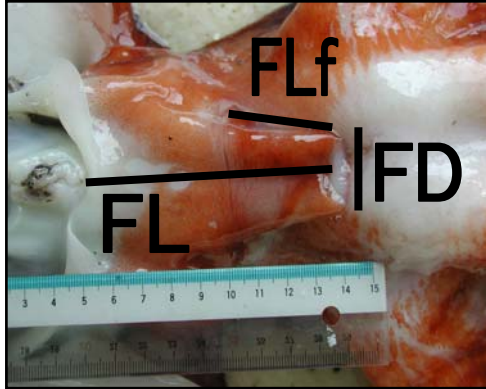
DML: dorsal mantle length

EWi: Eye width including the two eyes

EWb: Eye width between the two eyes

Fig 1.2 b, c, d - Dissection measurements

B

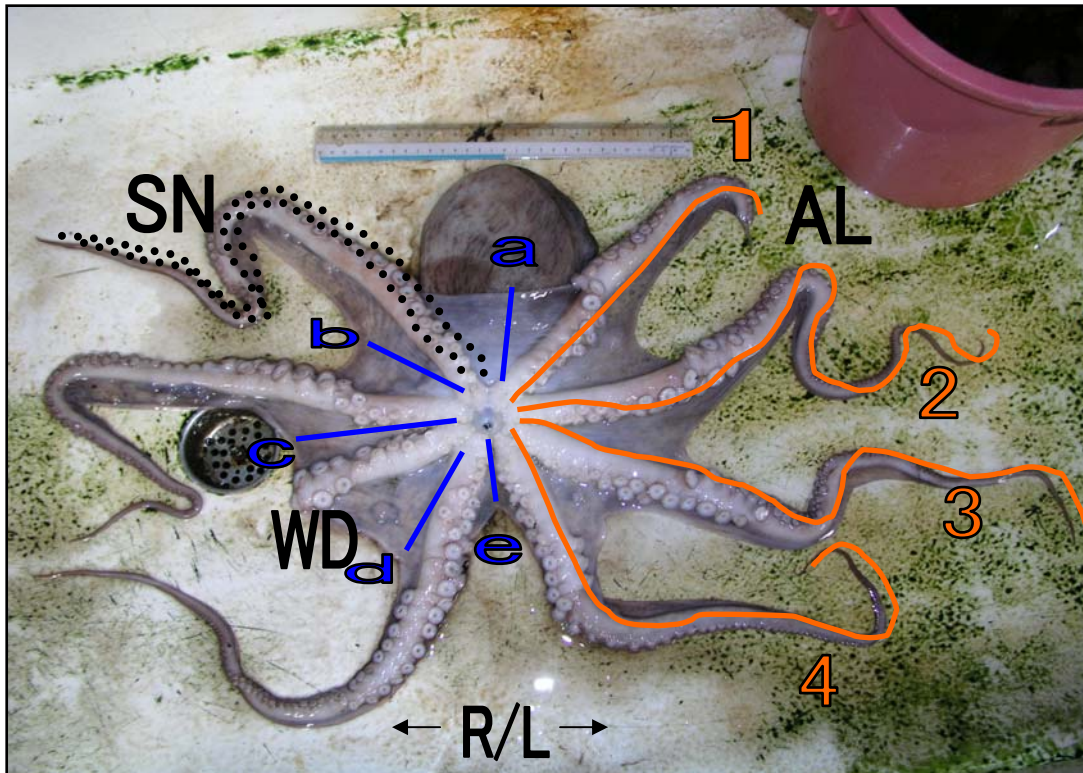


D



FL: Funnel length; FLf: Length of unattached funnel; FD: Funnel diameter; LL: Ligula
Length (tip of male R3)

C



SN: Number of suckers; AL: Arm length (number 1-4); WD: Web depth (sections a-e);

R: Right side; L: Left side

Results

The specimens used ranged in size from 100 g to 13 kg making raw measurements an impractical way to display the data. I have chosen to show instead how the measurements relate to each other. The following takes much from the protocol outlined by Pickford in 1964, and Robson 1945 and displays the means of the indices calculated and their standard deviation.

Table 1.1 Indexes means and standard deviation

Indices	Average (SD)
ALO - Arm Length Order: The sequence of arm length on the left side (Pickford 1945).	2 / 1 > 3 / 4
AWO - Arm Weight Order: The sequence of arm weight on the left side.	3 / 2 / 1 > 4
ALI - Arm Length Index: length of longest arm as a percent of the total length (Robson 1929).	74% (± 3)
AWI - Arm Weight Index: weight of heaviest arm as a percent of the total weight.	10% (± 1)
HAI - Hectocotylized Arm Index: length of hectocotylized arm as a percent of the length of its mate on the opposite side	76% (± 3)
LFI - Ligula Fold Index: length of ligula as a percent of the length of the hectocotylized arm	8% (± 1)

MAI - Mantle Arm Index: Ventral mantle length as a percent of the total length of longest arm (Pickford 1945 used mantle length, here the Ventral Mantle Length is used against the length of the longest arm).	24% (± 4)
HMI - Head Mantle Index: Head circumference as a percent of the mantle circumference (Width has been used in the past but to avoid mistakes of measurement I choose to measure circumference).	52% (± 4)
WDO - Web Depth Order: the sequence of web depth on the left side (Pickford 1945).	D/C/B>A/E
WDI - Web depth index: depth of deepest sector as a percent of the total length of longest arm (this is used by both Robson and Pickford).	29% (± 3)
WSI - Web Sector Index: depth of shallowest sector as a percent of the depth of deepest sector (Pickford 1964).	44% (± 4)
MI - Mantle Index: Ventral Mantle length as a percent of the Mantle Circumference (Circumference has been here instead width against mantle length).	59% (± 3)
EWI - Eye Width Index: Distance between eyes as a percent of the distance including eyes.	26% (± 1)

Table 1.2 - Arm details

arm	L1	L2	L3	L4	R1	R2	R3	R4	Male R3
number of suckers	207	253	267	304	214	231	233	289	96
% of complete (undamaged)	44%	72%	88%	88%	44%	66%	38%	100%	75%
arms									

The total weight and length comparisons of the immature octopuses are best fit by a logarithmic trajectory given by the ventral mantle length vs. total body weight ($y = 4.6237\ln(x) - 18.102$, $R^2 = 0.9077$). Neither the line drawn between the total weight vs. total body length ($y = 29.284\ln(x) - 128.37$ Log. $R^2 = 0.7212$) nor dorsal mantle length ($y = 0.0017x + 12.47$, $R^2 = 0.3988$) are as robust (Fig 1.3 - Weight vs. Length).

An average of 79.8% of the total body length is taken up by the arms (Fig. 4 - Mantle vs. Arm length) but they only average 68% of the total body weight. The rest of the body weight is divided on average in the internal organs (19%) and by the mantle (muscle and skin its attached skin - 12%) (Fig. 1.5 - Comparison of body part weights).

The mantle circumference, at its widest point is greater than the sum of the dorsal and ventral lengths (Fig 1.6a – Mantle measurements). The depth of the mantle is not apparent while the animal is stretched out on a dissection table where it can be falsely elongated. The fullness of the mantle can be best appreciated when the animals are alive (Fig 1.6 b).

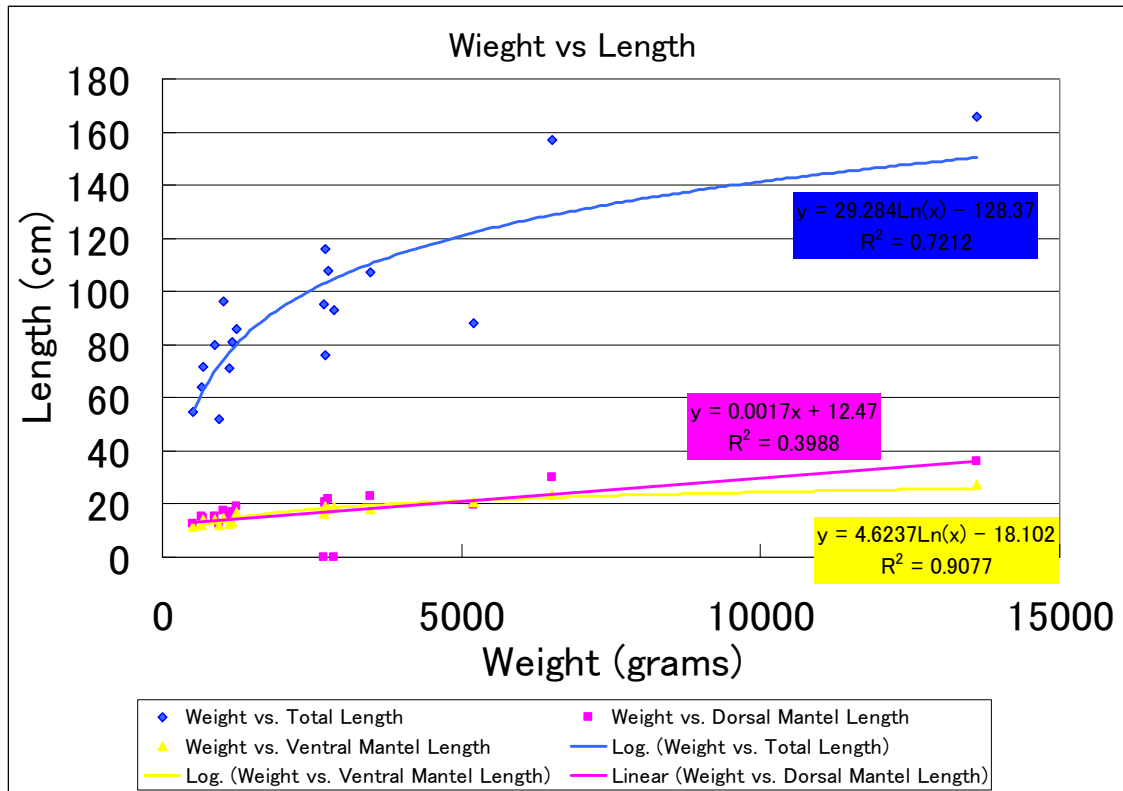
There were a considerable number of octopuses with out a complete set of 8 arms. 85% of the octopuses dissected had at least one leg at some stage of re-growth (Fig 1.7 a - c - Arm). Arms 1 and 2 were most frequently missing while the forth leg was most frequently present; this was not significantly correlated to either side.

Webs showed a biased of increased length on side; either the left or the right side had deeper webbing (Fig 1.8 - Web, one sided bias). The average length of the web is equal to

25% of the complete arms (Fig 1.9- Percent of arm covered by web). Some arms were damaged so as to extend as far as the edge of the web, no arm was found cut above this edge.

Smaller octopuses (10 – 700 g) have 4 identifying patches; 2 located just below both eyes on the upper arms and 2 behind the eyes not quite mid way down the mantle (Fig 1.9 a, b – Characteristic patches). These patches were easily mistaken for scars or skin imperfections however they were found on every octopus captured. They range between light brown and white and looking like areas where the chromatophors have been damaged. All four patches are visible to the naked eye on young octopus until the longitudinal grooves on the mantle darken (app. TW 500 – 700 g) after which the two on the mantle become difficult to discern. The scars on the upper arms (lower head between the eyes) occasionally morph in to one large light coloured patch and were viable on all octopus under 6 kg. Similar marks have been noted on other octopus and termed leucophore patches (Packard and Hochberg 1977; Messenger 2001).

Fig 1.3 - Weight vs. Length



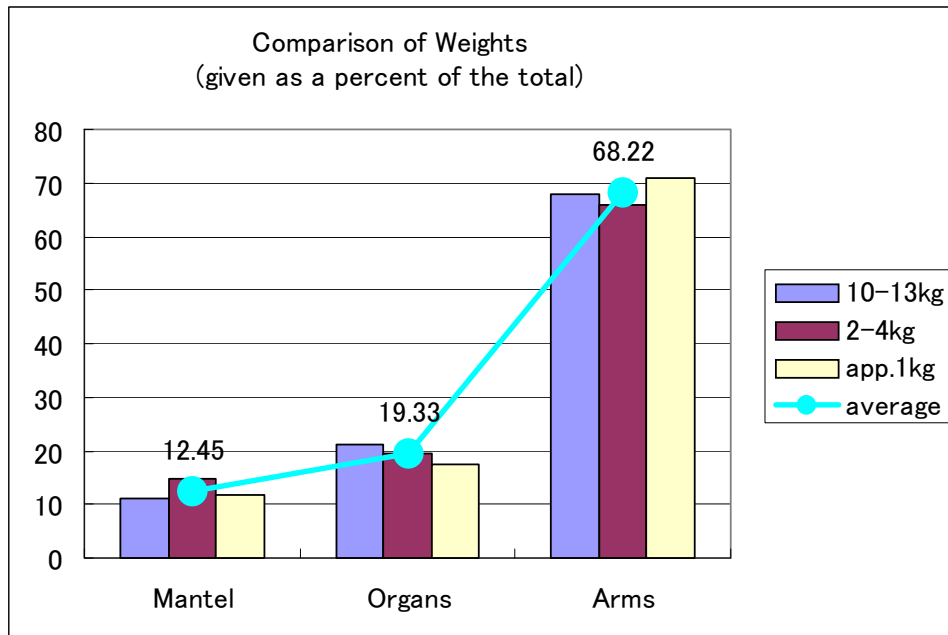
The total weight and length comparisons of the immature octopuses is best fit to a logarithmic trajectory given by the ventral mantle length vs. total body weight

Fig. 1.4 - Mantle vs. Arm length



An average of 79.8 % of the total body length is taken up by the arms. Only 18 octopuses are displayed here, as total length was only obtainable from octopuses with at least one complete right or left arm number 1 or 2.

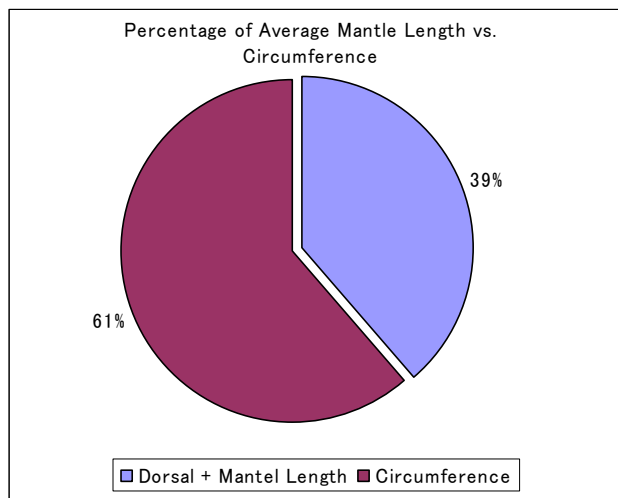
Fig. 1.5 - Comparison of body part weights



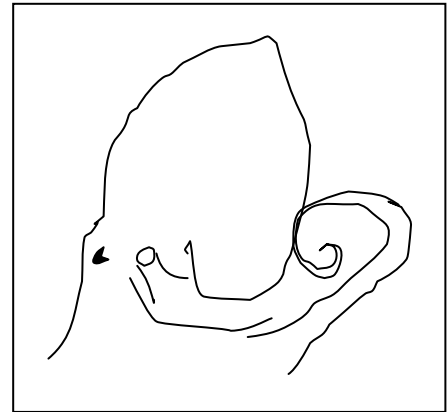
An average of 68 % of the total body weight is taken up by the arms, the rest is divided between the internal organs at 19 %, and the mantle (muscle and skin) at 12 %.

Fig 1.6 – Mantle measurements

A

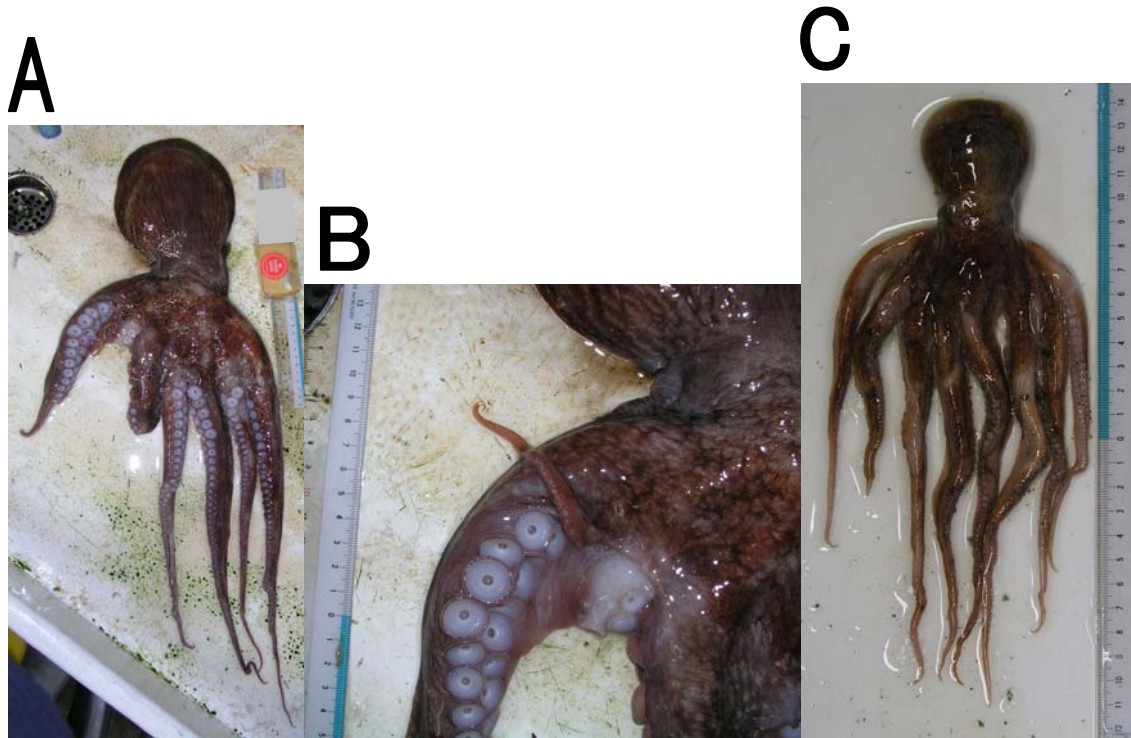


B



The mantle circumference, at its widest point is greater than the sum of the dorsal and ventral lengths, giving considerable depth to what can appear to be an elongated sphere.

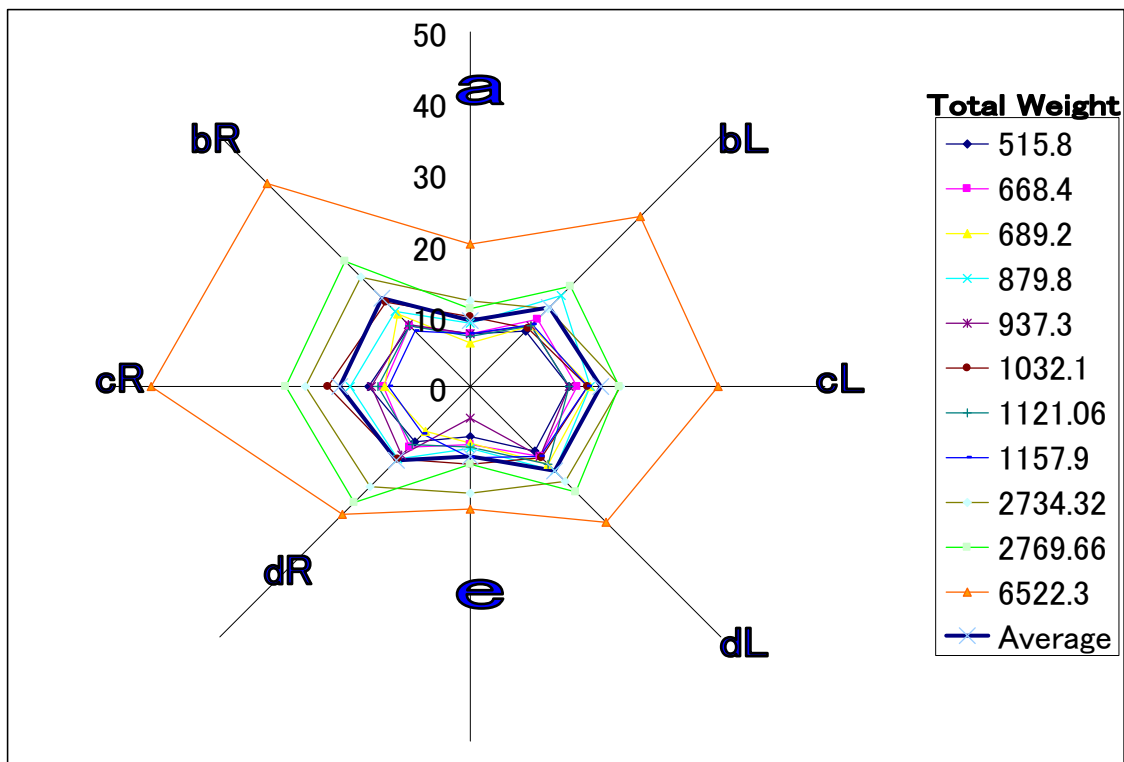
Fig 1.7 – Arms



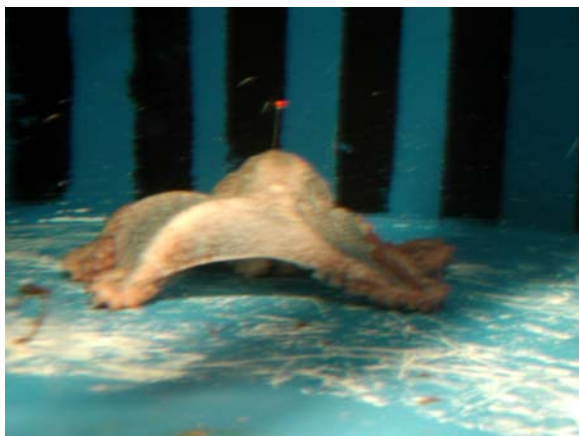
A total of 85% of the octopuses used as specimens had at least one noticeable damaged arm. The octopus shown on the left (a) is an extreme with only the four left arms undamaged. Re-growth of the right arms is at various stages and along various point of the arm including from the edge of the web (b). The photo on the right (c) shows the complete set of arms of a juvenile (122 g) octopus (Note that the lingua is already apparent).

Fig 1.8 - Web, one sided bias

A

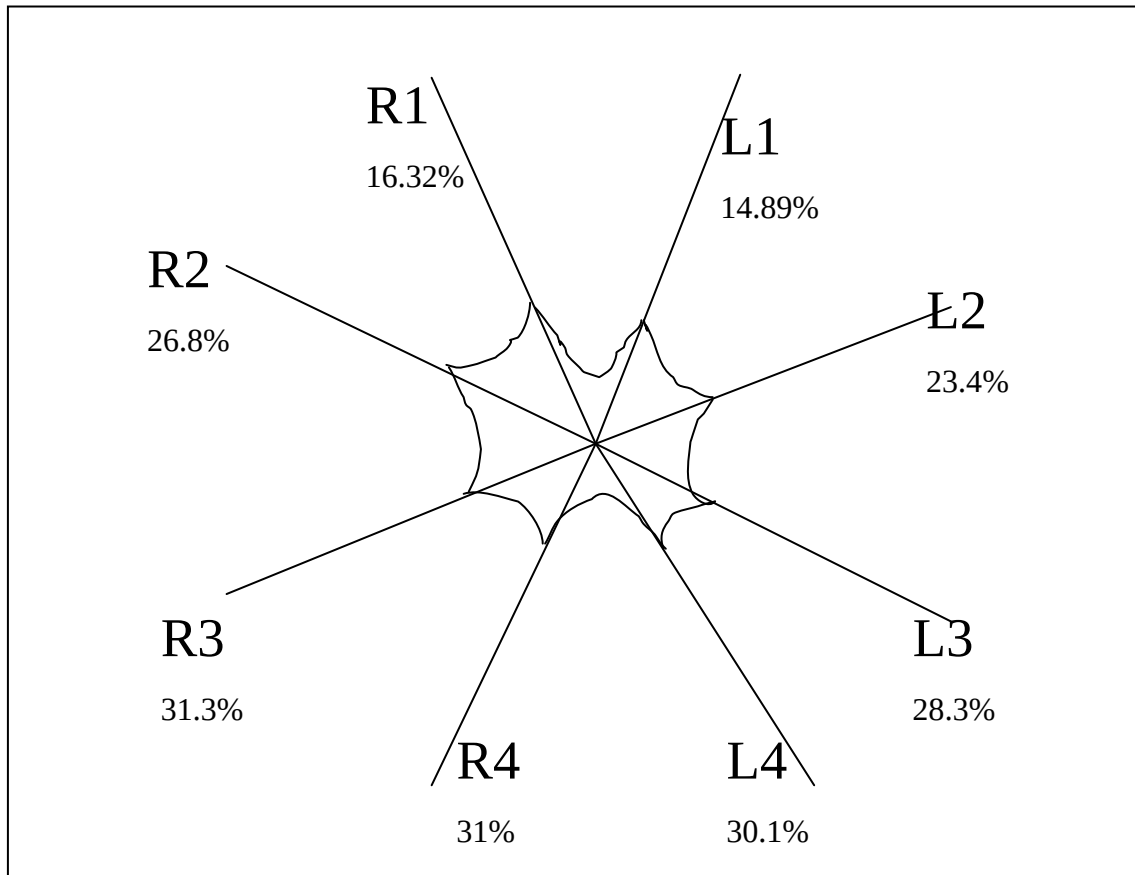


B



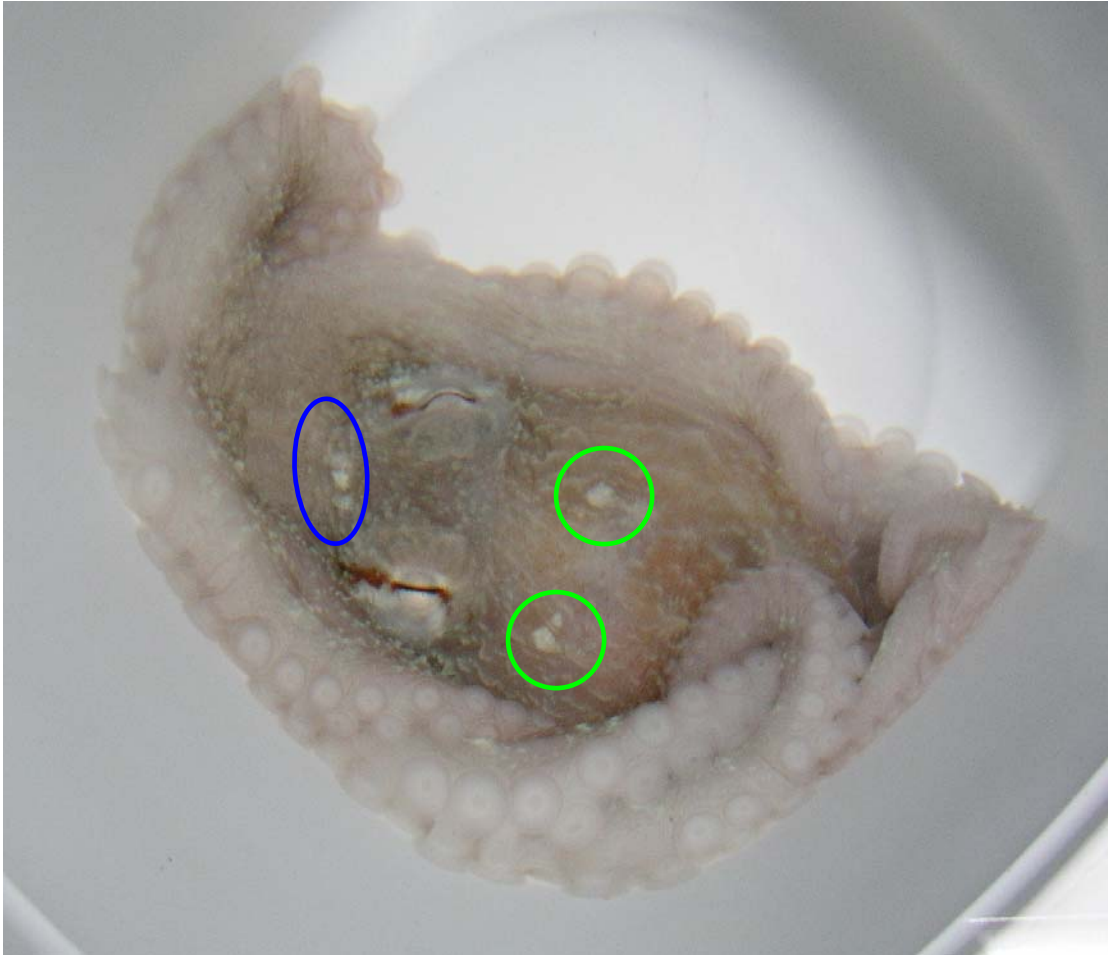
Interbrachial webs showed one sided bias; either the left or the right side had deeper webbing possibly caused by more use of one side than the other as would happen if there was a preference for facing predators or foes from the left or right.

Fig 1.9 – Web depth as a percent of arm length



Interbrachial webs are approximately 25% of length of complete arms. Web depth was measured at the deepest point (Fig 1.2 b) and so direct measurements displayed on a circular graph could be misleading (Fig 1.8 a). The average web depth has been drawn here in respect to the legs it is found between; legs are all drawn as equal (although this is not so Fig 1.7 c) so as to represent 100%.

Fig. 1.10 a- Characteristic patches



All four characteristic patches can be seen on this 53 g *E. dofleini*. The 2 located just below both eyes on the upper arms are circled in blue, the 2 behind the eyes not quite mid way down the mantle are circled in green. These spots are easily mistaken for scars or skin imperfections (as if the skin chromatophors have been marred) and range between light brown and white

Fig. 1.10b- Characteristic patches



This is a 57 g *E. dofleini* showing two characteristic patches located just below both eyes (blue circle) on the upper arms, notice how they have already started to morph in to one large spot that is visible on *E. dofleini* until at least 6 kg.

Discussion

Externally immature *E. doffeini* appear similar to the adults minus the long ligula, darken longitudinal grooves along an orange/red mantle and small non protruding eyes.

Ligulas were present but were 8 % not 15 % of the length of the hectocotyli. Visible ligulas have been used to suggest maturity of males, this should not be continued as males as small as 100 g (total length 25cm had ligulas of over 1 cm accompanied by the shortening of the 3rd arm), and the reproductive organs of these animals were not nearing maturity.

Skin colour changes with age. Paralarvae are transparent. Settled octopuses of app. 3 g are opaque and slowly develop darker skin as they grow. Octopuses of 100 g are no longer transparent however they are much lighter than octopuses over 500 g. The longitudinal grooves on the mantle of octopuses less than 2 kg are less apparent than on octopuses above 2 kg. The lack of distinguishing dark mantle grooves was relieved by the identification of 4 patches on the immature octopuses that match the description of leucophore patches; 2 located just below both eyes on the lower head/upper arms and 2 behind the eyes not quite mid way down the mantle. These patches look like scars or skin imperfections and range between light brown and white. They are apparent on young octopus above 10 g until the longitudinal grooves darken. As the longitudinal grooves darken the 2 below the eyes (occasionally appearing as only one just below the center of the two eyes) are still apparent, although the ones on the mantle become difficult to distinguish.

The eyes of small octopuses protrude (Fig 1.11). Eyes do not grow proportionally to the rest of the body and as octopuses grow the eyes appear to protrude less and less however in juveniles the eyes are prominent (in any position) and in small immature animals they are still not as 'relatively small' as in adults.

The shape of the adult *E. dofleini* mantle has been described as almost spherical; being only slightly longer than it is wide. In the immature specimens many octopuses appeared to have elongated mantles when placed on a dissection table (and underwater when loose skin billows behind them). However when mantle circumference and ventral plus dorsal length was compared, circumference proved to be the greater of the two and 23% larger than the sum of both mantle lengths.

The measurement of web depth is rather misleading. The WDI measures the longest arm by the deepest web. But the longest arms are usually the first 2 and the deepest web is usually sectors B, C, or D. Thus web coverage is better illustrated by the mean average of web depth as a total of the arms to which it is attached, this is 25%. The one sided biased in the length of *E. dofleini* web could be linked to greater use of one side than the other. Recent reports of lateralized eye use in *Octopus vulgaris* (Byrne *et al* 2003) suggest that octopuses do have preferred sides to which they move or attack. It would be interesting to study this further and see if the soft web is stretched by preferential use.

High predation has been linked to arm loss and scaring (Hartwick *et al* 1988). Only 15% of the octopuses dissected here had all 8 arms. Interestingly the front arms (arms 1 and

2) are much more frequently damaged than the back arms (arms 3 and 4). This is true for *O. digueti* as well (Voight 1992) and may tie in with the way the different arms are used (see searching and walking behaviors in Chapter 3 part A).

It is quite understandable why immature octopuses have been confused with *Octopus conispadiceus* (as has been pointed out by Sasaki, 1917 and Glendal 1993) due to their lighter colour, small lingua and less prominent warts and grooves. However, careful attention to the skin texture (wrinkles rather than goose bump like protrusions), the four characteristic patches, and other features that are common to both mature and immature animals such as the numerous cuticles allow external verification of the species, which is important for experiments using live animals.

Fig 1.11 - Protruding eyes



Eyes do not grow proportionally to the rest of the body and as octopuses grow the eyes appear to protrude less and less however in juveniles the eyes are very prominent as can be seen here in a photo provided by Y. Iwata.

Chapter 2

Physiology and Growth

Abstract

On the southern coast of Hokkaido, Japan where temperatures range between 1 and 20°C immature Giant Pacific Octopus *Enteroctopus dofleini* are found when the water temperature is between 5 and 14°C (Hokkaido Department of Fisheries 1937-2000; Fukuda *et al.* 1995). In most other octopus species the immature stage is thought to correspond with the first part of a two phase growth pattern that has been described as following first exponential and then logarithmic or power trends (Forsythe & Van Heukelem 1987; Forsythe & Hanlon 1988; Cortez *et al.* 1999; Segawa & Nomoto 2002). Details when the growth shifts between the two rates or what influences the transformation have not been clarified. Here the effects of temperature on the energy flow through the immature octopus system and growth rates of immature octopus in captivity were investigated. Part A discusses energy used in respiration (3-13°C), voided in waste (9.5°C), consumed and stored in somatic tissue (6-13°C) by captive octopuses ranging between 1-5kg was monitored. Results suggest that a mixed low lipid diet of approximately 3% body weight and temperatures of 7-9.5°C lead to more energy being available for growth and activity. Net Conversion Rates and efficiency of temperatures and diets are discussed in terms of importance to aquaculture and their implications for the behaviour of immature *E. dofleini*. Part B focuses on results from long-term maintenance of individual 5g-5kg *E. dofleini* kept in separate aquariums and feed *ad libitum* give a brief description of the growth rates of juvenile and immature octopuses.

Chapter 2 Part A

Growth Efficiency

Introduction

In Funka Bay, Hokkaido, where coastal temperatures range from 1 to 20°C, immature (3 g- 9 kg) individuals of the North Pacific Giant Octopus (*Enteroctopus dofleini*) occur at water temperatures between 5 and 14°C (Hokkaido Dept. of Fisheries 1937-2000; Fukuda et al. 1995). When coastal temperatures occur outside this range in winter and summer octopuses move offshore into deeper water, which results in a biannual inshore-offshore migration pattern (Kanamaru 1964; Kanamaru and Yamashita 1967). The effect of temperature on instantaneous growth and growth rates of cephalopods (Forsythe and Hanlon 1988; Cortez *et al.* 1999; Segawa and Nomoto 2002), its correlation to different sized eggs, yolks, hatchlings and cohorts (Sakaguchi *et al.* 2002; Vidal et al. 2002; Forsythe 1993), changes in food supply, migration and distance from shore (Hartwick *et al.* 1981; Hatfield and Rodhouse 1994; Sato 1996;), are well documented. Reports show that within the physiological limits of a species, higher temperatures result in higher growth rates, larger age at maturity and shorter life spans. However, to determine the role temperature plays in this biannual migration experiments must go beyond the direct result of temperature on growth rates and attempt to assess the effectiveness of energy flow at different temperatures (O'Dor and Wells 1987; Wells and Clarke 1996).

Here we look at the effect of temperature on energy input, somatic storage and expenditure by monitoring the food ingestion, growth and respiration of captive

immature octopuses. Three different experiments were conducted. The first tested the oxygen use of starved inactive acclimatized individuals in a dark flow through chamber. The second tested the effectiveness of different diet regimes by measuring intake, growth and waste of captive octopuses fed different amounts and types of food. The third experiment estimated the growth at 7, 9.5 and 12°C by measuring intake and growth, and using the results of the previous two experiments to supply basic metabolic rates and appropriate diets.

Methods

Octopuses were collected from the southern part of Funka Bay, Hokkaido by SCUBA and box-trap fishing. They were kept separate and monitored for 48 hours before use in the following experiments. Filtered water from Funka Bay was circulated through a temperature controlled aeration system that supplied a 13 ton raceway tank, where individuals weighing 2-4 kg were kept separate in perforated 72 l containers during the feeding, growth and waste experiments, and a covered 2 ton directional water tank where flow through chambers were submerged during the metabolism experiments. A 12h:12 h dark-light cycle was maintained using indirect lighting during the day. Octopuses were weighed before and after all experiments.

Measuring metabolism

Experiments were conducted using octopuses between 5 and 5000 g at temperatures between 3 and 13°C. Octopuses were kept at the same temperature for at least 7 days after which food was withheld for 3 days, and then individuals were placed in a darkened flow through chamber (Fig. 1). A 100 l chamber was used for octopuses over 1

kg, and a 2 l chamber was used for those under 1 kg. An oxygen sensor monitored the outflow continually (inflow was checked every few hours). Measurements of sedentary activity, made after water in the occupied chamber had turned over twice (i.e., 200 l or 4 l of water depending on chamber size), were used to estimate the metabolic rate. Respiration rates per gram at each temperature were calculated by multiplying the change in inflow and outflow oxygen by the rate of flow through the chamber and dividing the result by the average of weights of the octopus at the beginning and end of each trial.

Measuring growth efficiency of different diet regimes

Experiments were conducted at $9.5 \pm 0.5 \text{SD}^{\circ}\text{C}$ and lasted an average of 10 days. Feces and cell debris were removed twice daily, and feces were frozen for later caloric assessment. Octopuses were fed every three days according to one of four diet regimes; normal (3% body weight) or excess (4.5% body weight) amounts of a mixed diet (*Todarodes pacificus*, *Cololabia saira* and *Hemigrapsus sanguineus*), or 3% body weight of a single food diet, squid (*Todarodes pacificus*) or fish (*Cololabia saira*). The amount and type of food were recorded, and uneaten food was removed. Caloric values for 1 g dry weight of food items, somatic tissue and feces were measured using a digital bomb calorimeter (Yoshida Seiskusho Co. Ltd.) and converted to calories per 1 g wet weight.

Energy entering (calories consumed daily per gram - C), voided by (calories excreted daily per gram - F) and stored by (calories gained in somatic tissue daily per gram - G) the octopuses were measured directly. Energy used for basic metabolism (calories used in respiration daily per gram - R_{Basic}) were calculated by multiplying the metabolic rate

(determined in the previous experiment) by the caloric value of 1 mol of O₂ (4.8 cal. mol⁻¹), and hours per day (24 hrs.). A practical measure of the energy used by the octopuses ($C \cdot G \cdot F = R_{\text{Practical}}$), gross conversion rates ($G/C = \text{GCR}$), net conversion rates ($G/C \cdot R_{\text{Basic}} = \text{NCR}$) and practical conversion rates ($G/C \cdot R_{\text{Practical}} = \text{pNCR}$) were calculated for each octopus, see Table 1 for details of equations used.

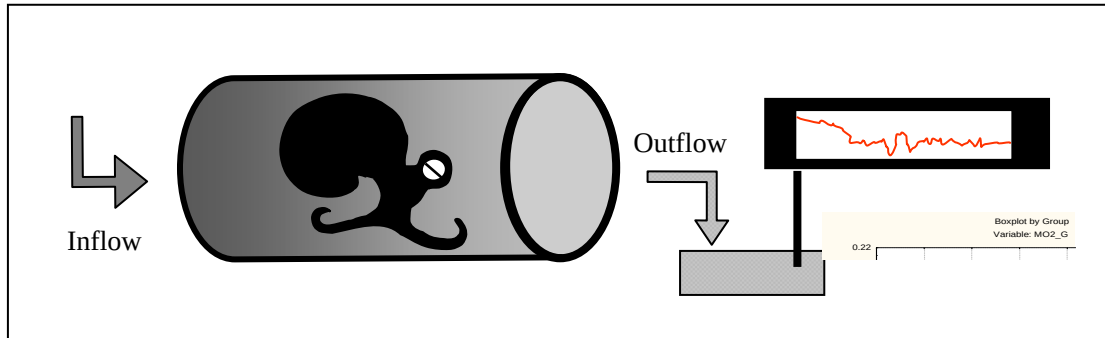
Measuring growth efficiency at different temperatures

Experiments lasted an average of 24 days and were repeated at $7 \pm 1\text{SD}$, $9.5 \pm 0.5\text{SD}$ and $12 \pm 1\text{SD}^\circ\text{C}$. Waste and cell debris were removed daily, animals were fed a mixed diet (*Todarodes pacificus*, *Cololabia saira* and *Hemigrapsus sanguineus*) of approximately 3% body weight every three days, the amount and type of food consumed were recorded, and uneaten food was removed.

Energy entering (calories consumed daily per gram $\cdot C$) and energy stored by (calories gained in somatic tissue daily per gram $\cdot G$) the octopuses were measured directly.

Energy used for basic metabolism (calories used in respiration daily per gram $- R_{\text{Basic}}$) was calculated using the same equation as above. Gross conversion rates ($G/C = \text{GCR}$) and net conversion rates ($G/C \cdot R_{\text{Basic}} = \text{NCR}$) were calculated for each octopus, see Table 1 for details of equations used.

Fig. 2a.1 - Measuring metabolism



Starved acclimatized octopuses were placed in dark, size relative flow through chambers where an oxygen sensor continually monitored the outflow. Measurements used to calculate respiration rates (MO_2/g) were taken of sedentary activity after water in the occupied chamber had changed over twice.

Table 2a.1 – Energetic equations

Respiration	MO₂/g	Flow Rate (O ₂ (INFLOW) - O ₂ (OUTFLOW))
Rate per gram	mol /g	(W ₁ +W ₂)/2
Caloric	cal	Dry cal (W _{wet} / W _{dry})
Values		
Energy	C	$\sum W_i \times \text{Cal}_i$
Consumed	cal/day/ g	(W ₁ +W ₂)/2
Basic	R_{Basic}	MO _{2(C)} × 4.8 cal mol ⁻¹ × 24 hours
Respiration	cal/day/ g	
Energy	G	Cal _{Octopus tissue} $\left(\frac{(W_2 - W_1)}{\text{days}} \right)$
Stored	cal/day/ g	(W ₁ +W ₂)/2
Energy	F	$\left(\frac{W_{\text{dry}} \times \text{cal/g}}{\text{days}} \right)$
Voided	cal/day/g	(W ₁ +W ₂)/2
Practical	R_{Practical}	A - G
Respiration	cal/day/g	
Gross Conversion	GCR	$\frac{G \times 100}{C}$
Rate	%	
Net Conversion	NCR	$\frac{G \times 100}{C - R_{\text{Basic}}}$
Rate	%	
Practical Net	pNCR	$\frac{G \times 100}{C - R_{\text{Practical}}}$
Conversion Rate	%	

W: weight, 1: initial, 2: final, *i*: food item, cal: calorie

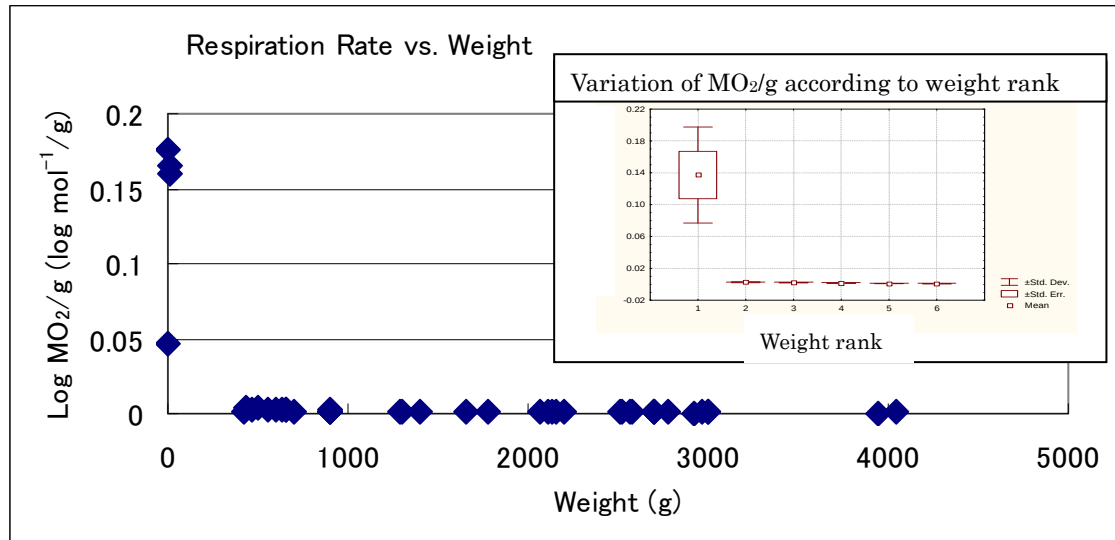
Results

Measuring metabolism

There was a significant difference between respiration rates per gram (MO_2/g) of octopuses above and below 100 g body weight (Kruskal-Wallis test $H(1, N=41) = 10.57$, $p = 0.0011$), Fig 2a.2. Octopuses under 100 g had significantly higher respiration rates per gram and more variation between individuals than octopuses above 100 g. There was a significant difference between the respiration of octopuses over 1 kg when they were grouped in seasonal temperature averages; winter, $4 \pm 1\text{SD}^\circ\text{C}$; spring/fall, $8 \pm 2\text{SD}^\circ\text{C}$; and summer, $12 \pm 1\text{SD}^\circ\text{C}$ (Kruskal-Wallis test $H(2, N=41) = 8.427$, $p = 0.0148$). Between $6\text{--}13^\circ\text{C}$ respiration rates increased with changes of temperature from a low at $7/8^\circ\text{C}$. Below 6°C respiration was severely reduced and larger animals showed greater reduction per gram than smaller animals, Fig 2a.3.

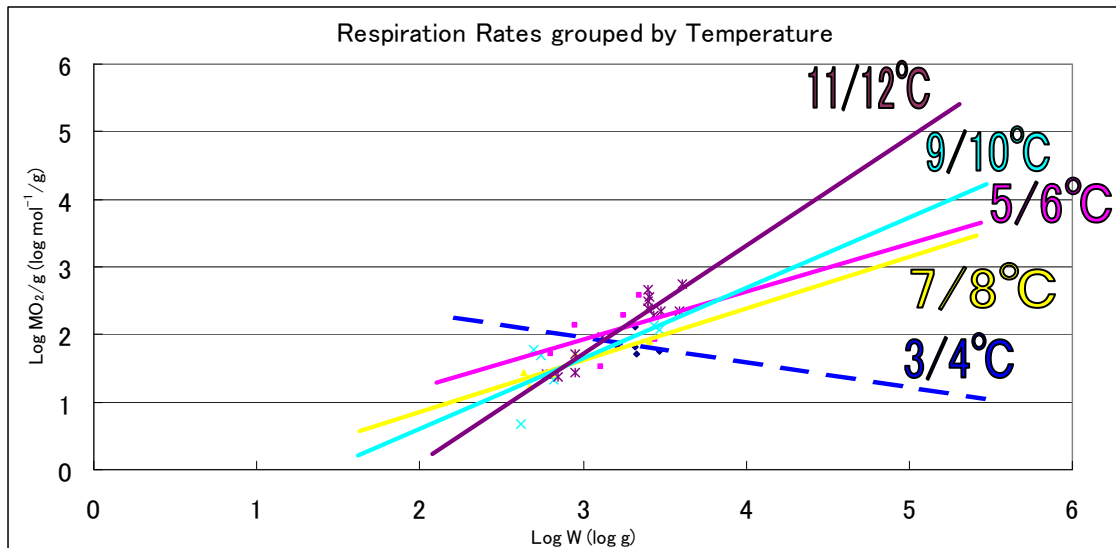
Comparing respiration rates per gram for octopuses at $7 \pm 1\text{SD}$, $9.5 \pm 0.5\text{SD}$ and $12 \pm 1\text{SD}^\circ\text{C}$ (temperatures that matched the feeding and growth experiments) a significant difference was seen between octopuses under and over 1kg at $12 \pm 1\text{SD}^\circ\text{C}$ (Kruskal-Wallis test $H(1, N=14) = 7.21998$, $p = 0.0072$).

Fig. 2a.2 - Respiration in small and large octopuses



There was a significant difference between respiration rates per gram of octopuses above and below 100 g body weight, those under 100 g had significantly higher respiration rates per gram and more variation between individuals than octopuses above 100 g. The graph shows the logarithmic scale of respiration rates against weight, the insert is a box pot of the same, with the weights grouped in ranks; 1 = 1 - 99 g, 2 = 0.1 - 0.9 kg, 3 = 1 - 1.9 kg, 4 = 2 - 2.9 kg, 5 = 3 - 3.9 kg, 6 = 4 - 5.9 kg.

Fig. 2a.3 - Respiration rates grouped by temperature



Between 6-13°C respiration rates (MO₂) increased with changes of temperature from a low at 7/8°C. Below 6°C basic metabolism was severely reduced and larger animals showed lower respiration rates per gram than smaller animals.

Measuring growth efficiency of different diet regimes

Caloric values for wet weights decreased in the following order: fish, squid, octopus, and crab (Table 2). Caloric values for crab were consistently less than half those of the other food items. More energy was consumed per day per gram (C) on the mixed diets than the single food diets, however there was no significant difference in consumption within the two single diets or two mixed diets (Table 3; Fig. 4). More energy was used for respiration ($R_{\text{Practical}}$) during excess feeding than normal feeding. More energy was voided (F) during consumption of the fish diet than the squid diet.

Gross, net and practical net conversion rates showed similar patterns. Conversion rates were higher during normal than excess feeding, a result of the increase in energy used to metabolize food. Conversion rates were lower during consumption of the fish than squid diet, a result of the increase in energy voided from the system and the greater amount of energy available for storage.

The benefit of moderation and variety was seen in the assessment of all four diets (Fig. 4). Although single food diets used less energy ($R_{\text{Practical}}$), the energy stored in growth (G) while on the mixed diets was greater. At the normal feeding rate, the mixed diet had a significantly higher efficiency (GCR, NCR, and pNCR) than the other diets.

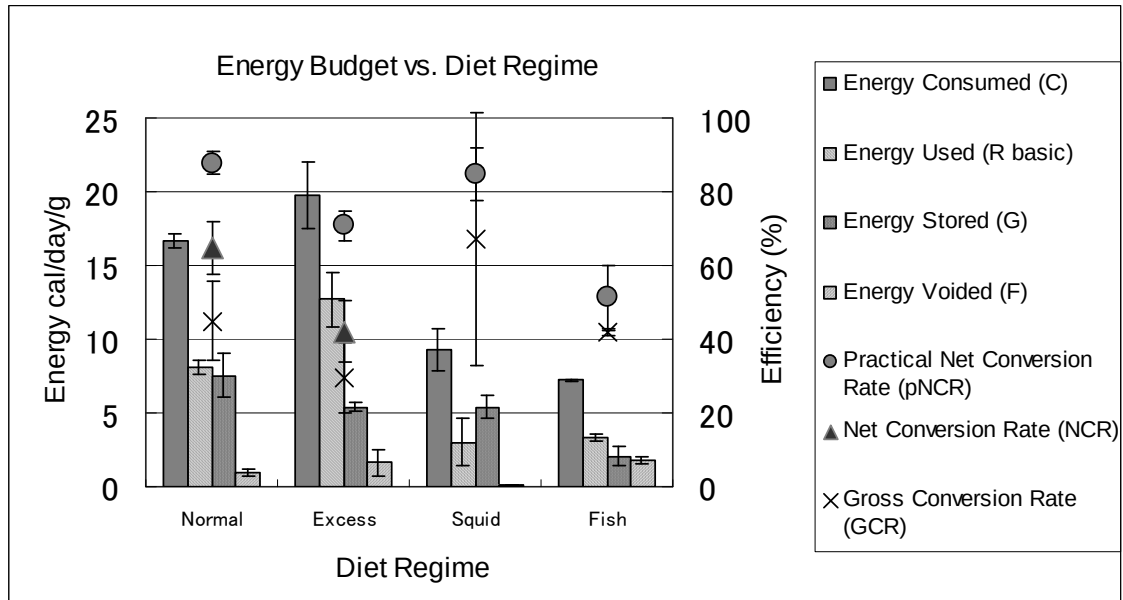
Measuring growth efficiency at different temperatures

There was a significant increase of net conversion rate (NCR) with temperature caused by slight changes in feeding, storage and use (Fig 5).

Table 2a.2 - Caloric values with standard deviation

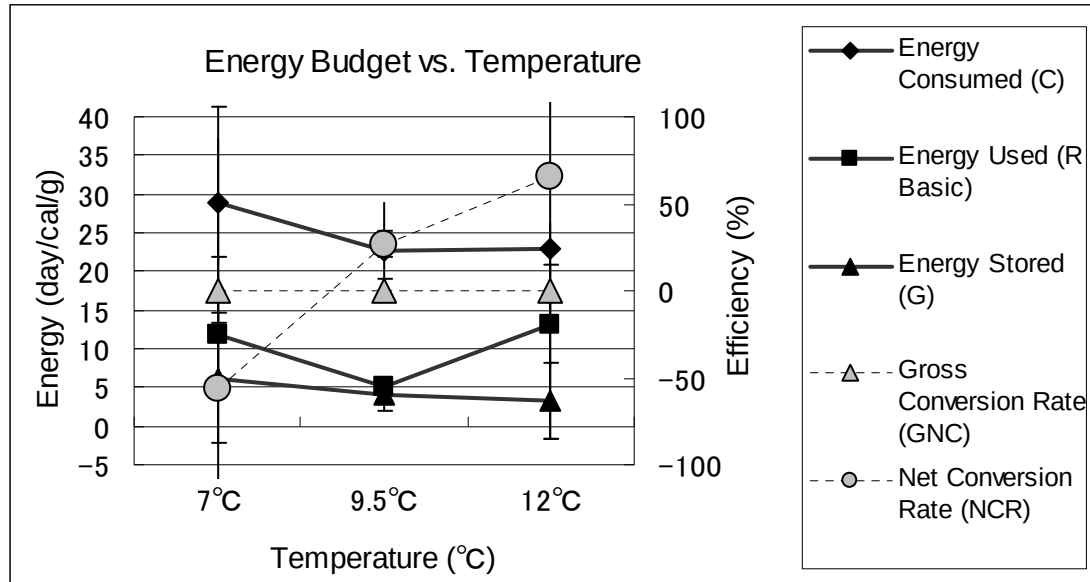
		Calories/ dry g	Calories/ wet g
Octopus	<i>E dofleini</i> (immature)	4680.84 ± 98.69	781.808 ± 16.57
Food	<i>Todarodes pacificus</i> (mature)	5424.13 ± 42.76	1311.26 ± 10.34
	<i>Cololabia saira</i>	7064.48 ± 52.35	1962.18 ± 14.54
	<i>Hemigrapsus sanguineus</i>	1680.16 ± 217.89	521.31 ± 67.61
	Normal (3% body weight)	5174.20 ± 1855.67	1759.29 ± 414.22)
Waste	Excess (4.5% body weight)	7294.07 ± 934.79	2949.70 ± 1087.51
	Squid Only	5627.065 ± 606.74	1781.19 ± 1267.36
	Fish Only	7649.42 ± 39.59	3401.45 ± 50.18

Fig. 2a.4 - Energy budget vs. diet regime



Energy in daily food (C), growth (G) and waste (F) were recorded at four diet regimes; normal (mixed diet at 3% body weight), excess (mixed diets at 4.5% body weight), squid only (3% body weight) and fish only (3% body weight). The excess feeding rate was less efficient than the normal feeding rate as more energy was used to process the excess food. The squid diet was more efficient than the fish diet as more energy was voided from the squid diet.

Fig. 2a.5 - Energy budget vs. temperature.



Energy in daily food and growth were recorded at three different temperatures; low $7(\pm 1\text{SD})$, medium $9.5(\pm 0.5\text{SD})$ and high $12(\pm 1\text{SD})^\circ\text{C}$. Basic metabolic rates and appropriate diets were supplied by the two previous experiments. The slight differences in feeding, storage and use resulted in a significant increase in the efficiency (NCR) with temperature.

Table 2a.3 - Results of measuring efficiency of different diet regimes and at different temperatures

	Energy Consumed (C) cal/ g/ day	Energy Respired (R _{Basic}) cal/ g/ day	Energy Used (R _{Function}) cal/ g/ day	Energy Stored (G) cal/ g/ day	Energy Voided (F) cal/ g/ day	Gross Conversion Rate (GCR %)	Net Conversion Rate (NCR %)
Normal	16.641(+/-0.94)	-	8.146(+/-0.94)	7.557(+/-3.04)	0.942(+/-0.40)	44.826(+/-0.11)	64.749(+/-7.04)
Excess	19.749(+/-4.49)	-	12.715(+/-3.71)	5.412(+/-0.69)	1.624(+/-1.79)	29.475(+/-0.09)	42.101(+/-8.37)
Variance of Normal & Excess	<i>n.s.</i>	-	$H(1, N=36) = 11.03604$ $p = 0.0009$	<i>n.s.</i>	<i>n.s.</i>	$H(1, N=36) = 6.56756$ $p = 0.0104$	$H(1, N=72) = 14.83562$ $p = 0.0001$
Squid only	9.32(+/-2.85)	-	3.031(+/-3.11)	5.389(+/-1.48)	0.138(+/-0.05)	67.016(+/-0.34)	312.435(+/-159.98)
Fish only	7.206(+/-0.13)	-	3.370(+/-0.47)	2.078(+/-1.34)	1.759(+/-0.52)	41.96(+/-0.01)	161.333(+/-26.77)
Variance of Squid & Fish	<i>n.s.</i>	-	<i>n.s.</i>	$H(1, N=8) = 5.6000000$ $p = 0.0180$	$H(1, N=8) = 5.6000000$ $p = 0.0180$	<i>n.s.</i>	<i>n.s.</i>
Variance of All Diet Regimes	$H(3, N=48) = 26.44898$ $p = 0.000$	<i>p</i>	$H(3, N=48) = 32.69898$ $p = 0.000$	$H(3, N=16) = 9.642858$ $p = 0.0219$	<i>n.s.</i>	$H(3, N=48) = 13.22449$ $p = 0.0042$	$H(3, N=96) = 49.50000$ $p = 0.0000$
Variance of PNET & NET	$H(7, N=444) = 0.$						
Variance of PNET & NET & GROSS	$H(9, N=480) = 358.71$ $p = 0.000$						
7°C(s.d.+/-1)	22.269 (+/-8.12)	11.706(+/-8.23)	-	6.180 +/-8.46)	-	28.97(+/-0.38)	-56.40 (+/-162.46)
9.5°C(s.d.+/-0.5)	19.394(+/-6.19)	5.134 (+/-0.89)	-	4.064 (+/-2.06)	-	22.73(+/-0.13)	27.01 (+/-7.47)
12°C(s.d.+/-1)	15.013(+/-3.46)	12.982(+/-5.34)	-	3.301 (+/-4.84)	-	22.82(+/-0.30)	65.05 (+/-149.73)
Median Test of Temperatures	<i>n.s.</i>	<i>n.s.</i>	-	<i>n.s.</i>	-	<i>n.s.</i>	chi sq= 11.61618 df 2 $p = 0.0030$

Discussion

Maintenance and metabolism

To measure the energy required for internal maintenance alone (protein turnover, circulation, etc.) octopuses must be anaesthetized. Unfortunately the results of anaesthesia vary according to method used, animal size, and age which results in considerable variation in any consequent equations. Measurements on stationary animals are often considered to be repeatable and representative of resting metabolism (Barnes *et al.* 1989). However, octopuses quickly adapt to constant solitary conditions seen readily in *E. dofleini* by the loss of eye horns, skin folds and papillae. The (probable) corresponding decrease in neural activity implies that what is being tested is less than standard metabolism. Calculating metabolism using starved acclimatized octopuses in dark, size relative chambers ensured repeatable measurements of basic metabolism (R_{Basic}).

System maintenance is multifaceted, and a great amount of energy required by the system is not captured by the measurement of basic metabolism (R_B), such as the; energy used to metabolize (R_M), and handle food (R_H), or used during growth (R_G), production (R_P), and activity (R_A). Further energy lost in secretion (U) and cell exfoliation is also part of basic system maintenance. Ideally the total energy used could be measured directly by monitoring all energy gates (points where energy is lost, used or changed). However, in the current experimental set up this was not possible. Thus the assimilated energy ($C - F = A$) not used in growth (G) was calculated as a practical measure of the energy used for maintenance and given the acronym $R_{\text{Practical}}$. When this practical assessment was used in place of the R_{Basic} in the net conversion rate (NCR)

calculation the rate was renamed practical net conversion (pNCR).

There was a large difference between the energy budgets for the mixed diets and the single-food diets. The greatest difference was in the energy required to handle and process food. In the single food diets energy use ($R_{\text{Practical}}$) was lower than the basic metabolism (R_{Basic}) that was recorded at the same temperature. This confirms that the recorded basic metabolism (R_{Basic}) was not basal metabolism (R_B), and supports the theory that octopuses can suppress their metabolism. Octopuses have been reported to reduce their basal metabolism (R_B) during starvation which is thought to be a factor in the slow weight loss and muscle degradation seen during extended maternal egg care (Forsythe and Toll 1991). Metabolic rates were not significantly different between octopuses starved for 3 or 5 days (Rigby, unpublished data) and suppression was not considered to be a factor in the experiments where respiration rates were measured directly, it may however have occurred during the long term consumption of the single food diets. Seasonal variation in respiration has been linked to a composite of factors including food type (Clarke 1993), and although calories were available (to the extent that they were voided), metabolism may have been suppressed due to the lack or similarity of nutrients in the diet. This hypothesis is further supported by the lower growth rates during consumption of single-food diets vs. mixed diets, even though considerably less energy was used.

Respiration

The difference in respiration values between large (>400 g) and small (<400 g) octopuses suggests this rate might change through the life cycle. Many octopuses go through two

phases of growth; the first is exponential, and the second has been described using either logarithmic or power patterns (Forsythe and Van Heukelem 1987; Forsythe and Hanlon 1988; Cortez *et al.* 1999). It is probable that the changes in growth rates can be used to define the split between juvenile and immature phases; the juvenile phase taking up the entire exponential growth spurt and the immature phase being the first part of the logarithmic or power function phase where energy is stored in somatic rather than reproductive tissues. It is not clear whether the growth rate change is related to age, size or an environmental signal but the 0.4-1 kg *E. dofleini* used here showed similar growth and metabolic patterns to octopuses between 1-5 kg, suggesting that those weighing 0.4-1 kg may be at the beginning of the second growth phase. It would be of interest to see if there is a change in respiration as energy begins to be stored in reproductive tissue (i.e., at the onset of vitellogenesis). It may be that growth rates are maintained by a rearranging of the energy budget through a decrease in activity. This would help explain reports of how during the later growth phase gonad production starts without arresting somatic growth (Clarke *et al.* 1994; Sato 1996) as well as why adult *E. dofleini* do not migrate inshore or over long distances like the immature stages (Sato 1990; Fukuda *et al.* 1995).

The effectiveness of different diet regimes

Unlimited food and the resulting constant feeding activity can strain filtration systems with excess debris and waste, often severely reducing water quality. In captive trials it has been noted that octopuses seemed to benefit (i.e., remained healthy and showed stable feeding and activity) from a mixed diet of approximately when fed 3% body weight every three days (P. R. Rigby unpublished data). The four diets (mixed 3%,

mixed 4.5%, squid 3% and fish 3%) were used to clarify the energetic reasons for this finding by relating the amount and type of diet to growth rates and efficiency. Removal of the organs, appendages, skin and bones of squid and fish decreased the variation in the weight and caloric value of the intake assessment. However, the availability of calories for use depends on the ability of the octopus to digest the food consumed (Penry 1993). The significant difference between the energy voided under the two one-food diets may be due to the fact that *Cololabia saira* has higher lipid content than *Todarodes pacificus*. Comparison between the high caloric value of the abundant oily buoyant waste produced by the *C. saira* diet and the low caloric value of the scant concentrated strings produced by the *T. pacificus* diet suggests the lipid content of the food may be a principle cause of this difference. The implication of this is that lipids, although shown to be vital to the growth of young cephalopods (Navarro and Villanueva 2000), may not be as easily broken down and used as other caloric sources.

The implication for aquaculture is clear. Feeding costs can be lowered without impacting growth rates by feeding octopuses a mixed low-lipid diet of approximately 3% body weight every three days and keeping the temperature around 9.5°C.

Growth efficiency at different temperatures

We calculated the inactive energy balance and growth efficiency after reducing activity by placing the octopuses in individual containers and preparing their food. Energy budgets showed more energy was available at temperatures under 10°C due to lowered energy use at 9.5°C or greater energy intake at 7°C. The extra available energy did not cause a matching jump in growth but rather a decrease in the efficiency of the net

conversion. However, because of the way that efficiency (NCR) was calculated, this loss in efficiency can be seen as a gain in extra energy, which could be essential for activity and presumably vitellogenesis.

The biannual migration of immature *E. dofleini* in southern Hokkaido can thus be reinteroperated in light of its energetic balance: during summer a combination of decreased feeding rates and increased maintenance costs created by high coastal water temperatures keep the octopuses offshore. In the late fall when coastal water temperatures decrease to 12°C, lowered maintenance costs benefit octopuses that migrate inshore. The octopuses stay inshore until the increasing costs of maintenance are no longer balanced by increased feeding (or through a combination of food availability and physical limitations they are no longer able to maintain sufficient energy inflow). From January to April, octopuses are found only offshore in deep waters where temperatures (app. 5 °C) are higher than they are along the coast. In spring when coastal temperatures increase above 5°C octopuses migrate inshore, staying until temperatures are no longer energetically friendly.

The imbalance of energetic costs caused by the extremes in seasonal temperatures keep immature animals from inhabiting the inshore area throughout the year. Adult octopuses however, are not seen on the coast of Funka Bay at any time of the year. It is feasible that mild temperatures (7-9.5°C) would benefit *E. dofleini* at all life cycle stages, but that maturing octopuses simply do not have the `extra` energy necessary to perform the biannual migration and take advantage of the benefits. Whether there are other factors and what role they play on immature *E. dofleini* life history choices may be best

assessed by further studies on food availability, social interaction and the role of social structure.

Chapter 2 Part B

Growth Rates

Introduction

The life cycle of *Enteroctopus dofleini* follows the pattern: egg (hatch) paralarvae (settlement), immature (vitellogenesis), mature (sexually active), death (delayed when brooding eggs). The change in growth rates that have been described for many octopus species (Forsythe & Van Heukelem 1987; Forsythe & Hanlon 1988; Cortez *et al.* 1999; Segawa & Nomoto 2002) occurs in *E. dofleini* during the immature phase and can be denoted by using the terms juvenile and immature. This switch occurs sometime between total body weights of 0.101-1 kg (Chapter3 part A) but what influences it (environment, weight, temperature, time, season) remains unclear. Both power and logarithmic curves have been used to describe growth in the immature phase. However, the difference between the two over the time span of a large cold water octopus like *E. dofleini* is considerable.

Immature *E. dofleini* move on and off shore, experience temperatures between 5 and 15°C and feed on various prey items. Growth rates in captivity at set temperatures and prepared diets as assessed here cannot replicate these conditions. The experiments described here were conducted to answer 2 questions 1) How different are juvenile and immature growth rates 2) how long does it take to reach maturity.

Methods

All octopuses were caught at bottom depths of less than 15 m on the rocky shore of Funka Bay (Usujiri, Minamikayabe). Temperature ranged between 10 and 13°C in the juvenile aquaria and was regulated at 7, 9 and 12°C in the immature aquaria.

Individual juvenile *E. dofleini* were kept in 50l circular tanks designed for high density fish aquaculture. A temperature controlled open water supply (filtered thrice, aerated twice) circulated from the top down in to 25cm of rocks and coral. There was an attempt to reproduce the substrate, lighting, and seasonal flora and fauna of Funka Bay.

Juveniles were supplied with live crabs (*Hemigrapsus sanguineus*) *ad libum* and occasional pieces of fresh squid (*Todarodes pacificus*). Crab carapaces were removed daily, counted, measured and replaced. Octopuses were weighed during the day every 10 days.

Three juvenile (less than 100g) *E. dofleini* were caught in Funka Bay. One was found March 2001 in the port area attached to wide frond kelp *Laminara sp.* that was being prepared for processing. The two others were caught during research dives in April 2003 and in October 2003. The two octopus found in the spring were under 3 g and grew to 26 and 116 g respectively. The one found during the fall was 98 g on capture and is currently 225 g.

Six immature *E. dofleini* were caught on the rocky shoreline of Funka Bay in September-December 2002, and April- June 2003 and kept in 60l perforated boxes that were allowed to float freely in a 13 ton race way tank supplied with water from a temperature

controlled open water filtration system (filtered twice, aerated once). Octopuses were fed a mixture of fish and squid that equalled 3% of their body weight every 3 days and live crabs twice a week. Uneaten fish and squid were removed after 4 hours (crabs were not). Octopuses were weighed before eating every 9 days.

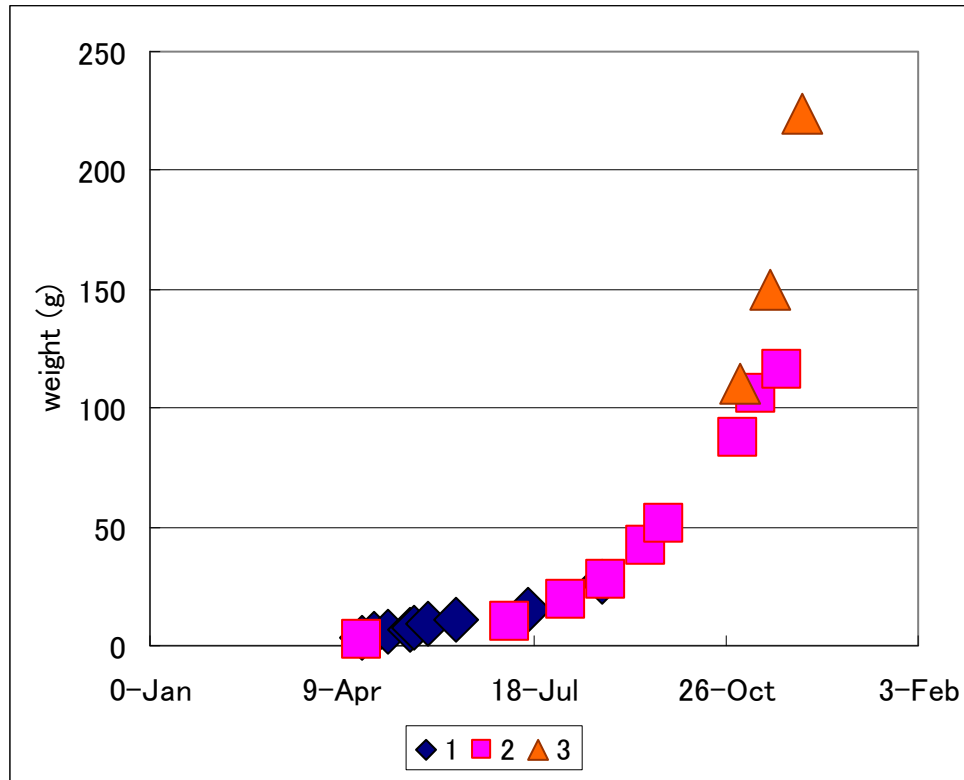
Results

The data from the three juvenile octopuses were kept separated. Two octopus had growth rates best described by an exponential curve (exp. $R^2 = 0.995$, 0.9963). The growth of the other octopus was described more accurately by a power curve (power $R^2 = 0.9797$ exp. $R^2 = 0.9511$). Extending the growth rates by exponential trajectories to predict when they were 1g gives potential settling dates of between January and March of year one (Figure 2b.2- Juvenile growth curves, Table 2b.1- Measurements of Growth) If exponential growth rates continue then 500g and 1kg would be reached early in the next year and during the next spring, respectively (Figure 2b.3- Projected juvenile growth curves Table 2b.2- Hypothetical settlement dates)

The median growth rate of the six immature octopus at 7, 9 and 12 °C was best described by a power curve at 7°C ($R^2 = 0.8204$) and a logarithmic curve at 9 and 12°C ($R^2 = 0.8807$ & 0.9538 , respectively). (Fig 2b.4 – Immature growth rates)

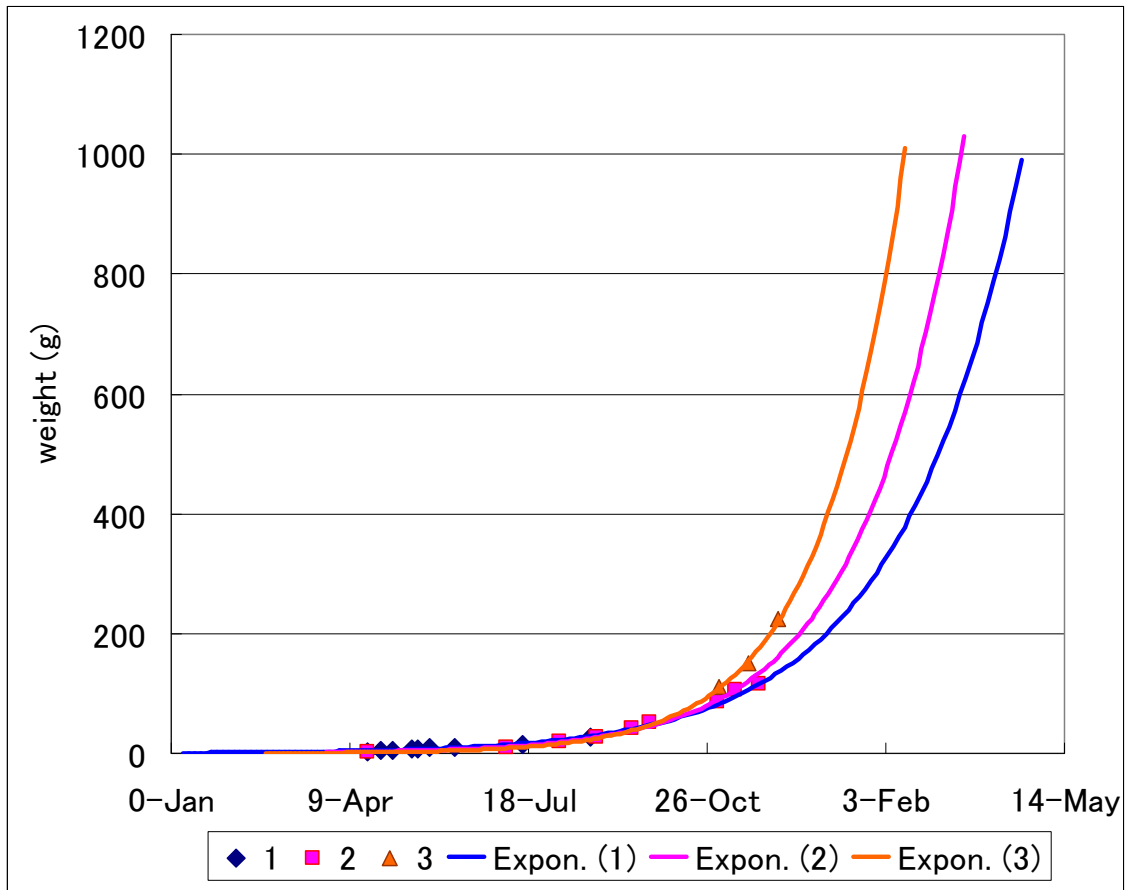
Time from settlement to potential maturity (1g -15kg) represented the transitional phase by calculating a switch between growth rates at i) 100g ii) 500g iii) 1000g. They differed depending on temperature (Table 3a.4- Growth rates) however in general maturity is reached within 1 or 2 years with power curves, 2 or 3 years with linear growth and 5 years with logarithmic growth curves.

Fig. 2b.1- Juvenile growth curves



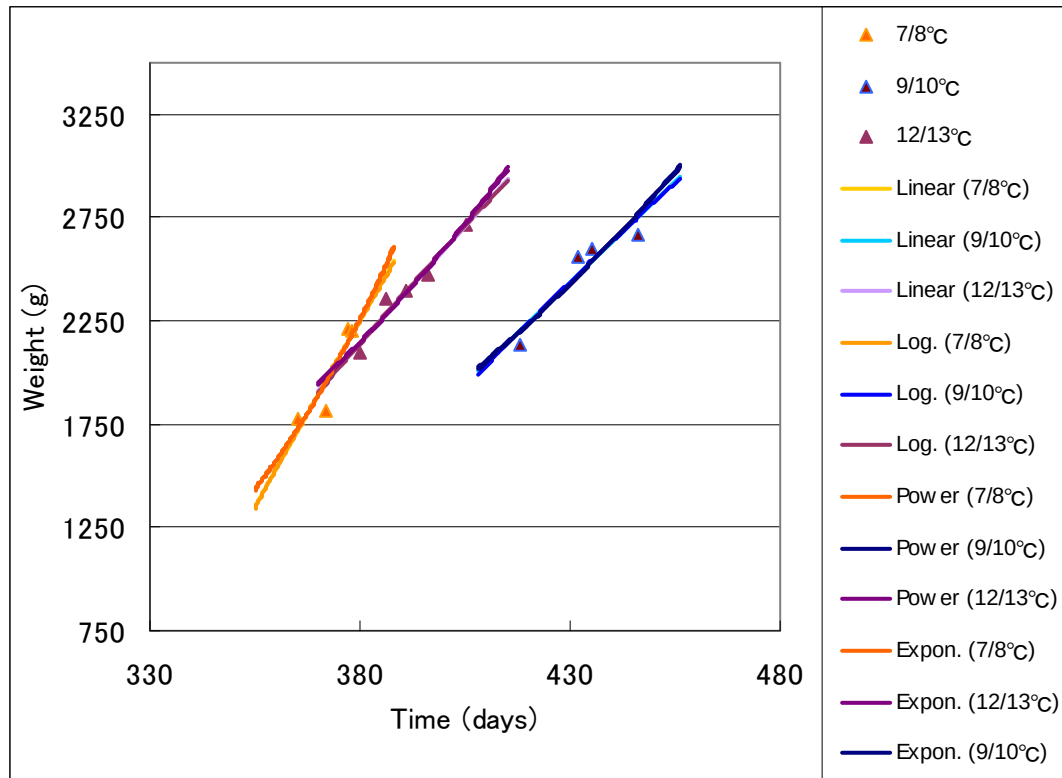
The growth rates of all three juvenile (less than 100 g) *E. dofleini* caught in Funka Bay between 2000- 2003 were exponential. The two octopus found in the spring were under 3g and grew to 26 and 116 g respectively. The one found in the fall was 96 g on capture and is currently 225 g.

Fig. 2b.2- Projected juvenile growth curves



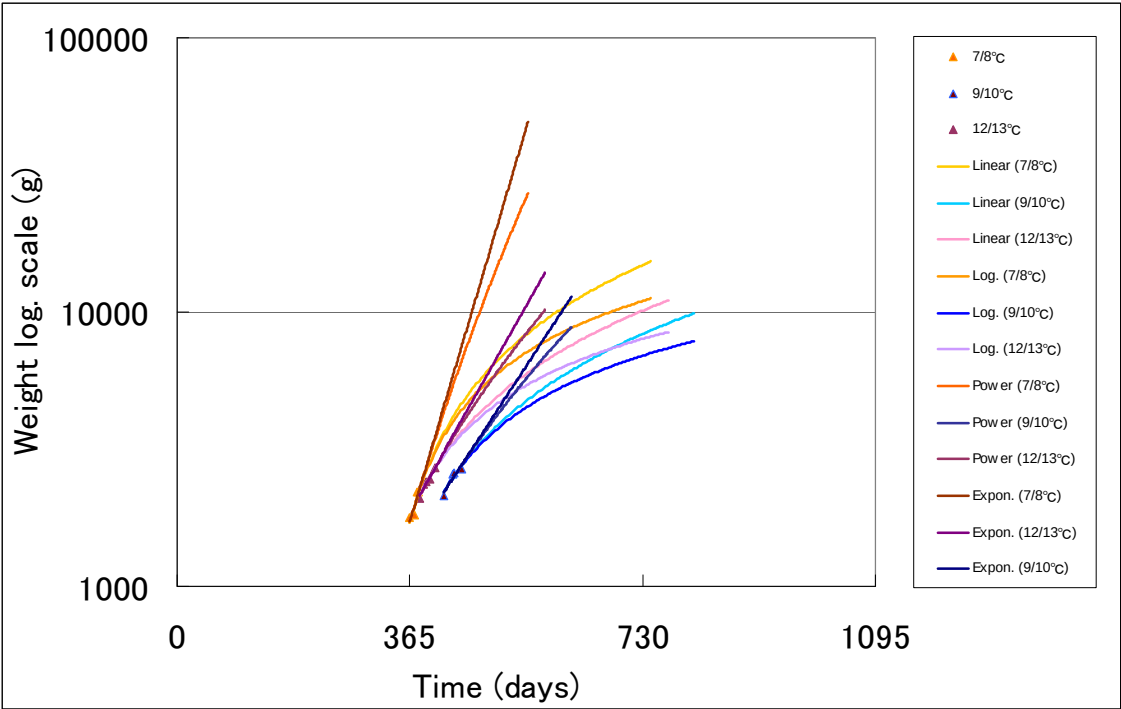
The exponential trajectories were used to predict: 1) when they were 1 g, show potential settlement dates between January and early March. 2) When they would be 500 g (early part of the next year, almost 12 months after settlement) and 1kg (the following spring, just over a year after settlement).

Fig. 3b.3 – Immature growth rates



All trends (linear, log & power) fit more than 80% of the data. However, best fits are found by power trends at 7°C and logarithmic growth at 9-12°C.

Fig. 2b.4 Extended Growth Rates



The difference that rates can make is shown here by extending the power trajectories by 6 months and the logarithmic and linear trends by 1 year from the end of the available data.

Table 2b.1 - Measurements of growth rates

Growth Rate	Juvenile		Immature	
	no.	Best fit	temp.	best fit
exp.	1	0.9511	7	0.8235
	2	0.995	9	0.8616
	3	0.9963	12	0.9411
linear	1	0.9708	7	0.8173
	2	0.8278	9	0.8734
	3	9756	12	0.9526
log.	1	0.9352	7	0.8142
	2	0.6816	9	0.8807
	3	0.9708	12	0.9538
power	1	0.9797	7	0.8204
	2	0.9738	9	0.8691
	3	0.9942	12	0.9432

Table 2b.2 - Hypothetical settlement dates

	Intercept	Slope	app. 1g	app. 100g	app. 500g	app. 1kg
1	0.9251	0.0147	6-Jan-02	14-Nov-02	4-Mar-03	20-Apr-03
2	0.3895	0.0177	23-Feb-03	9-Nov-03	8-Feb-04	19-Mar-04
3	0.1534	0.0214	28-Mar-03	29-Oct-03	13-Jan-04	14-Feb-04

Table 2b.3 - Temperature and growth rates: Power, Log or Linear?

Rate	Temp.	Intercept	Slope	app. 1g	app. 500g	app. 15000g
Linear	7	11415	35.972	318	332	735
	9	5991.5	19.602	306	332	1071
	12	6513.4	22.779	286	308	945
Log	7	76963	13335	8103	8156	9683
	9	49060	8492.9	5423	5479	7081
	12	51003	8946.3	5606	5661	7254
Power	7	1E-14	6.7127	1E+14	2.5239E+14	4.1891E+14
	9	0.000001	3.5539	1000000	5750000	14965000
	12	0.0000005	3.7245	2000000	10610000	26441500

Table 2b.4- Growth rates

Growth rate	temp.	octopus	Scenario								
			exp. growth rate from 1-100g			exp. growth rate from 1-500g			exp. growth rate from 1g-1kg		
			1-15kg (days)	Maturity*		1-15kg (days)	Maturity*		1-15kg (days)	Maturity*	
				day-month	year		day-month	year		day-month	year
Linear	7	1	727	2-Jan	2	826	11-Apr	2	859	14-May	2
		2	674	28-Dec	1	754	18-Mar	2	779	12-Apr	2
		3	629	17-Dec	1	694	20-Feb	2	712	10-Mar	2
	9	1	1073	14-Dec	2	1162	13-Mar	3	1184	4-Apr	3
		2	1020	9-Dec	2	1090	17-Feb	3	1104	3-Mar	3
		3	975	28-Nov	2	1030	22-Jan	3	1037	29-Jan	3
	12	1	967	30-Aug	2	1060	1-Dec	2	1085	26-Dec	2
		2	914	25-Aug	2	988	7-Nov	2	1005	24-Nov	2
		3	869	14-Aug	2	928	12-Oct	2	938	22-Oct	2
Log.	7	1	1882	2-Mar	5	1950	9-May	5	1944	3-May	5
		2	1829	25-Feb	5	1878	15-Apr	5	1864	1-Apr	5
		3	1784	14-Feb	5	1818	20-Mar	5	1797	27-Feb	5
	9	1	1960	19-May	5	2025	23-Jul	5	2017	15-Jul	5
		2	1907	14-May	5	1953	29-Jun	5	1937	13-Jun	5
		3	1862	3-May	5	1893	3-Jun	5	1870	11-May	5
	12	1	1950	9-May	5	2016	14-Jul	5	2008	6-Jul	5
		2	1897	4-May	5	1944	20-Jun	5	1928	4-Jun	5
		3	1852	23-Apr	5	1884	25-May	5	1861	2-May	5
Power	7	1	579	7-Aug	1	626	23-Sep	1	640	7-Oct	1
		2	526	2-Aug	1	554	30-Aug	1	560	5-Sep	1
		3	481	22-Jul	1	494	4-Aug	1	493	3-Aug	1
	9	1	865	20-May	2	873	28-May	2	860	15-May	2
		2	812	15-May	2	801	4-May	2	780	13-Apr	2
		3	767	4-May	2	741	8-Apr	2	713	11-Mar	2
	12	1	794	10-Mar	2	813	29-Mar	2	806	22-Mar	2
		2	741	5-Mar	2	741	5-Mar	2	726	18-Feb	2
		3	696	22-Feb	2	681	7-Feb	2	659	16-Jan	2

Discussion

Funka Bay does not have a large juvenile octopus population; the three used here were the only ones seen during 2001- 2003. The two spring octopuses were found at the same time of year the year (April 19th 2002, April 18th 2003). Both were very recently settled, lacked skin pigmentation (i.e. were see through) and refused anything but live crabs.

The octopus found in the fall was very similar to the longest living spring octopus end of its life. This results in the growth lines of the three octopuses to appear continuous.

Juvenile growth is best described by exponential curves. A power curve fits the raw data of the fall juvenile better than an exponential curve but not as well as the exponential curve fits the other two octopuses. Both linear and logarithmic curves describe immature growth well (linear at 7°C and log at 9 & 12°C). Power trends, which show octopuses maturing within a year, did not describe immature *E. doffleini* growth.

The length of the transitional phase may vary among octopuses however significant difference found in metabolism and metabolic response to temperature show focal points of 101 g and 1 kg (Part A). By using the focal points and the mid point between them (500 g) to calculate potential time spent between settlement and vitellogenesis it was apparent that there was no advantage seen in continuing the exponential growth rates between 101 g and 1 kg (exception: power trend at 7°C).

Hypothetical time from settlement to potential maturity (1g -15kg) differed depending on temperature. However in general *E. doffleini* growth as reported in the literature is best described by a linear growth rate in its immature phase, thus taking 2 or 3 years

(approximately 30 months) to reach maturity. This may change to a logarithmic growth rate after vitellogenesis but the 5 years that would be required to reach maturity by logarithmic growth is much too slow while the speed of a power curve, where the octopuses would mature within 1 or 2 years is much too fast.

Chapter 3

Behaviour in Captivity

Abstract

Immature *Enteroctopus dofleini* are exposed to a range of temperatures on the southern coast of Hokkaido. Here the behavioral response; defined as space use interactions and activity, to this range in temperature is investigated. In Part A descriptions of the behaviours seen in captivity are listed according to apparent energy expenditure. In Part B individual locations and interactions between, and the activity levels of groups of four or five octopuses kept in a 13 ton open water raceway tank were recorded on the hour for ten days at 2, 5 & 7°C by hand and video. The effects of temperature and relative size on space use and interaction, and change in activity level in the aquarium are reported. The resulting changes in social structure and its implications to the biannual migration of immature *E. dofleini* on and off the southern coast of Hokkaido are discussed.

Introduction

'Behaviour is the action or reaction [of an animal] under specified circumstances or the aggregate of the responses, reactions or movements made [by an animal] in any situation'.

(Oxford Word Net 1997)

Various aspects of *E. dofleini* behaviour have been documented; reproductive (Gabe 1975); foraging (Vincent *et al.* 1997), feeding (Harwick *et al.* 1978), and antagonistic

(Hartwick *et al.* 1979; Kyte and Courtney 1977). The description of octopus behaviour has been approached in two ways. The first is to describe body patterns by noting chromatic, textural, and postural components (Crowdy 1911; Packard and Sanders 1971; Packard and Hochberg 1977; Forsythe and Hanlon 1988; Mather and Mather 1994). The second is to describe activity by recording space use and interactions (Hartwick *et al.* 1984; Mather *et al.* 1985). The combination of these two styles has resulted in the listing of circumstances under which the given body patterns occurred (Mather and Mather 1994), or the creation of behavioral categories based on body patterns (Mather 1988).

In southern Hokkaido, immature *E. dofleini* migrate when seasonal temperatures drop below 5°C or rise above 12°C. Various environmental conditions may correspond to this behaviour but the finding that temperature directly impacts octopus physiology (Chapter 2 - Physiology and Growth) endorses the investigation of the relationship between temperature and behaviour.

Here, in Part A I have organized the behaviours seen in captivity into activity levels. The levels are scaled according to awareness, responsiveness, movement and (apparent) energy consumption. I believe that it is a necessary continuation of the compilation of the two traditional styles of behaviour description. And that it will benefit the description of social structure, temporal activity, and the comparisons of species and individuals.

In Part B I have described the movement, interaction and activity patterns of *E. dofleini*

in captivity. The work done for Part A on behaviour is used to comment on the differences seen in space use, activity and social structure at each temperature and the implication that behaviour has on the biannual migration of immature *E. dohleini*.

Chapter 3 Part A

Activity levels: Behaviour scaled according to energy use

Methods

Octopuses were collected from the Usujiri coast, southern part of Funka Bay, Hokkaido by box trap fishing from April to June of 2001. They were kept in a 13 ton raceway tank at the Field Station for Northern Biosphere Research, Usujiri, Hokkaido. The tank was equipped with an open water system fitted with cooler/heaters and a coral filter. A net covered the tank and a chair and desk were set next to one of the 3 under water windows for observations. Groups of 4 to 6 immature 1- 4 kg octopuses were allowed free access to box homes and each other. A 12:12 light dark cycle was set where indirect light was provided during the day. Octopuses were tagged with coloured beads attached by thread to their mantles that lasted approximately 3 days after which individuals were identified by size, scars and patterns of arm regeneration. Observations were carried out over three 9 day periods. A three day observation schedule was rotated where: 3 hours of observation time was spaced with 1 hour breaks for the duration of the light cycle on day one and the dark cycle on day 2, on day 3 observations were made for 30 min every 3 hours. Behaviours, activities and interactions were record by camera, sketched and described on the spot. In the time between experiments the results were condensed and ordered in to descriptions of activity levels that were then reevaluated during the next experimental time. The result is a list of levels scaled according to increasing awareness, responsiveness, movement and (apparent) energy consumption of the behaviours involved.

Results

Table 3a.1-Behaviour: Scale of activity

1. **Resting:** No movement, except of the mantle where due to light breathing. Skin is wrinkled but papillae and warts are not apparent. Arms are kept around the body and the resulting positions are best described as dense or compacted. Colouring depends on the surroundings, ex. animals near the surface of the aquarium were dark when seen from above and light when seen from below (Fig 3a.1).
2. **Monitoring:** Movement includes the repositioning of arms or mantle that does not result in a change of location. Skin is very wrinkled and papillae and warts are noticeable. The papillae on the top of the eye can extend up to 4 cm from their base. Arms are kept near the body but not piled on themselves (unless the den location requires it), and more surface (skin area) is exposed than during resting. Colouring is often molted but depends on the surroundings (Fig 3a.2).
3. **Shifting:** Shifting of arms and the mantle can result in movements of less than 0.5m. Skin is wrinkled, papillae and warts are present but not extensive. There is space between the arms and they are not held around the mantle. The most common position is best described as thick and long; arms1 and 2 are folded out in front of the head and arms 3 and 4 are folded or wrapped behind the mantle.
4. **Grooming:** Cleaning of mantle; the tips of the arms are trailed along the inside and out side of mantle cavity, water is forcefully blown out of the funnel a number of times. The skin is relaxed but not wrinkled and colouring is fairly light and even.
5. **Aware:** The octopus is visually attentive. Eyes follow movement of other octopus. The whole head (body) often ends up leaning towards the source of activity. Skin is not wrinkled but eye papillae are occasionally noticeable. The head and upper web

and front part of the mantle are usually light coloured (Fig 3a.3).

6. **Active Shifting/ Guarding:** Movement of arms results in repositioning of between 0.5m and 1.5m. This is usually a response to the presence or activity of other octopus. There are two options when an octopus is approached (by another octopus)
 - 1) The octopus moves away from the activity. In this case the octopus shifts so as to be out of the direct line of sight of the other octopus. Colouring remains light and wrinkles are not visible.
 - 2) The octopus makes itself apparent. In this case the octopus shifts in to direct line of sight and faces the opponent. The upper part of the arms R1 and L1 is straightened the front web and arms are lined with bands of light and dark colours and the skin has an a thick almost quilted appearance (Fig 3a.4).
7. **Cleaning:** One or two arms are inserted in to the mantle, there is extensive agitation and the ends can be seen briefly protruding out of the funnel. Arms are twisted in rotating circular movements. Often the twisting of one arm is followed by the twisting of all arms on the same side and then all eight arms at once. These circular twisting movements are followed by very forceful blowing through the funnel and contraction of the mantle cavity, while the arms are (shaken) loose and refolded in to position. The animal is often quite light, (almost white) during this whole time, which can last for up to five minutes. Although cleaning can occur at any time it is regularly done after feeding, or fighting and clouds of cuticle skin cells are often produced (Fig 3a.5).
8. **Feeding:** As these observations were conducted on animals in captivity the true nature of hunting was not seen and so this level only deals with feeding or the handling of food after the hunt or acceptance of food from the handler. A food bulge

under the upper arms and a bright red line that extended from both sides of the eye slit were the most conspicuous details of feeding octopuses.

9. **Ballooning:** Defensive actions. The web is stretched out (i.e., smooth) between the upper part of arms, which are raised vertically from the substrate, suckers on the arms at the base of the web (thus the largest ones on each arm) are attached to the substrate, the part of the arms that extend beyond the web depth are curl behind and away. There are a number different balloon positions. 1) The side closest to the opponent or adversary is raised. 2) This can be followed by the octopus turning to face its opponent and raising its front arms (R and L 1) vertically; the other arms remain down or curled behind the mantle. The web is crisscrossed with a pattern of dark lines that are patterned like burst capillaries, on a light background (Fig. 3a.6). 3) The next stage is for the back arms join in the balloon forcing the mantle up often vertical to substrate. In this pose the second arms create the widest point and all arms are bright white under a translucent mantle that is patterned with intense dark lines (in the same burst capillary fashion) (Fig 3a.7).
10. **Walking:** Linear movement is usually done in a forward motion, however can be in any direction. When all arms are held up off the substrate touching only at web edges movement proceeds rather slowly. This habitually happens when walking is done from the guard or ballooning poses. Slightly faster movement results when L1 and R1 are straightened out and used to explore the upcoming area and suckers at the web edge of L2, L3, R2 and R3 are attached and unattached from the substrate. L4 and R4 are not raised and seem to drag behind or occasionally act as weight bearing points (Fig 3a.8).
11. **Direct action:** When one octopus raises its arm (L1 or R1 but occasionally L2 R2)

towards another octopus (Fig 3a.8) it often stops after making contact with the opponent. If this occurs when the initiator is walking or searching and the receiver is not in its den or in more than an `active` state the touch is usually ignored and both octopuses continue to act as before it happened. However if the touched octopus is in its den and in a state of `active` or above then this is followed by either a fast retreat (Fig 3a.9) or escalates in to a fight.

12. **Searching/ Pacing:** This consists of fast paced walking and is done only in a forward direction. L1 and R1 are extended forward, L3 and R3 are attached and unattached from the substrate along the whole free length (the part below the main web), minus the ends which are kept curled and not used. L2 and R2 alternate between the activities of sets 1 or 3. L4 and R4 are held under the body although the upper part of the 4th legs is occasionally used to raise the entire body higher off the substrate. Searching behind or around objects often escalates to pacing, going back and forth between the same two places (A and B) in the aquarium. Pacing consists of walking in one direction (A to B) and slowly jetting in the other (B to A). On reaching one location (B) the octopus gathers in its legs, pushes up from the substrate and slowly jets back to the initial location (A) (Fig 3a.10). When reaching the initial location (A) the octopus spreads its legs, opening its web and parachutes down through the water column, after reaching the substrate the octopus then walks back to the destination point (B). This activity was recorded for up to an hour and a half and was never performed by two octopuses at the same time (in fact A and B were boundaries set by the presence of other octopuses – usually just out of arm length of where they were located). Skin is smooth. Upper and extended arms, eyes and dorsal mantle are dark. The ventral mantle and the cuticle side of the arms are

slightly lighter.

13. **Jetting:** Quick contraction of the mantle muscle forcing water through a tightened funnel corresponding with all legs pushing off of the substrate results in an upward movement in the opposite direction of the funnel focus. Movement is maintained by a streamlining of the body where mantle and arms elongate lengthwise and occasionally further contractions of the mantle muscles. Skin is very smooth and dark however the area near the eyes and the whole length of second arm can appear lighter (Fig 3a.10).
14. **Inking:** Ink is released from the ink sac attached to the stomach and discharged through the funnel. *E. dofleini* ink contains mucous like strands that appear to hang in the water even after most of the ink has dissipated. Ink is released just before the octopus jets out of the area *E. dofleini* were not seen to hide behind an ink screen.
15. **Fighting:** From the balloon position octopuses approach each other with arms raised and undersides bared. The struggle seems to consist of getting suckers attached to the opponent's web or mantle. Octopuses approaching a fight have bright white and tan coloured areas (app. 2cm²) connected by fine light coloured lines on dark red background. Small almost black splotches of colour are interspersed with this pattern. The full length of the web is stretched out along each arm. During fights both octopus are very dark with tight smooth skin (Fig 3a.11).
16. **Continued Fighting:** If during a fight one octopus is flipped over by a successful hold on the mantle by the suckers of its opponent then this octopus is enfolded in the opponent's web. The arms of the visible octopus create a chokehold around neck of the engulfed octopus. In the aquarium octopus were of similar size, and after

considerable twisting the engulfed octopus always untangled itself and escaped.

Discussion

Captivity severely decreases the variation seen in the skin patterns of *E. dofleini* and renders any comments made here on colour and texture inadequate. However, one point of interest about colour is that the immature octopuses did not regularly turn white during the observed altercations. Turning white has been described as indication of fear and has been reported to happen during fights or when octopuses are disturbed (Kyte and Courtney 1977; Hartwick *et al.* 1979). However during the fights observed here octopuses alternated between very dark or molted colouring. There was only one observation of an immature octopus turning white. The observation was made when a resting octopus was pulled out its den by slightly larger octopus. The victim of this altercation was pure white and took a considerable time to return to its regular pattern of behaviour. Juvenile octopuses (under 400 g) however regularly lightened, becoming almost transparent when their tanks were being cleaned.

In the field investigation has revealed smaller *E. dofleini* under the webs of larger ones. The larger octopuses seem to be feeding on the small octopus (all small octopus found to date in this circumstance have been dead). It is possible that the small octopuses died after being engulfed and suffocated as has been reported to be the outcome of altercations (Kyte and Courtney 1977). However the greatest percent of altercations in the aquarium were between similarly sized animals and seemed to be conducted until dominance was established.

Two octopuses were never found in the same area at the same time (unless fighting), and homes were spaced so the octopuses were as far as possible and out of the direct line of sight of each other. However when two octopuses came in to contact with each other there were various results: if both animals were searching the result was usually a fight, which was won by either the larger or more aggressive octopus (the octopus that initiated the fight). If one octopus approached another's den or intentionally made direct contact with another responses ranged from the fleeing of either octopus to continued fighting between them. When one octopus became aware of the presence of another animal (usually due to the increased activity of the other animal) they often moved out of sight. This was done by moving to the other side of a rock, pipe or wood box or by retreating in to a den. On a number of occasions octopus physically pushed rocks in to the opening of the wooden box dens and retreated behind this barrier. However octopus did not simply ignore, retreat or fight when confronted with the activity of others. There seemed to be some amount of acceptance of the presence of others. This was most noticeable in the reaction of the octopus when one of them began to pace. The presence of other octopus set the boundaries for pacing area but this area was usually set after rearrangement of the octopus. For searching to escalate in to pacing the boundary octopuses needed to 'give way' during the initial searching period. Octopus on the wall moved up or down (depending on if pacing was done at the surface or along the bottom) and octopus in boxes retreated deeper inside that allowed the pacing octopus greater freedom. If this did not happen then searching ended with out escalating to pacing or fights resulted.

Fig 3a.1 Resting

A 3 kg octopus rests
out in the open



Fig 3a.2 Monitoring

A juvenile octopus monitors from its den



Fig 3a.3 Aware

A 2.3 kg octopus becomes
aware and leans towards the activity



Fig 3a.4 Guarding

A 2 kg octopus stands guarding its den

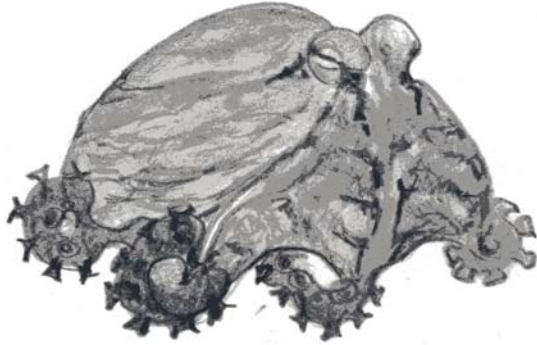


Fig 3a.5 Cleaning

An immature octopus pales as it
twists its arms as it cleans

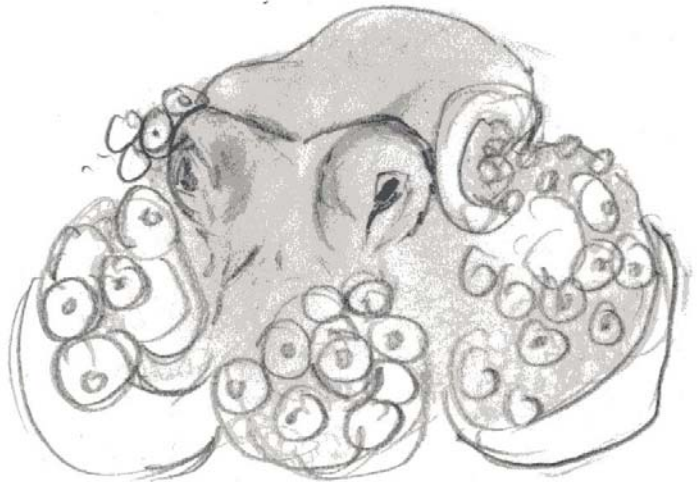


Fig 3a.6 Ballooning

A 3kg octopus faces its opponent and raises both dorsal arms

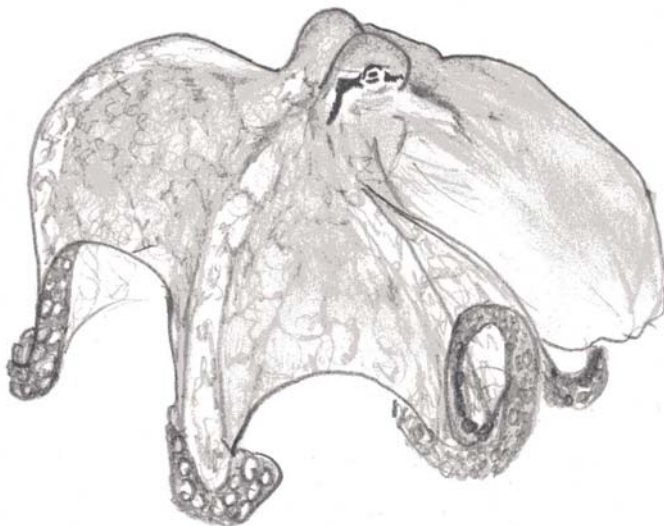


Fig 3a.7 Full Balloon

A 2 kg octopus goes in to a full balloon by spreading out all its arms

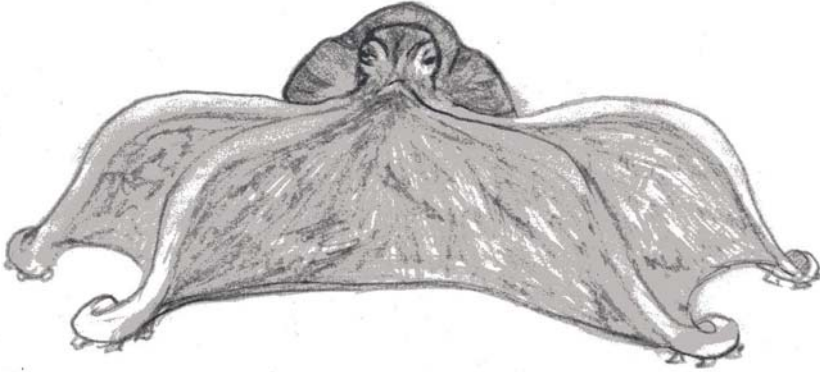


Fig 3a.8 Walking, reaching

A immature octopus reaches out its dorsal arms as it walks forward

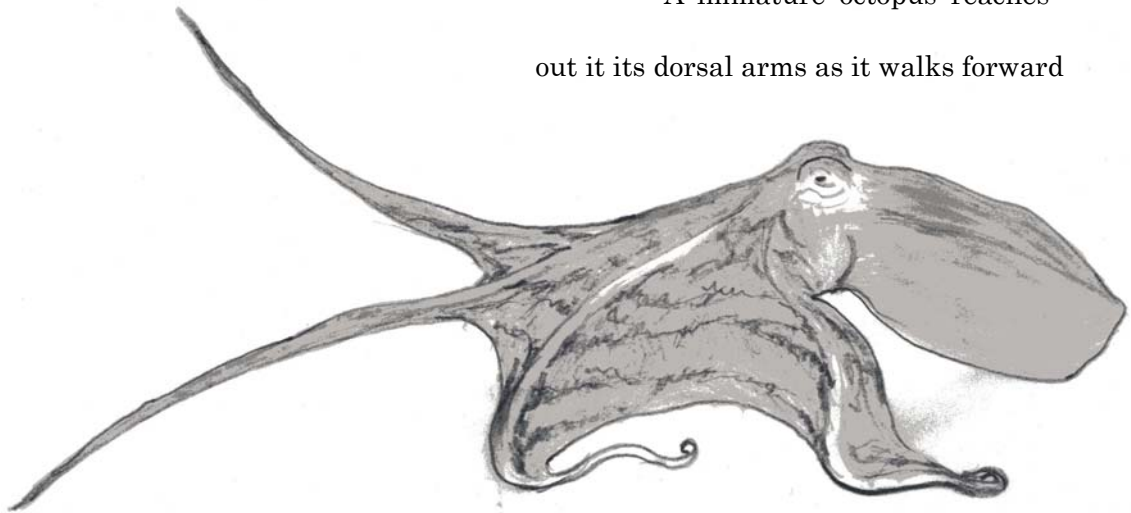


Fig 3a.9 A fast retreat

A octopus turns almost maroon as it flees after reaching out to an unfriendly foe

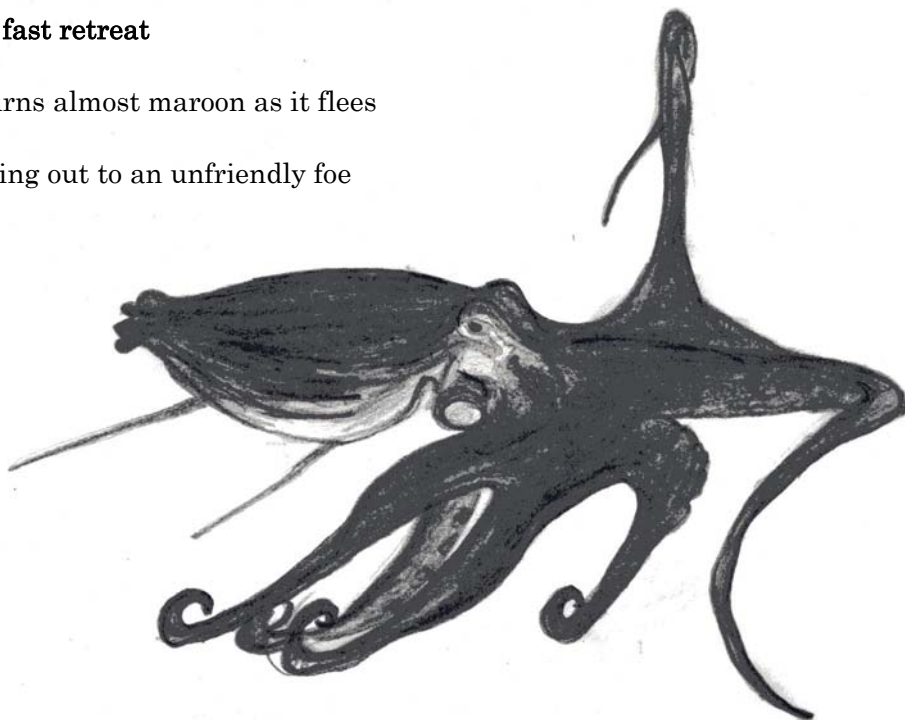


Fig 3a.10 Jetting

Pushing off from the substrate a 3.5 kg octopus jets up and away

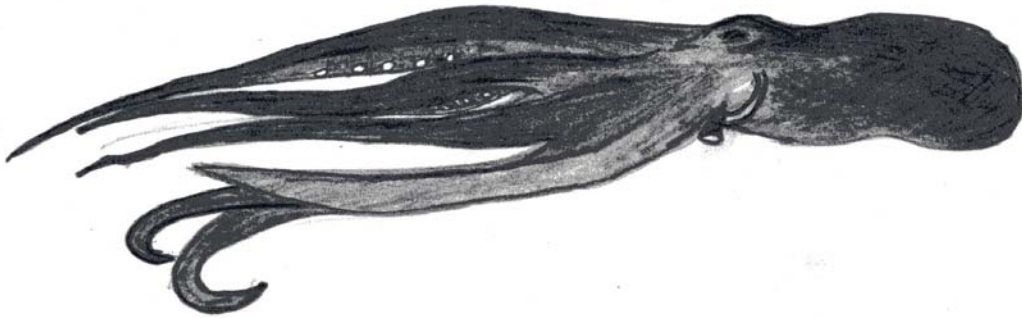
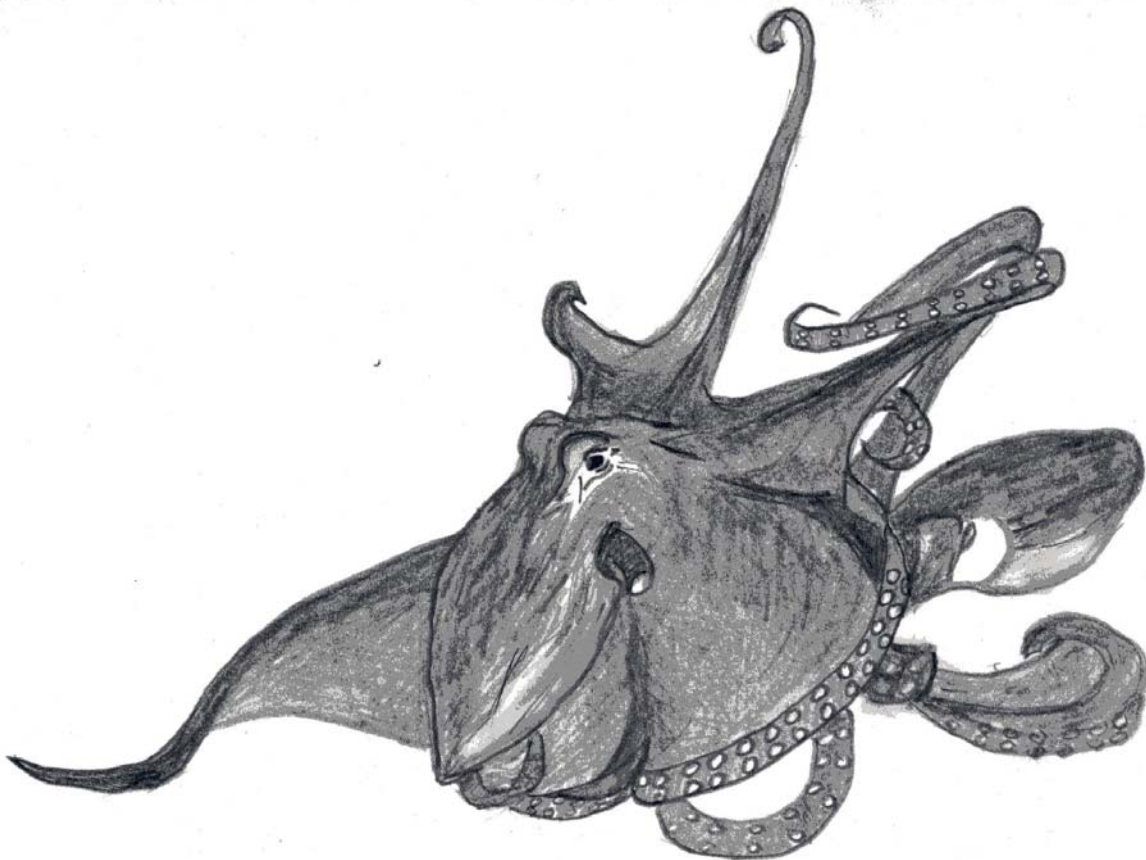


Fig 3a.10 Fighting

16 arms stretch out across the aquarium as two immature octopuses fight



Chapter 3 Part B

The effects of temperature and relative size on space use and interactions

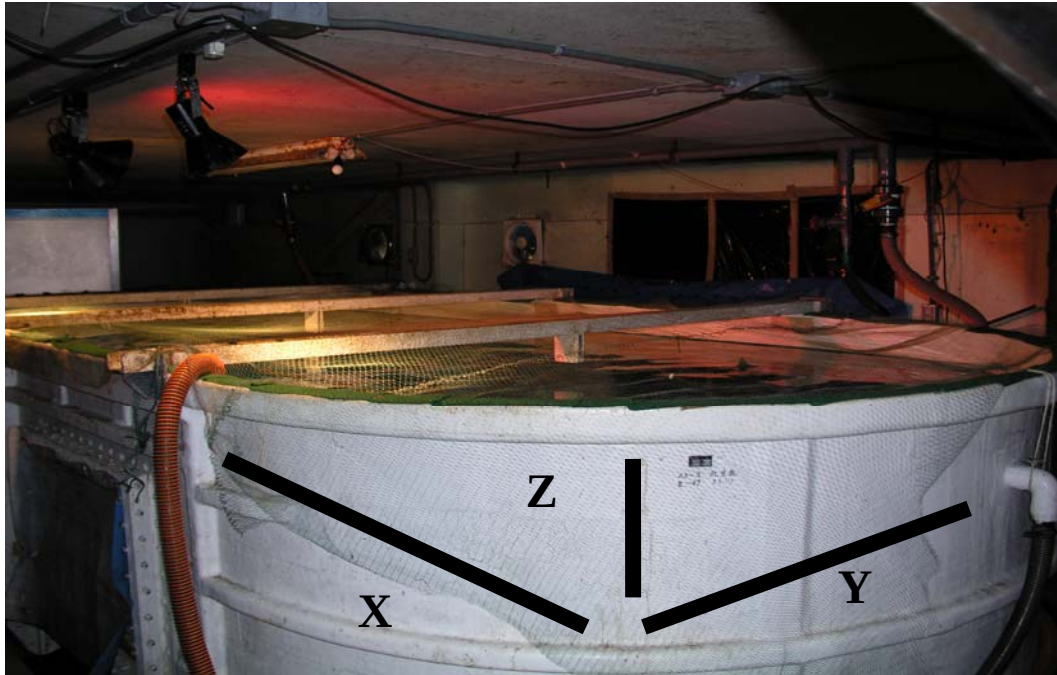
Methods

Octopuses were collected from the coast of Usujiri, in the southern part of Funka Bay, Hokkaido by SCUBA and box trap fishing from October to December 2001. They were kept separate and monitored for 48 hours before being used in the following experiments. An open water system fitted with cooler/heaters and a coral filter tank supplied a 13 ton raceway tank housed in the center on northern biosphere research, Usujiri, Hokkaido (Figure 3b.1- Raceway tank). The tank was covered by a net and a video camera recorded individual locations and clear fights between octopuses on the hour for 10 days at 2, 5 and 7°C. Clear fights were considered to be altercations that involved only two octopuses and resulted in an obvious win/loss (Figure 3b.2a-c- a clear fight). Groups of 4 and 5 octopuses were allowed free access to box homes and each other. A 12:12 dark light cycle was set by lights that provided indirect light during the day. Octopuses were weighed before and after all experiments.

Space use was broken down in to graphable components of X, Y and Z (Fig 3b a, b). Lateral positions were recorded by X and Y and used to define prime locations, and where the most time was spent. The .03m locations were ranked according to the relative percentage of time spent in each. The area where the octopus spent the most time was considered to be the 1st home. The area with the next highest frequency of time spent at it was considered to be the 2nd home. Time spent away from these two areas was considered time spent away from home. Vertical movements or positions given by Z were used to find how time was spent in vertical space. Time spent on the

bottom was compared to time spent at the surface or in the water column. Interactions were defined by the number of fights between octopuses. Interaction potential was assessed by calculating the distance between 1st homes. Activity levels were assigned according to the scale given in Part B. Daily averages of activity in the aquarium were calculated by averaging the activity of all octopuses in the aquarium during each of the 10 days of the experiment. Finally statistical tests combined all the data to assess the interaction between all factors.

Fig. 3b.1 - Race way tank



The 13 ton raceway tank, used for captive experiments. Located in Usujiri, Hokkaido at
The Center for Northern Biosphere Research,

Fig. 3b.2 - A clear fight

A

Approach;

The initiator

is seen here

on the left



B

The fight;

Arms are raised,

suckers are exposed,



C

Winners Circle

The winner stays in area

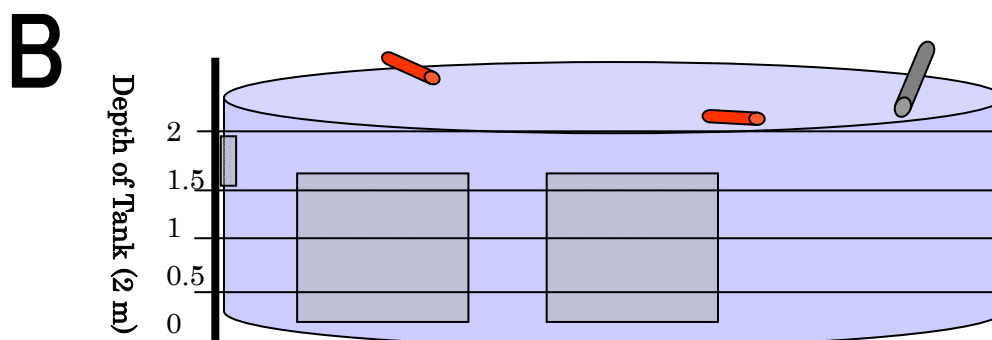
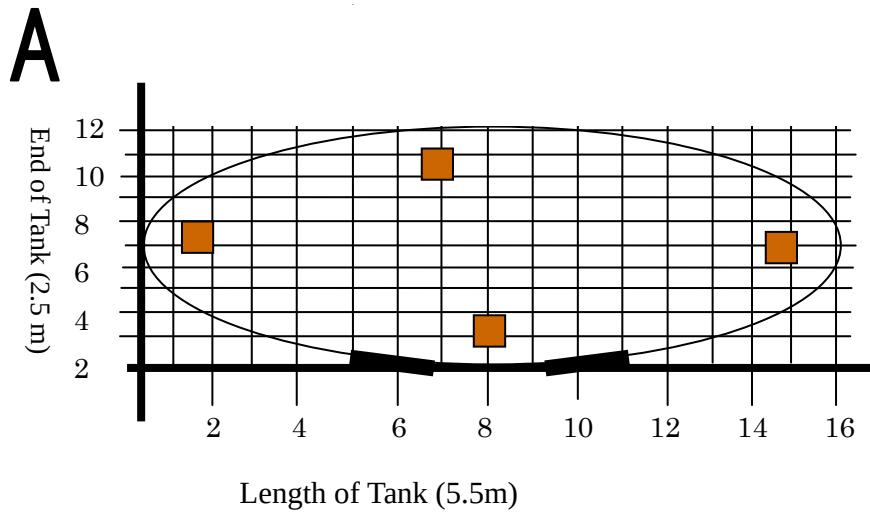
where the fight was.

Spreading out its arms as

if to claim the new area



Fig. 3b.3 – X, Y and Z in the aquarium



The tank was partitioned in to areas of approximately 0.3 m². Octopus locations were specified according to X (length of aquarium), Y (end of tank) and Z (depth of tank). The yellow boxes in A represent empty wood boxes (used as homes). In B the red pipes represent inflow water, and the grey pipe overflow drainage (all pipers were covered by netting as was the entire tank to prevent octopus from escaping).

Results

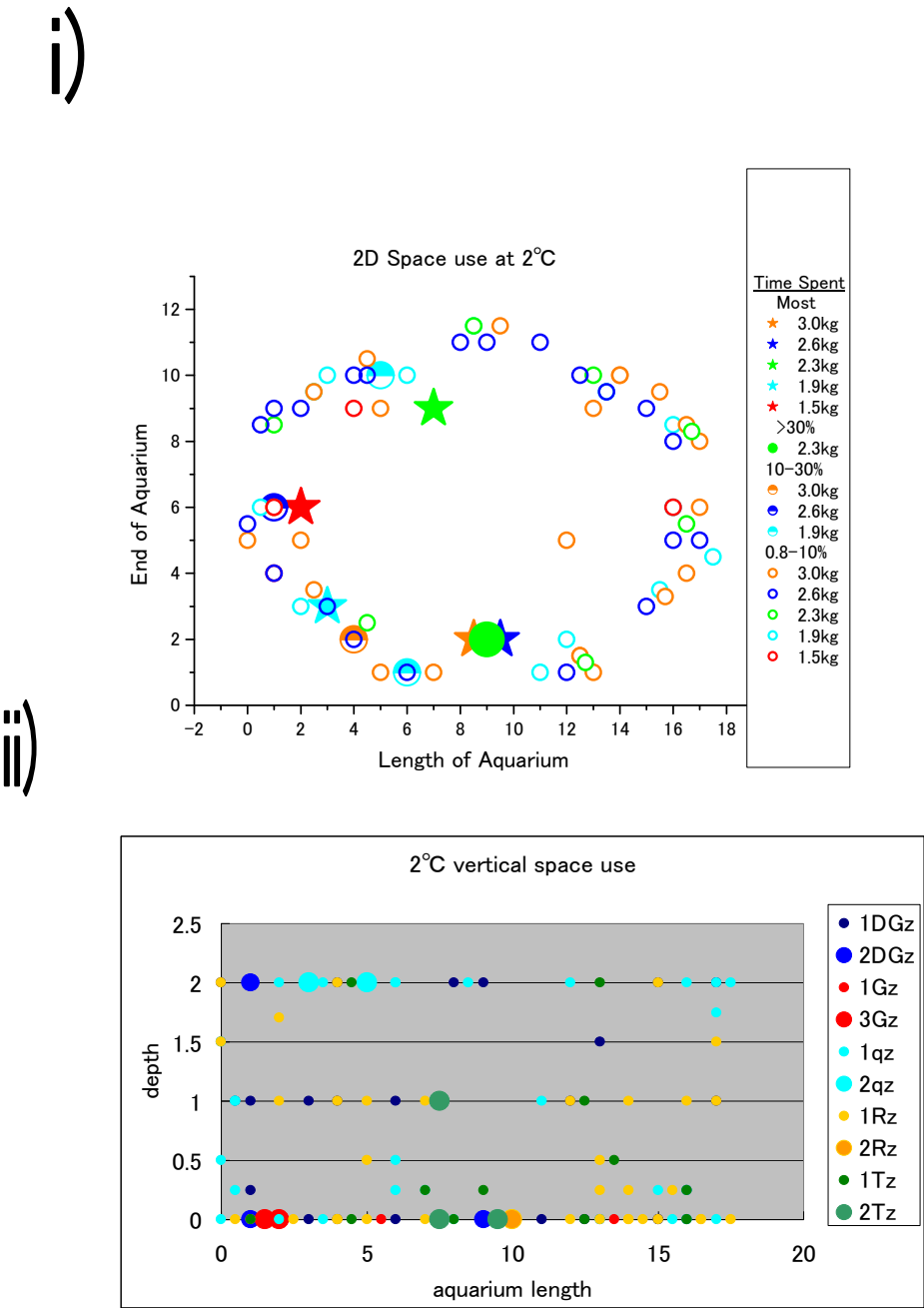
Positions that equaled more than 0.8 % of the total time recorded for the octopuses at each temperature is shown in Fig. 3b.4 a i) ii), b i) ii), and c i) ii). At all temperatures octopuses spent more time in protected areas (i.e. along the walls on, in or near boxes) than in the open (Protected avg. 94.31 ± 6.86 SD, Open avg. 5.68 ± 6.86 SD, Friedman $p < 0.00018$). The lower the temperature the more time octopuses spent at home. However, this was only proved between 5°C and 7°C (5°C avg. 25 ± 4.1 SD 7°C avg. 46 ± 16 SD, Kruskal Wallis $p = 0.0468$) as the variation at 2°C made the combination of all temperatures insignificant.

The larger the animal the more time spent on the bottom (Freidman $p < 0.00742$). As the temperature increase more time was spent at the surface (Freidman $p < 0.02014$).

However the greatest difference was with smaller animals (Freidman $p < 0.00571$) and was most prominent at 7°C (Wilcoxon $p = 0.027715$).

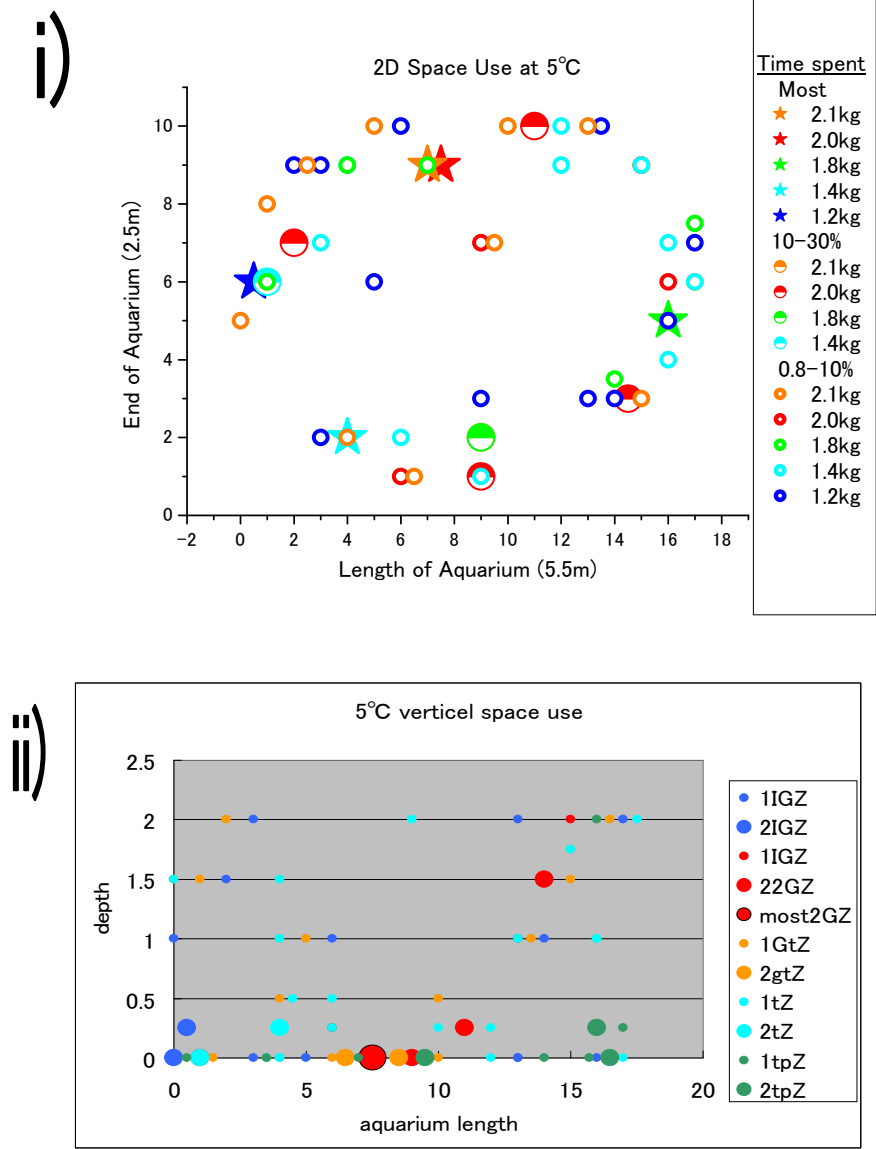
An increase in temperature coincided with an increase in number of fights per animal (Kruskal Wallis $p = 0.0080$). At 7°C octopuses were equally spaced out in the aquarium (Wilcoxon $p = 0.592984$). This was not so at 2 and 5°C where unequal spacing lead to significant difference between the size of individuals and distance of next 1st home (Wilcoxon 2°C $p = 0.027715$, 5°C $p = 0.035700$). Activity lessened over time in captivity. Increased with temperature and peaked during feeding. After 4 days activity levels stabilized, there were fewer interactions and general activity decreased (Fig 3a.5). The higher the temperature the more fights and the greater the distance between 1st Homes (Freidman $p < 0.01730$).

Fig. 3b.4a - Space Use in Captivity at 2°C



The positions that equaled more than 0.8 % of the total time recorded for the five octopuses kept at 2°C. Stars in (i) show core areas where the most time was spent. Note the variation, the 1.5 kg octopus (red) stayed in a den almost the entire time and the 2.3 kg octopus (green) spent considerable time moving around.

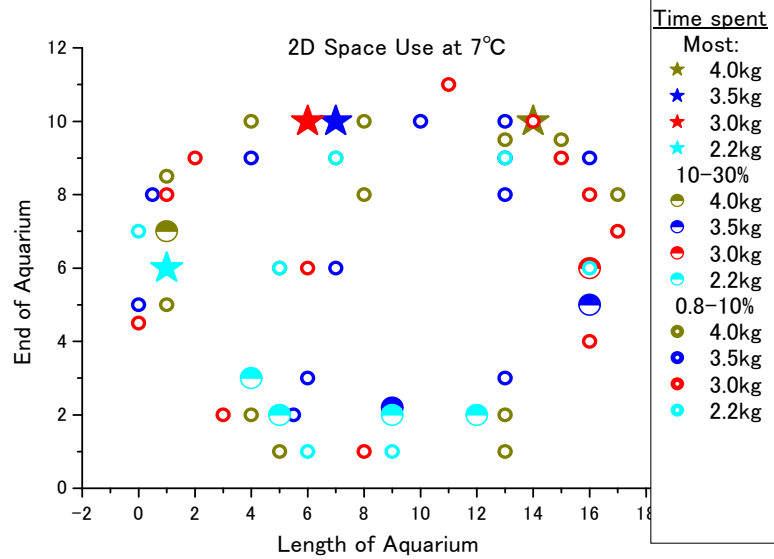
Fig. 3b.4b - Space Use in Captivity at 5°C



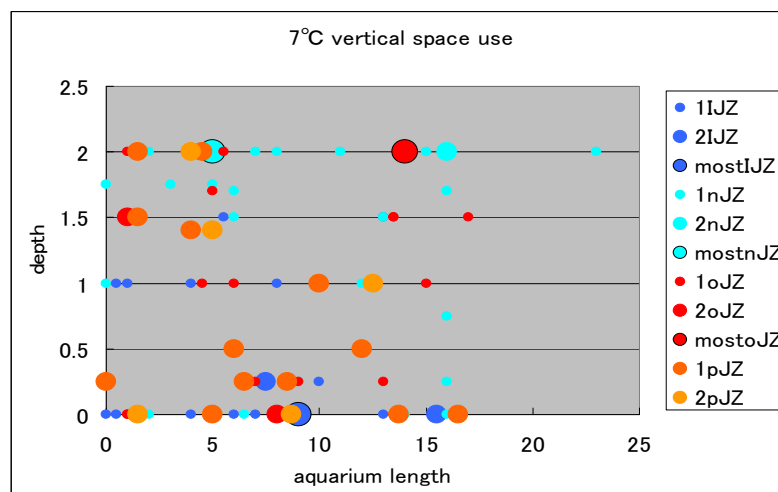
The positions that equaled more than 0.8 % of the total time recorded for the five octopuses kept at 5°C. Stars show core areas where the most time was spent.

Fig. 3b.4c - Space Use in Captivity at 7°C

i)

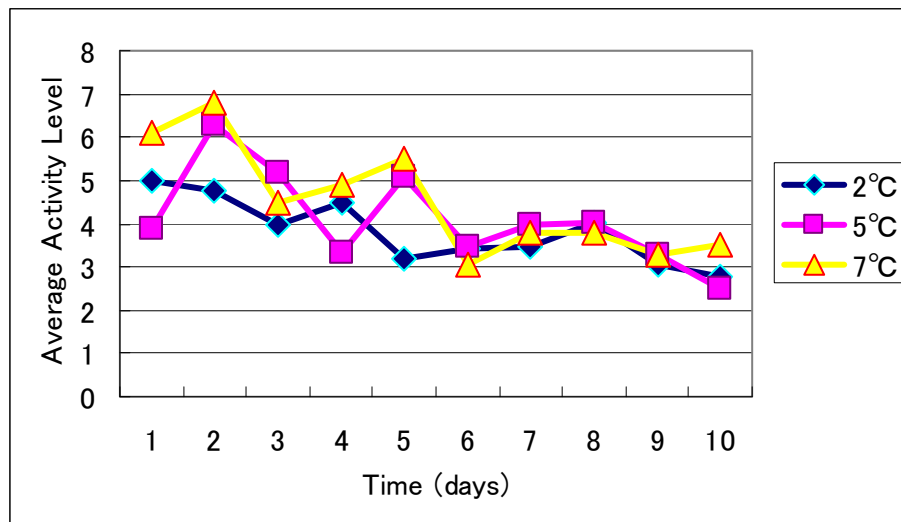


ii)



The positions that equaled more than 0.8 % of the total time recorded for the four octopuses kept at 7°C. Stars show core areas where the most time was spent. Note that no two animals considered the same space as their first home (an increase in the distance between first homes).

Fig. 3b.5 - Average daily activity levels in captivity



Activity was given value according to the scale presented in Part A and calculated for the aquarium per day. At all three temperatures activity decreased over time, possibly due to acclimation (to the surroundings and other occupants). Increases in activity were seen with temperature and feeding.

Discussion

Octopus are not found in Funka Bay at 2°C. The extreme low temperature was used to see what would happen below the natural limit of distribution. The highly individual behaviour seen here may have been a result of the stress put on the animals by the unnatural temperature condition.

In captivity it is difficult to draw a line between territoriality and hierarchy. Regardless of temperature two octopuses were never at the same place at the same time unless they were fighting. This confirms that they are solitary animals. Octopus remained out of direct line of sight of each other, unless entering in to an altercation. However at lower temperatures octopus seemed to time-share favored locations. This sharing was done less as temperature increased. This time-sharing or toleration of the presence of other octopus may have been an artifact of supplying ample food and not of social acceptance. In the field where food is not supplied and may be less abundant the presence of others may be less tolerable.

Temperature was shown to influence space use that in turn influenced interactions between octopuses. Size was more important and territorial behaviour; fights between octopus and spacing of first homes, increased with temperature. Showing that even with only a 2° change in temperature there is increase size defined dominance. Seasonal migration patterns in southern Hokkaido where small octopuses come into shore earlier and stay later may be a reflection smaller octopus pushing their physiological limits further as they try to escape interactions that they are sure to lose.

Chapter 4

Space use and activity of immature *E. doffleini* in the field

Abstract

The space use and activity of *E. doffleini* in Funka Bay was tracked by radio acoustic pingers (RAPT system, Vemco Ltd) that reported positions within a 3.14km² area. Here the home ranges, daily activity patterns and the temporarily localized social structure are reported.

Introduction

Mather *et al.* (1985) advocate the detailing of movements and interactions of an animal as fundamental to describing its way of life. Studies of space use and activity of individuals reveal social structure by detailing the size and time spent in individual home ranges. The social structure of solitary animals has been describes as a choice between territorial and dominant (Mather *et al.* 1985). Reports have shown a range between patterns; territorial (Woods 1965), non interactive (Altman 1967, Kayes 1974) and dominate (Yarnall 1969, Mather 1980).

The three social patterns suggested by the few experiments conducted with octopuses Asocial, Territorial and Dominant, are distinguished by their differences in spatial organization and intraspecies relations. A-Social patterns occur when awareness of another's presence leads to an establishment of area boundaries. Asocial animals neither interact nor share space. Territorial and Dominant based structures require the communication of the underling factors on which they are based. Factors for the bases

of social structure can be individual animal size, strength, ability or age (this is not a complete list by any means). Territorial patterns are spatially similar to Asocial patterns however area boundaries are enforced through direct interaction. Territorial animals interact so as to not share space. Dominant patterns occur when animals of different rank live in a common space. Dominant animals interact (in order to define rank) and share space (Nobel 1939; Mather 1980; Mather *et al.* 1985; Stamps 1994).

Regardless of social structure the area used by an individual octopus, referred to as a home range depends on an individual's size, food availability, the availability of appropriate dens, and environmental conditions. The amount of space used by octopus depends on the individual's food needs. Large individual size or scarcity of prey items lead to larger foraging areas. Geographical and environmental features of the area influence spacing and behaviour. Fewer possible den sites lead to longer residence times and greater protection of inhabited dens. Behaviour and growth, both influenced by water temperature affect the residence time of a given den. Activity levels influencing the percent of time spent moving and growth rates dictating the speed with which a larger den is required. Life stage may have the strongest influence on space use; Paralarvae do not require a den, exponentially growing juveniles quickly need a new one. Immature migrating animals may hold dens for a very short period of time, while breeding females occupy a single den as they care for attached eggs until they die. In Asocial and or Territorial structures the ability to avoid others while moving within an area would also be of influence as would any thing that would change the clarity of their presence or defense signals such as visibility (Nobel 1939; Mather 1985; Stamps 1994).

Enteroctopus dofleini reside in dens to which they may show short (Mather *et al* 1985) or long term (Hartwick *et al* 1981) fidelity. From these dens individuals may conduct foraging expeditions returning with food items thus lessening time in the open and creating midden piles at the entrance of the dens (Vincent *et al.* 1998). *E. dofleini* has been described as a refuging predator, basing its activity on a fixed point in space (Hamilton and Watt 1970; Hartwick *et al* 1981) and a solitary, temporarily - localized predator (Mather *et al* 1985).

Here the space use and activity of *E. dofleini* in Funka Bay was tracked by Radio Acoustic (RAPT) pingers that reported locations within a 3.14km² area. The time spent in and sizes of home ranges are discussed, as are the daily activity patterns, and the implication this has on social structure of the octopus in Funka Bay.

Methods

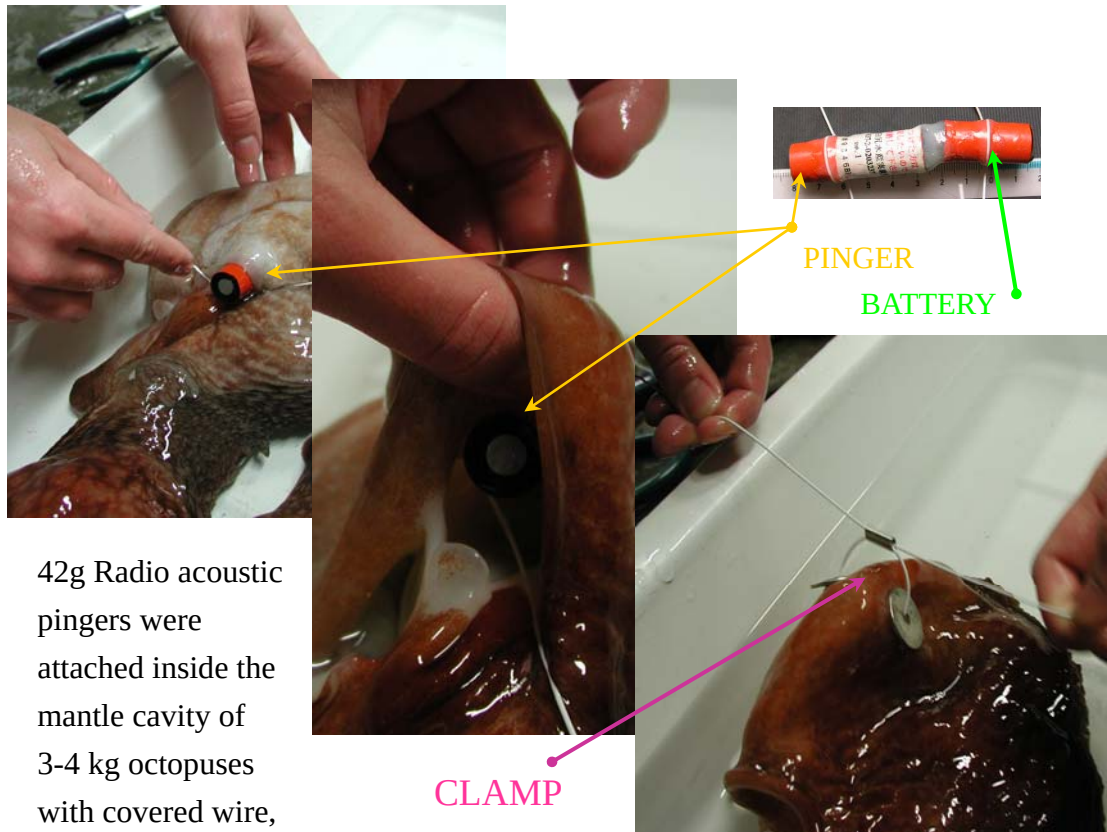
Anaesthetized in 2% alcohol (in aerated sea water) 3-4 kg immature *E. dofleini* were tagged with 42 g acoustic pingers. The pinger (radio transmitter and lithium battery) was inserted in to the mantle cavity and attached by piercing the muscle with covered wire. A circular loop was created with the pinger on the inside of the mantle and a clamp on the outside. Wire reinforced silicon washers were replaced with full metal washers after laboratory tests showed the degradation of the silicon with a combination of the octopus movement and salt water (Fig. 4.1- Octopus and Tag).

Octopuses initially attempted to dislodge the tag and refused proffered food for an average of 24 hours. Pre-tagging behaviour patterns and feeding levels resumed within 3 days and lasted for at least 2 weeks in the laboratory. After 2 weeks in captivity,

activity decreased and feeding levels dropped from average laboratory feeding rates of 3.25 % to 1.85 % (% of body weight fed every 3 days). This coincided with ever enlarging holes around the tag fastenings. No octopus died during the month that it took before tags dropped out. Holes in de-tagged octopus healed within 2 months. However skin did not close over these wounds and the white mantle muscle visible until the end of the experimental time. Activity and feeding returned to normal laboratory levels within 48 hours. Eight locally caught (by SCUBA and box trap) immature octopuses were successfully tagged. Each octopus was released in to the field separately (Funka Bay, Hokkaido, Japan) after a 24-48 hour recuperation period.

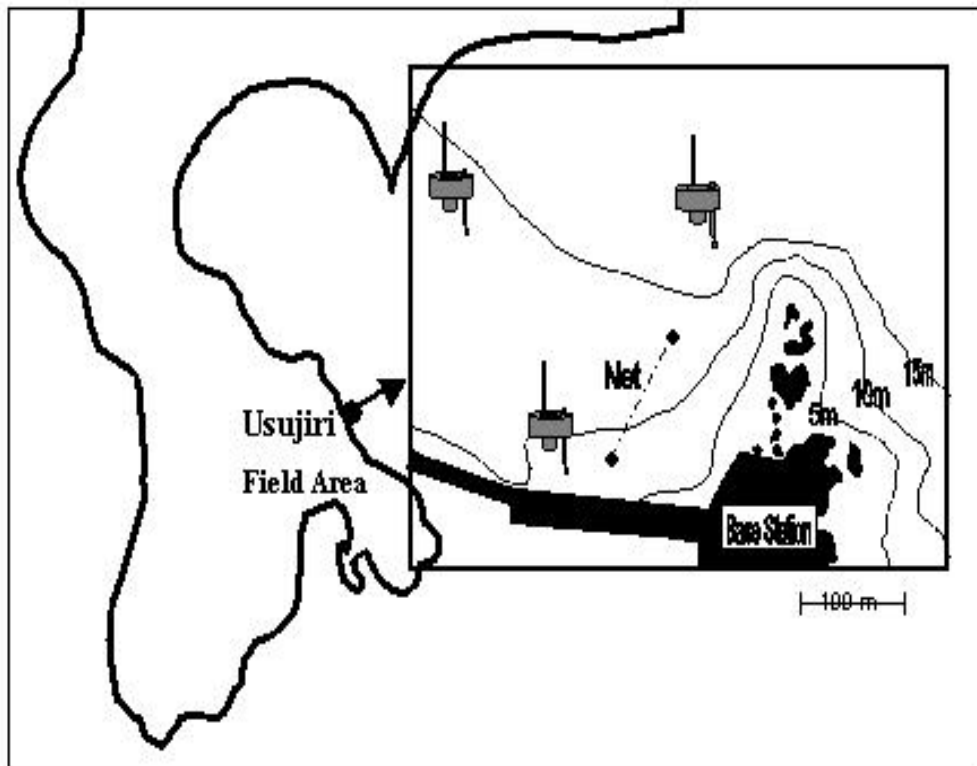
Three radio acoustic buoys (Vemco Ltd.) were set in a 300 m equilateral triangle less than 500 m offshore (Fig 4.2 - RAPT Field). The buoys were moored with lengths of 3 inch rope that matched low tide height. The central part 1.5 m of rope was removed and replaced with 2 separate elastic cords (bungee cord) that is sold to tow cars of up to 1.3 tons. The bungee cord stretched to accommodate high tide. This set up reduced lateral movement that would have been caused inelastic moorings measured to high tide height (Fig.4.3 - Buoy anchoring). Buoys recorded and uploaded pinger arrival times to the base station constantly for the duration of the field experiment, which ran from April 8th to June 23rd 2003. Data showed octopus positions within a 1 km radius of the centre of the triangle.

Fig. 4.1- Octopus and Tag



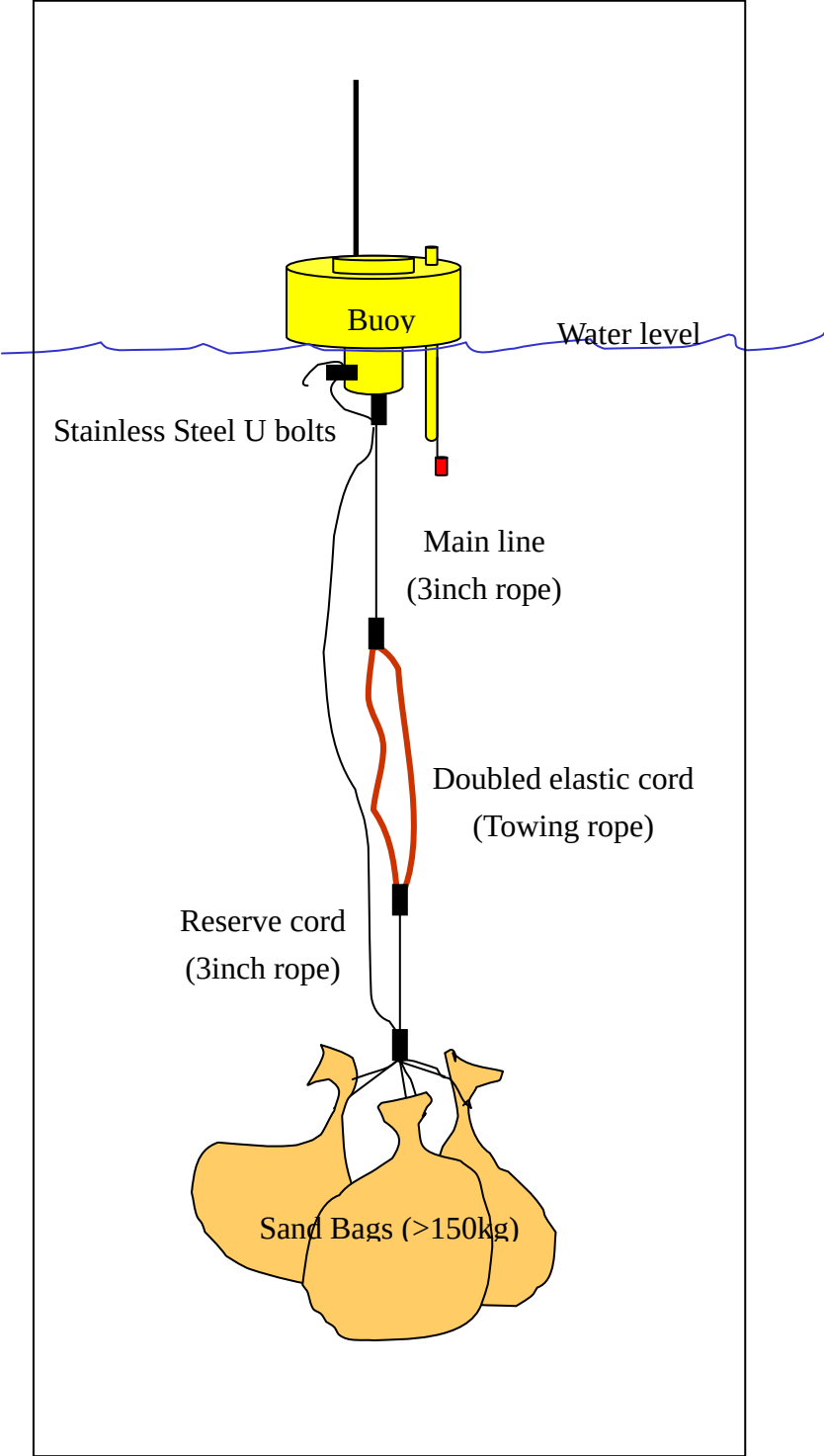
42g Radio acoustic pingers were attached inside the mantle cavity of 3-4 kg octopuses with covered wire, clamps and washers

Fig. 4.2 - RAPT field



Three radio acoustic buoys (Vemco *Ltd*) were set in a 300 m equilateral triangle less than 500 m off the coast of Usujiri.

Fig. 4.3 - Buoy anchoring



Results

The released octopuses initially stayed in the rocky shoreline moving down and in range of the hydrophones within days. Seven of the eight octopuses stayed in the 3.14km² area around the buoys the first two weeks after being released (Figure 4.4 Composite of the initial locations).

Clear consistent tracks from five octopuses showed that individual octopuses spent an average of 83% of the recorded time within areas of 250m² and 35% in much smaller 46 m² areas (Table 4.1- Home and core areas; Appendix A- Home and core areas). All five octopuses showed very different movement patterns (Table 4.2- Summary of octopus movement; Table 4.3 - Significance of movement between octopuses). Significant difference was seen between time of day and week of experimentation (Table 4.3 -Significance of movement vs. day and week) resulting in a division between week one and two and time of day; represented as Dawn (3:00-7:00), Day (7:00-16:00), Dusk (16:00-21:00), Night (21:00-3:00). The percent of time spent by individuals in home ranges did not differ significantly between weeks one and two. There were examples of home ranges growing smaller, larger or staying the same during the second week. The average time may have been reduced by octopus that had more than one home range, or by those that spent time in one area before settling in another range. Individual octopus moved around their home ranges differently however they all had areas of peak use, referred to as core areas. There was a significant difference in the percent of time spent in the core areas between week one and two. Daily activity pattern changed considerably in the second week, resulting in a general increase in the time spent in the core area during dawn, and less during the day and dusk. Octopuses were most active

and moved around the greatest distances during the day (Table 4.5 - Home ranges and core areas; Fig 4.5 – 3D plots of octopus movement; Appendix B).

The depth plots of three of the octopuses (Fig. 4.6 - RAPT depth plot) showed movements (of less than 3 m) out of sequence with the tide and each other occurring at the same time for a mean of 4 consecutive days. Observations using SCUBA revealed that the octopuses were moving toward a gill net strung soon after the experiment began. The octopuses were observed to climb the net and eat fish entangled in it. Three of the five octopuses that stayed in the area for the first two weeks after being tagged fed on the net for more than 3 days, and one octopus continued this behaviour for the duration of the tagging experiment (Fig 4.7a,b- Octopus on a gill net).

Table 4.1 – Mean home and core areas and the percent of time spent in them

region	week	area (SD)	%(SD)
Home	1	279.97 (174.77)	83.31(9.67)
	2	224.95 (219.45)	84.39 (8.32)
	both	252.46 (197.65)	83.86(8.92)
Core	1	42.49 (81.88)	39.26 (18.12)
	2	49.52 (147.68)	30.74 (14.28)
	both	46.01 (117.84)	35.00 (16.66)

Table 4.2 - Summary of octopus movement

1	By the 2 nd day the octopus had moved to 10 m, it took consistent dusk walks to 8 m coming back each night, on the 10 th day it moved to 12 m.
2	By the 6 th day the octopus was at 5 m. On the 7 th day it moved to 6.5 m where it stayed within 50 m where it moved around mostly at night and in the early morning
3	By the 4 th day the octopus was at 8 m. It may daily migrations up the water column to 6 m for 5 days, after this they occurred with less regularity
4	On the 3 rd night the octopus moved to 10 m. there was very little x or y movement however each of the first 4 days of being at 10 m it migrated back to 7m
5	On the 3 rd evening the octopus moved to 7 m from which it only moved around during the day. There was an absence of signals during the night, likely due to the octopus retreating in its den.

Table 4.3 – Octopus movement vs. Each other

	Dawn	Day	Dusk	Night
x	H (4, N=1659) =86.1119, p=0.000	H (4, N=3450) =352.0132, p=0.000	H (4, N=1414) =54.33974, p=0.000	H (4, N=1930) =20.0279, p=0.0005
y	H (4, N=1659) =197.2141, p=0.000	H (4, N=3450) =638.6220, p=0.000	H (4, N=1414) =193.6979, p=0.000	H (4, N=1930) =58.4834, p=0.000
z	H (4, N=1659) =102.6053, p=0.000	H (4, N=3450) =331.4897, p=0.000	H (4, N=1414) =104.7607, p=0.000	H (4, N=1930) =55.7912, p=0.0005

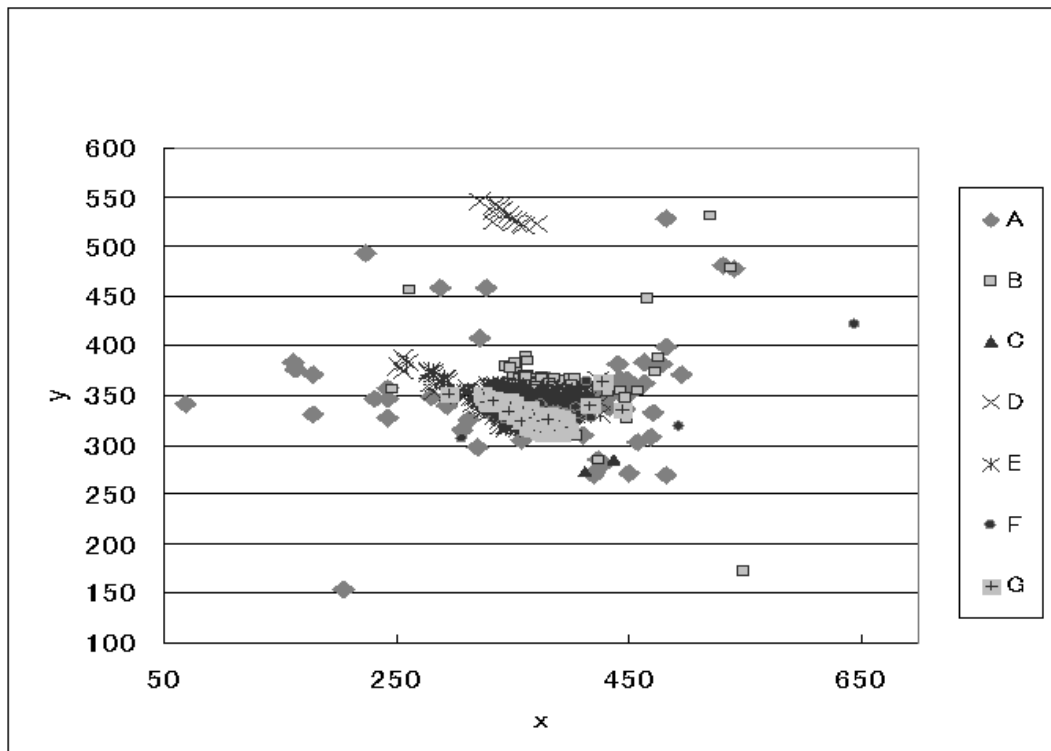
Table 4.4 Octopus movement vs. Day and Week

	41	45	48	49	50
movement in the X plane vs. Time	n.s	H (3, N=1515) =65.526, p=0.000	H (3, N=1658) =18.47952, p=0.0004	H (3, N=2746) =48.29413, p=0.000	H (3, N=2121) =49.0961, p=0.000
movement in the Y plane vs. Time	n.s	H (3, N=1515) =136.7576, p=0.000	H (3, N=1658) =115.3085, p=0.000	H (3, N=2746) =59.79351, p=0.000	H (3, N=2121) =44.66372, p=0.000
movement in the Z plane vs. Time	n.s	H (3, N=1515) =32.51210, p=0.000	H (3, N=1658) =15.57799, p=0.0014	H (3, N=2746) =90.57761, p=0.000	n.s

Table 4.5-Home ranges and core areas

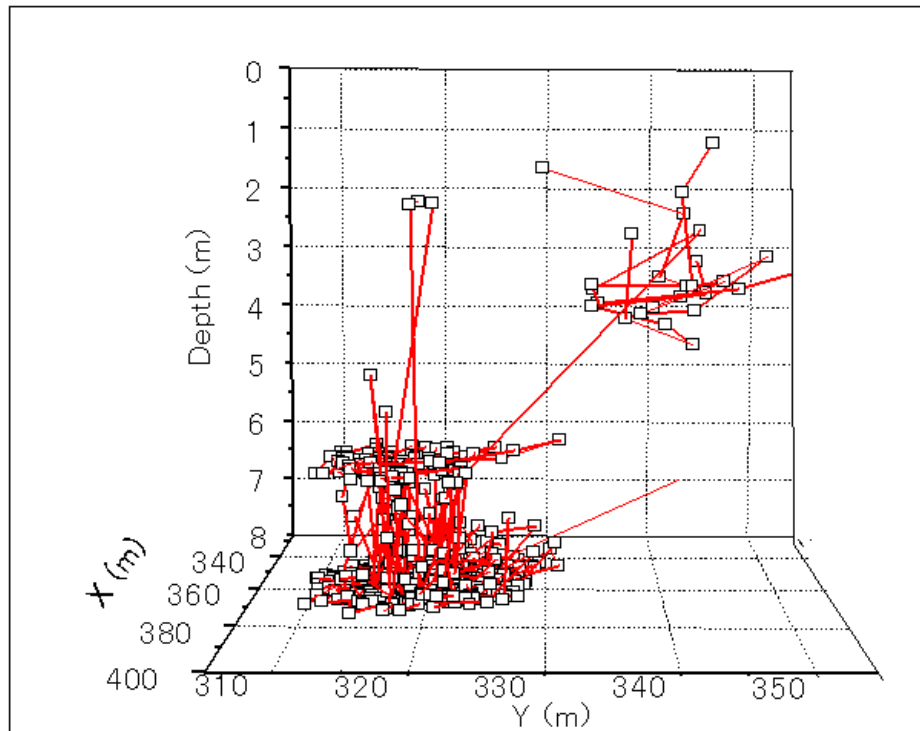
	41		45		48		49		50		Average	
region	area	%	area	%	area	%	area	%	area	%	area	%
home	181.427	64.5	131.947	74.45	106.814	84	164.934	76.5	379.347	67.8	192.894	73.45
core	4.71239	24	23.5619	52.25	9.42478	39.3	7.85398	36.25	3.92699	25.15	9.89602	35.39
home	339.292	62	75.3982	86	183.783	86.5	47.1239	73.5	94.2478	82	147.969	78
core	81.6814	40.5	18.8496	50	4.71239	22.5	2.35619	30	2.35619	36	21.9911	35.8
home	765.763	75	208.916	90.5	289.027	91	339.292	90	534.071	87.4	427.414	86.78
core	259.181	56.85	51.8363	57.5	18.8496	40	47.1239	46	31.4159	44.45	81.6814	48.96
home	842.732	76.85	296.881	93.5	226.195	92	132.732	89.5	122.522	82	324.212	86.77
core	98.9602	32.15	11.781	34.95	3.92699	23.8	0.7854	12.4	0.7854	11.4	23.2478	22.94
home	559.989	68	172.788	95.5	150.796	84	240.332	87	282.743	91.85	281.33	85.27
core	21.9911	31.55	9.42478	46.5	11.781	37	3.14159	24.7	282.743	91.85	65.8164	46.32
home	775.973	72.2	164.934	92	200.277	91.45	125.664	89	133.518	81.95	280.073	85.32
core	648.739	27.5	6.28319	25	3.14159	22.65	0.7854	11.1	0.7854	15	131.947	20.25
home			112.312	94.5	175.929	89.5	197.92	92	325.155	79.5	202.829	88.875
core			4.71239	20.5	3.14159	26.65	11.781	37.5	0.7854	8	5.10509	23.163
home			141.372	93.25	201.062	87.5	49.4801	84.35	120.951	88	128.216	88.275
core			32.9867	58	5.49779	30.8	11.781	57	4.71239	43.35	13.7445	47.288

Fig. 4.4 - Initial location of tagged octopuses



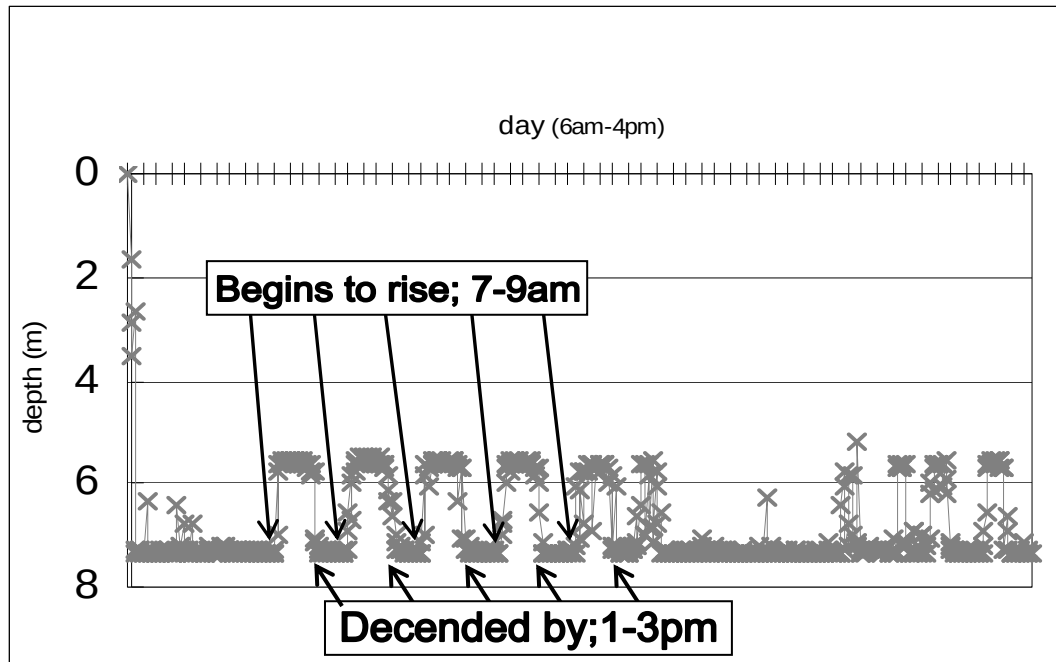
A composite of the X and Y positions of the first two weeks of the octopuses (a-g) tagged with RAPT pingers. Octopuses were released separately between April and June and did not all overlap in time, just choice of initial location.

Fig. 4.5 -3D positions of a tagged octopus



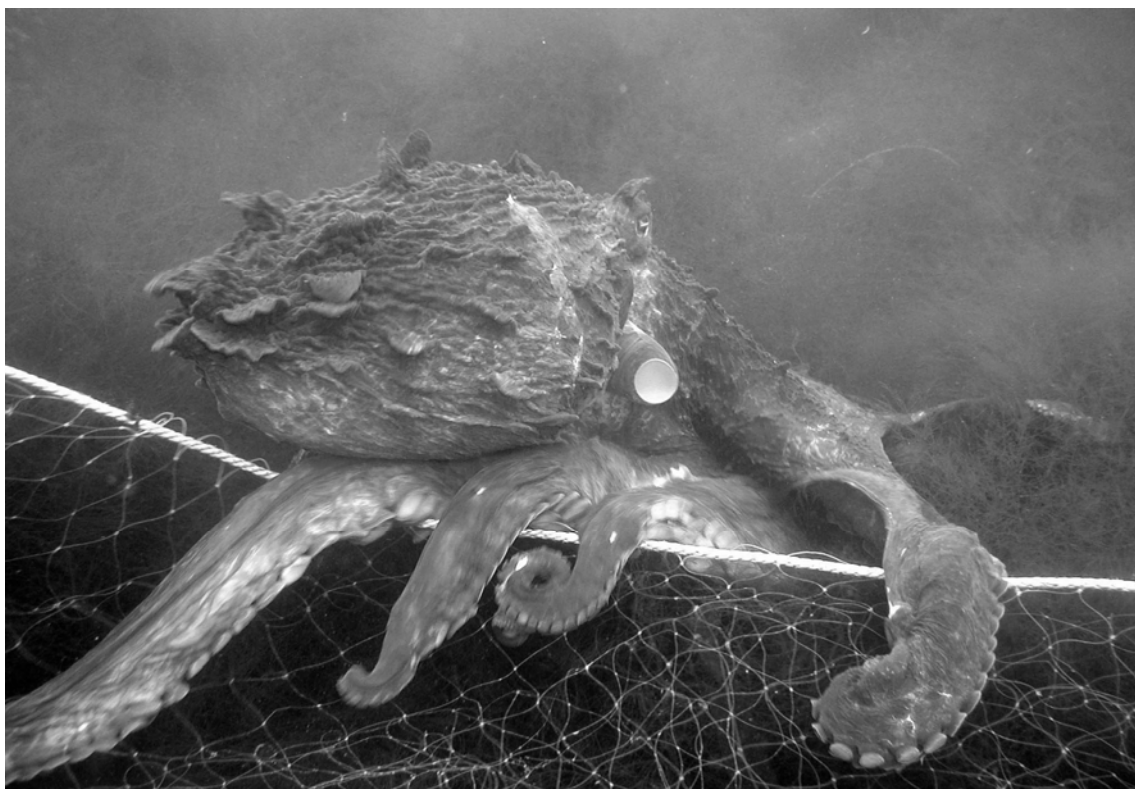
Movement of a tagged octopus (octopus G from fig. 4.4) seen here during the first month of tagging. Initially moving towards the buoy array (away from shore) finding a home range and moving up and down the water column with limited X and Y movement. More 3D plots can be seen in Appendix B.

Fig.4.5 - RAPT depth plot



Daytime (6-4 pm) depth data (z only) from one octopus (octopus G from fig 4.4) over the first two weeks of the tagging experiment. Seen the clear cyclical patterns showing the octopus ascending in the water column for extended time periods, confirmed by SCUBA to be feeding trips `up` a gill net

Fig. 4.6a - Octopus on a gill net



Both tagged and non-marked octopuses were observed to climb gill nets in the area and eat fish entangled in them. Picture provided by T. Abe.

Fig. 4.6b - Octopus on a gill net



A non-tagged octopus weighs down an empty gill net. Photographed at 6 m on the shore of Usujiri by T. Abe

Discussion

The tags were adapted to by the octopus. They are not thought to have caused individuals severe stress nor alternated the octopuses behaviours considerably (comparisons between tagged and untagged octopuses in the laboratory). Only the first two weeks of the data was used in this work due the increasing probability that the tags would have fallen off. Tags 'fell out' via the holes created in the mantle, caused by abrasion of the muscle against the wire. The inability for skin to cover the resulting wounds in the laboratory may have been due to secondary infection. Bacterial infections in the aquarium have been shown to delay skin for closing over wounds and to leave the muscles in the infected area with slightly less ability to constrict (Bullock *et al.* 1987).

Tagged octopus showed movement patterns that suggest foraging occurred within a 250 m² area and that 42 % of that time was spent in a core area. Any area where over 5 % of time is spent is considered a core area (Mather *et al.* 1985). However with octopus it is usual that the den is found in the core area and time spent in that one location were significantly higher than traditional core area use. The time spent away from the den was most likely spent foraging. The lack of large fish, mammals and crabs in the immediate area may have resulted in a greater amount of time spent out in the open than would be seen in an area with higher risk of predation (Mather and O'Dor 1991).

The 17 % of recorded time spent away from home ranges reflected the time taken to settle into a home range and the possession of more than one home range. When consecutive points were plotted it is obvious that this is not a result of leaving the home range and returning after long distance foraging trips. There were no tracks into or out of home ranges (except for when they were being established) that would have

suggested this behaviour. It is possible that the tags reduced forging trips as seen by other researchers (Mather and O'Dor 1991; Mather *et al.* 1985; Hartwick *et al.* 1984). However the lack of extensive hunting trips might also be explained by the fact that other experiments recorded animals that were not in the immediate vicinity as out of the home range, or that the home ranges on the Usujiri coast provided ample prey items (and well stocked gill nets).

The occupation of two home ranges within the experimental time suggests that home ranges are temporary. The small movements seen here are best described as an elastic movement of boundaries, where small changes in home and core regions produced a fluid like linear flow from one home range to the next. Whether this elastic boundary phenomenon is interrupted at some point was not investigated. It is possible that environmental signals speed up this movement of boundaries thereby encouraging migration. Of course the other possibility is that actual migrations are made and not simply directional movement along a continuum. Octopuses are known to prefer protected areas to rest in (Chapter 3 section B) and migrations may well be a series of very temporary core areas.

Territories are defined as 'any defended area' (Nobel 1939) or as 'areas that are held for an appreciable period of time as opposed to the defence of localized but ephemeral resources' (Stamps 1994). Home ranges overlapped spatially but not temporally. Core areas did not overlap spatially or temporally. There was a lack of evidence to claim that home areas were territories. However, the clear separation of core areas suggests that they may fit the definition of a temporary territory (matching finding in the laboratory).

Thus *E. dofleini* were shown to forage in overlapping ranges while defending core areas, and avoiding intraspecies interactions.

The finding that most activity occurred during the daytime is contrary to the belief that *E. dofleini* is nocturnal (Hartwick 1981; Mather and O'Dor 1991). In fact the complete loss of signals from one octopus during the night suggests that it may have remained concealed in its den through out the night.

The distinct cyclical patterns that were first noticed in the depth graphs only to be confirmed as regular feeding off a gill net were a unique finding. Octopuses cause no damage to gill nets when feeding, so there is no way of knowing just how significant an aid nets might be to the local octopus population. Octopuses generally prefer (Rigby unpublished) and are healthier when consuming (Garcia and Gimenez 2002) a crustacean diet. The choice of the high fish diet that the net would have provided may have been influenced by increased energy cost or inconvenience of hunting with the attached acoustic pinger. However untagged octopuses are a by-product of the gill net industry and as they are not caught in the net it may be that they are caught while feeding off of it.

Conclusion

Immature and juvenile *E. dofleini* are different than mature *E. dofleini*. The most conspicuous difference is that they are smaller. Being smaller means that they fit in to the ecological web and the species' size dominate social structure differently. They are more susceptible to predators and are at the bottom of the social hierarchy, which translates in to being at the losing end of more fights. Size also makes a difference to *E. dofleini* physiology. Smaller animals have higher respiration rates and so metabolize food faster than larger animals. This means they are less able to go for long periods of time with out feeding. Small immature octopuses consume small accessible food items (crabs) more regularly than prey that is difficult to open (bivalves) or hard catch (pelagic fish). Immature and juvenile octopuses use energy differently; although both their energy budgets are geared towards somatic growth. The young octopuses, not yet burdened with storing energy in reproductive material use spare energy to explore, learn, move and migrate.

Difference is not limited to the corporal or structural. Life is a progress of (mainly) physiological changes thorough, which behaviours must adapt. Behaviours play out in the visual arena but they are the outcome of external factors working on internal structures. The seasonality of *E. dofleini* around southern Hokkaido as noted in local seasonal culinary specialties, caught in fishermen's nets and the reported in the scientific literature: Kanemaru and Yamashita 1969; Kanemaru 1964; Hartwick *et al* 1978; Yamashita 1975, is shown in these chapters to be a behavioral response to physiological changes brought on by changes in water temperature.

While studying the juvenile and immature stage *E. dofleini* that migrate inshore biannually the difference between studying populations and individuals and the importance of observation (and its techniques) were highlighted. The advantage of the

seasonal fluctuations, rocky shore, low visibility, and ample small prey items found at the entrance to Funka Bay were also made apparent.

Live *E. doffleini* are distinguishable from other octopus of the same size found in the same region. Juveniles are identified by four leucophore patches and numerous (>200) suckers on each arm (not including the hectocotylized arm). Immature octopus are identified by two leucophore patches on the web of the upper arms, numerous (>200) suckers on each arm (not including the hectocotylized arm), and dark longitudinal lines that run lengthwise down the mantle.

By comparing the work on population (chapter 1) and growth (chapter 2) it is clear that population demographics do not show how an individual animal grows. The logarithmic curve seen in the weight vs. length chart made from the dissections was not replicated by the experiments on growth. *E. doffleini* showed exponential growth during the juvenile stage and linear growth during the immature phase. Calculations using these growth rates show that it takes between 2 or 3 for an octopus to grow from settlement to potential maturity (1g -15kg).

Previous field studies have reinforced the belief that many octopus species are nocturnal (*O. joubini* Mather 1982; *E. doffleini* Hartwick 1981; *O. vulgaris* Kayes 1974). The finding in captivity that these patterns are malleable (*O. vulgaris* Wells *et al.* 1983) has been considered a by product of keeping octopus in aquarium. Here the highly individual activity patterns seen in the laboratory were correlated in the field. The summary of the movement patterns of the tagged octopus shows greater movement during the day. These results suggest that octopus in Funka Bay are not nocturnal. This conclusion is possible due to the constant remote observation enabled by the radio acoustic tags.

Seasonal temperature fluctuations regulate the inshore/offshore migration. Summer and winter temperature extremes keep immature animals from being inshore throughout the year. Spring and fall migrations are made to take advantage of the coastal temperature range of 7-9.5°C. Mature octopuses restricted by size and energy constraints to water of year round habitability do not make this trip resulting in an absence of paralarvae and mature stages from the area.

This thesis dealt with the response to seasonal temperature but salinity also fluctuates. The cold water from the North Pacific brought in to the area by the Oyashio current that encroaches on the southern Hokkaido shoreline in February and March of each year has a low salt concentration. Salinity has been correlated to the abundances of Canadian populations of *E. dofleini* and migrations have been recorded to happen just before annual lows (Hartwick et al. 1984). The low salinity of the cold water may enhance the spring emigration from the coast of Funka Bay. The association of extreme temperatures and low salinity on monthly *E. dofleini* catches cannot be corroborated by fisheries data. Before 1985 the Hokkaido department of Fisheries grouped all octopus catches (i.e., there was no distinction made between *E. dofleini* and *O. conispadiceus* hauls). Unfortunately during this time the Dounan area has been under the influence of a warm temperature regime. During a warm regime salinity fluctuation is not as apparent as during a cold regime, when the Oyashio current sweeps in for months at a time as it did before 1988 (Kodama 2004).

The thick vegetation and numerous crevices along the Funka Bay shore provide numerous den opportunities and reduced direct lines of sight and visibility. This environment benefits the immigrant octopuses that set up of small temporally localized home ranges. The small-scale movements detailed by the RAPT test were analogous to the movements seen in captivity and to the portrayal of populations on the eastern

Pacific (Hartwick *et al.* 1984; Mather *et al.* 1985). In the field and in captivity *E. dofleini* were seen to be solitary, with a combination of behaviours that could be described in turn, to be: A-Social, Territorial or Dominant.

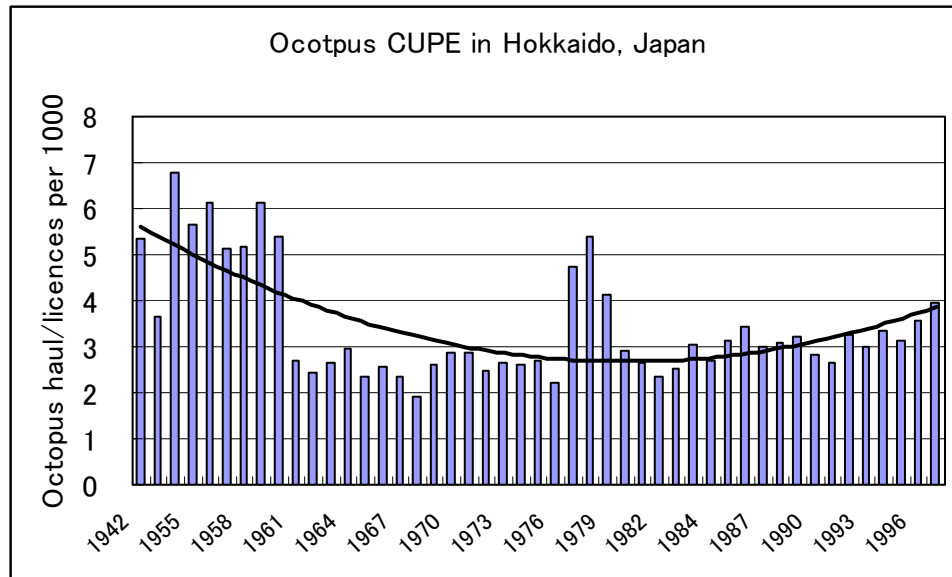
The octopuses prefer protected areas away and out of the line of sight from others. Other octopuses are not permitted to enter these areas or the immediate vicinity. Both in captivity and in the field this core area was physically defended. Octopuses do not spend all their time in these small territories. There is a general area, outside of the core that octopus move around in very individual temporal patterns. In the laboratory activities outside of the core included searching and foraging, but there was no correlation to aggression. This area primarily used for foraging represents what other researchers have termed 'home range'. There were no obvious trips out of the home range as has been reported by other methods of tagging. This difference could be explained as either an artefact of research method or location. The common practice of recording animals that are not in the immediate vicinity, as out of the home range was avoided by the continual monitoring by the radio tags. Usujiri coast may provide ample evenly distributed prey (and well stocked gill nets). These home areas are not exclusive, and both field activity patterns and captive experiments suggest that there may be a certain amount of time sharing involved in their use and maintenance. The boundaries of home ranges appeared to be elastic. This elasticity was shown by an extension of the home range, followed by a movement of the core area and a reestablishment of individual patterns and home range boundaries that lasted anywhere from one week to one month.

Funka Bay is home to a number of prey species of immature *E. dofleini*. The bay has numerous crustaceans (*Hemigrapsus sanguineus*, *Erimacrus isenbeckii* and benthic fish (*Bero elegans*, *Pleuronectes yokohamae*) that *E. dofleini* is known to feed on however

the consumption of fish (primarily *Hexagrammos otakii*, *Pleurogrammus azonus*, *Myoxocephalus stelleri raninus*, *Sabastes taczanowskii* and *Sabastes nivosus*) caught by gill net was a startling find. Local fishermen consider *E. dofleini* a profitable by-catch of running a gill net in public waters and so catch is not recorded. However, unlike the fish caught in the net the octopuses are not entangled. From this study it is apparent that they are likely caught while feeding. I believe that this can be taken as an example of flexibility that has been alluded to as adaptability or intelligence by studies on octopus neurology and behaviour (Young 1961; Mather 1994; Yamazaki *et al.* 2002).

Most marine populations are in decline (Pauly 2000; Pew 2003; Myers & Worm 2003) however, around Hokkaido Island octopus catch per unit effort (CPUE) and *E. dofleini* catch rates have been relatively stable (Fig c.1 - Hokkaido Fisheries Annual Report 1937-2000). Decreasing inter-specific competition for food, fewer large predators, positive environmental shifts and successful life history strategies have been proposed as reasons for this stability (O'Dor 1998; Chotiyaputta *et al.* 2002). I believe that the results from this thesis show that behaviour should be considered to be a factor in the stability of the *E. dofleini* population.

Fig. c.1 – Octopus CPUE since 1937 in Hokkaido, Japan



Current increasing octopus Catch Per Unit Effort around Hokkaido Island Japan (bar graph/ 1000t) supports reports of compensation by cephalopods for decreasing fish stocks. A polynomial spline highlights the original decrease in the 1960s and the recovery since. Gaps in years are due to incomplete records of total octopus haul (kg) and number of licences (people) recorded annually since 1937 by provincial and local governments.

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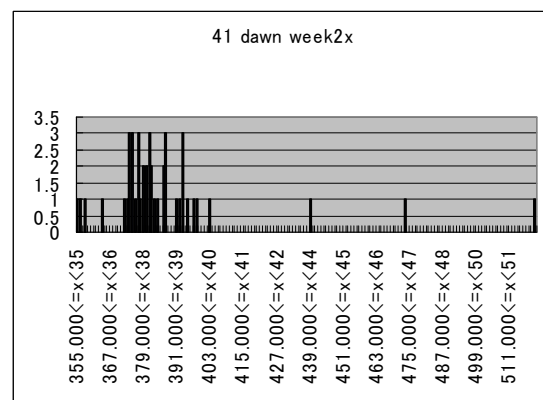
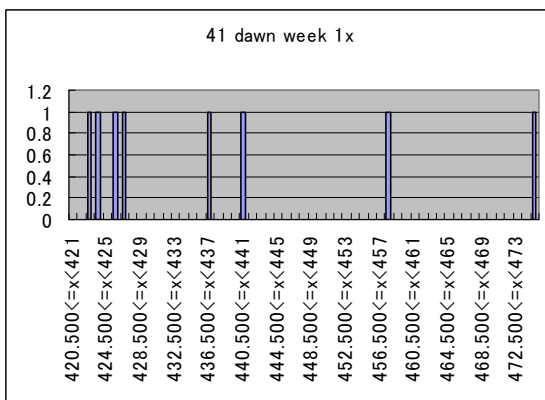
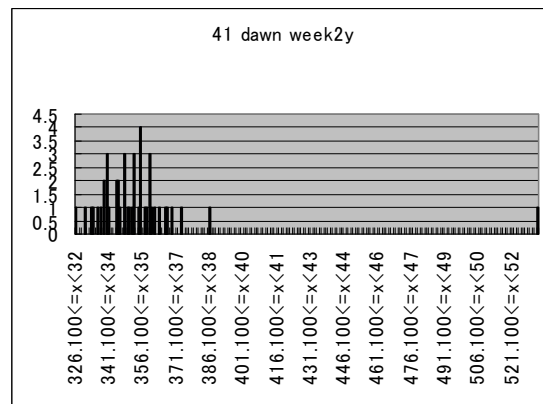
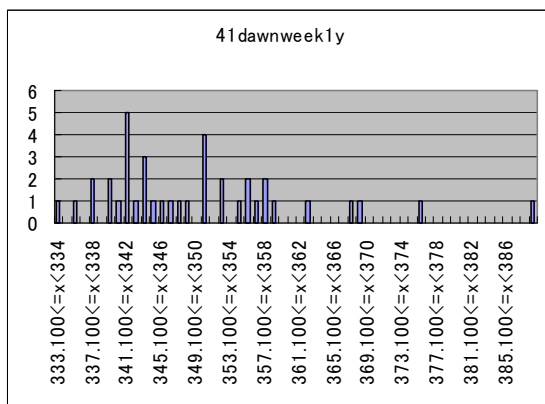
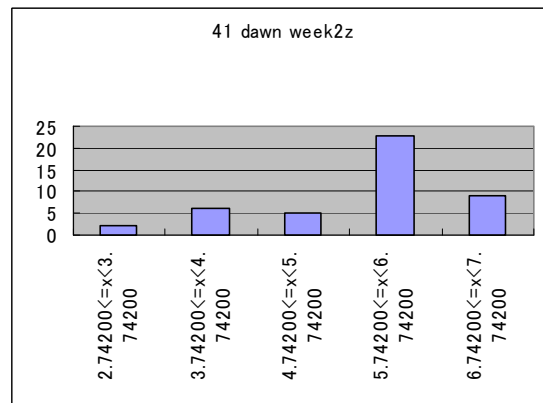
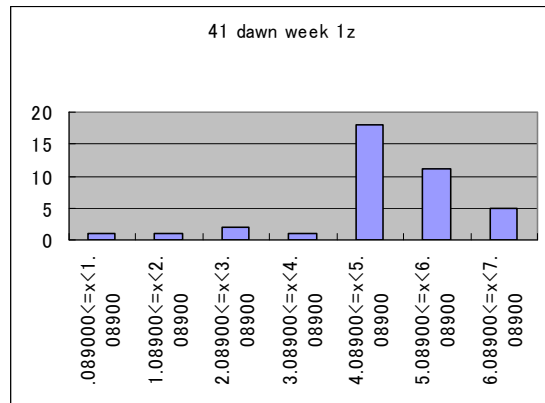
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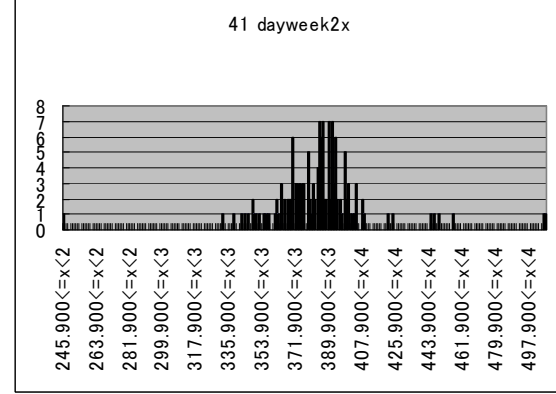
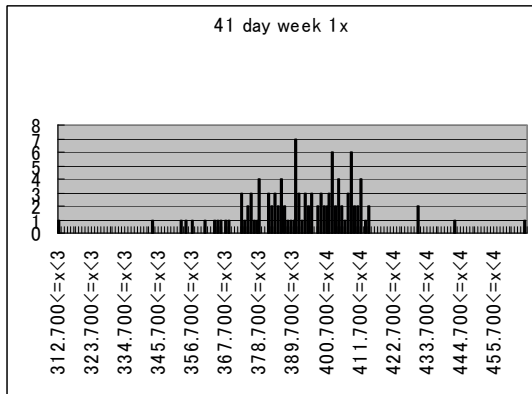
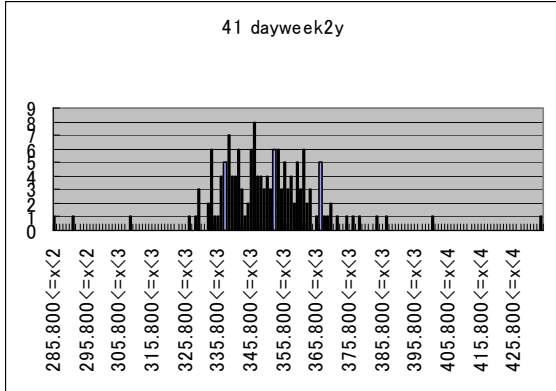
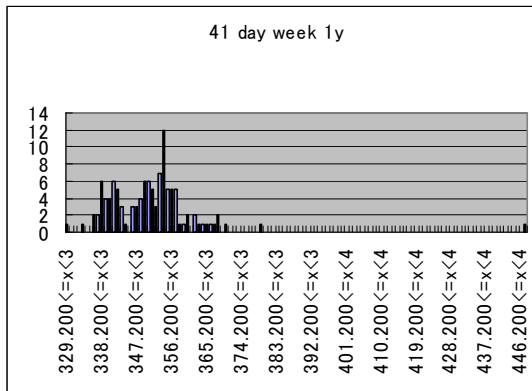
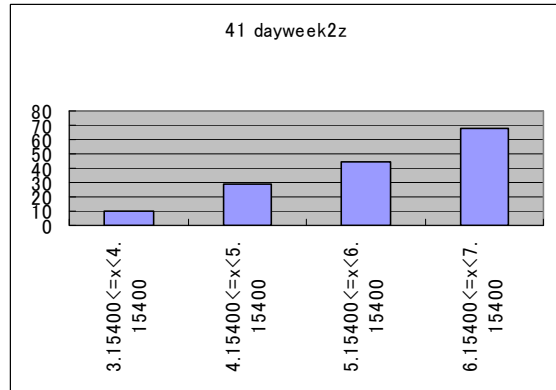
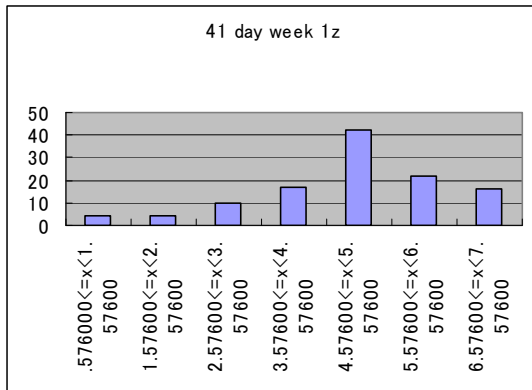
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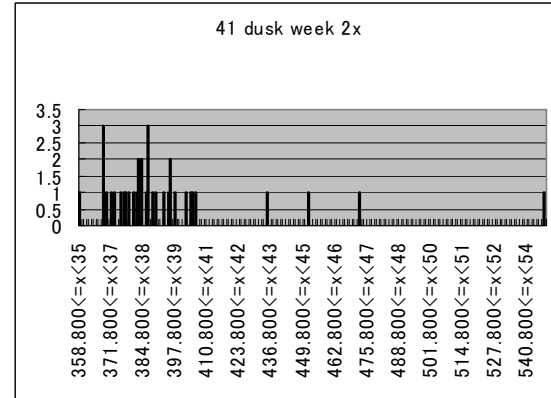
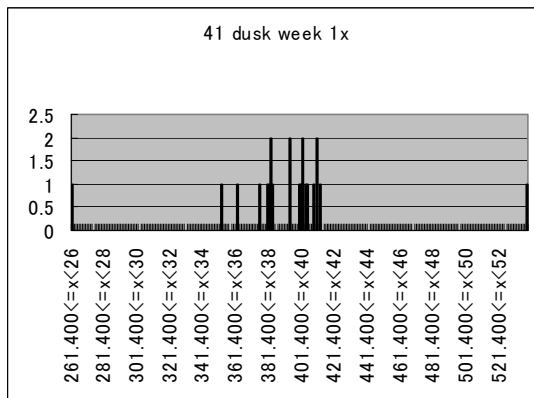
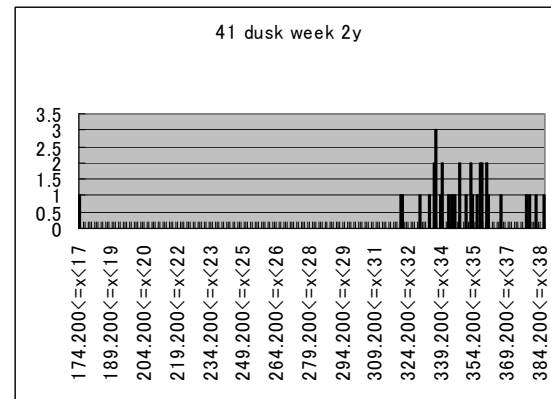
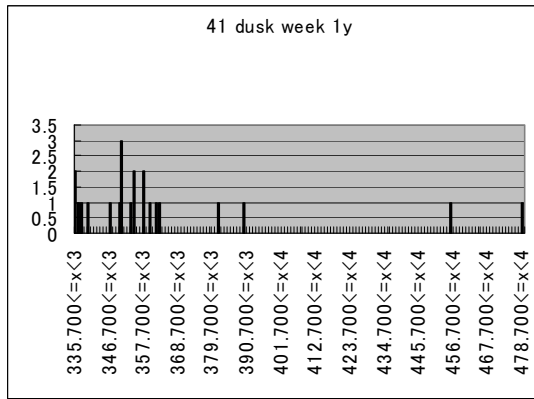
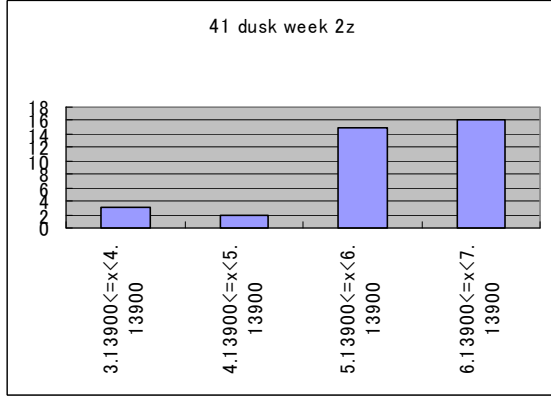
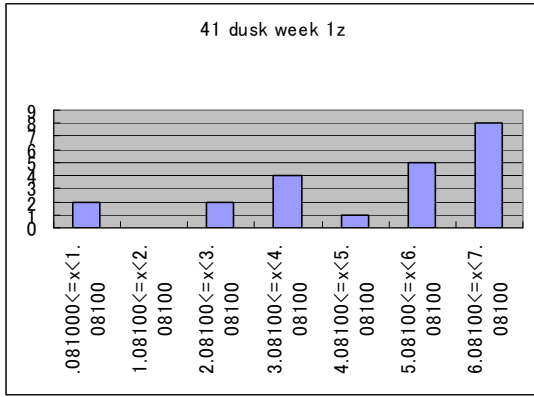
Appendix A

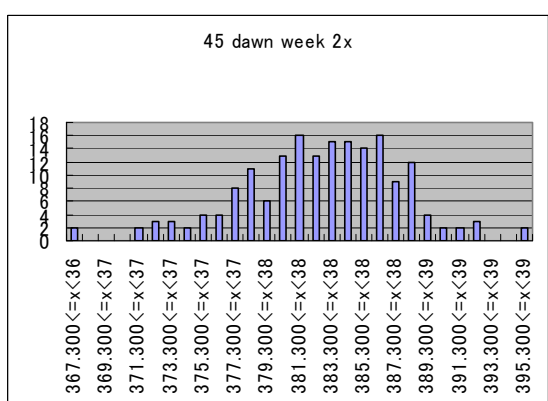
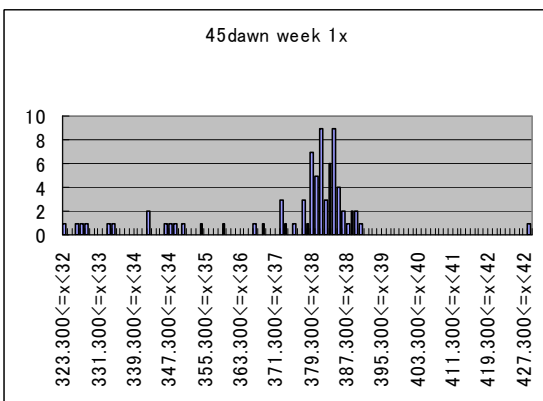
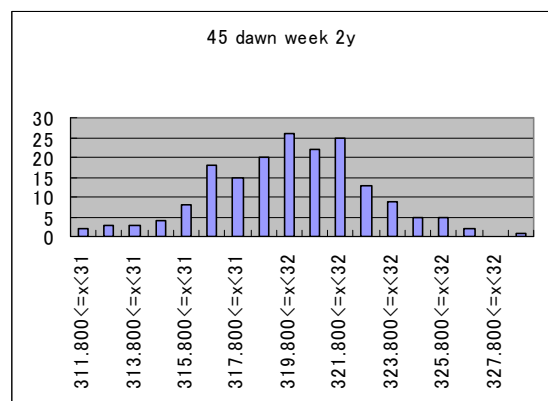
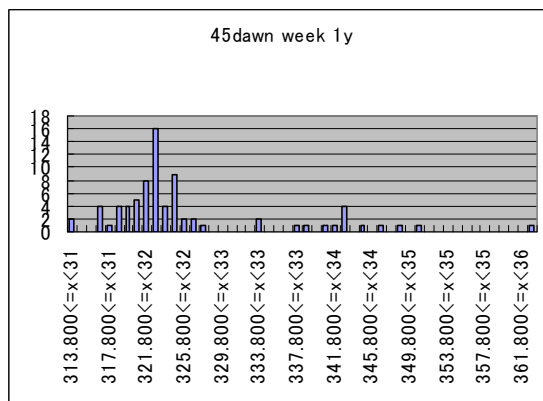
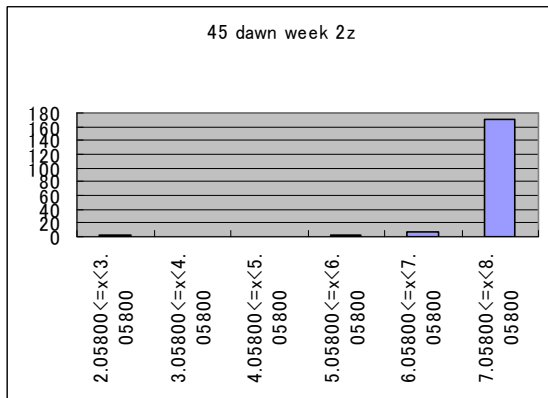
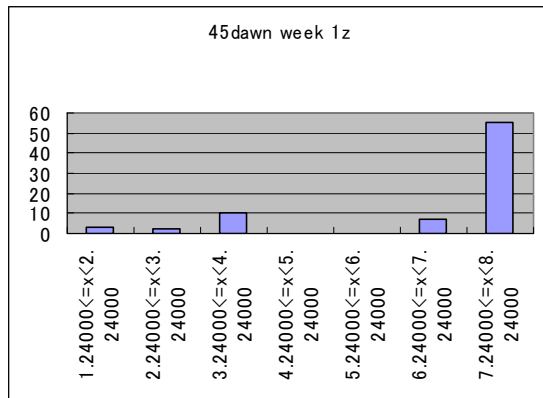
Home and Core Areas

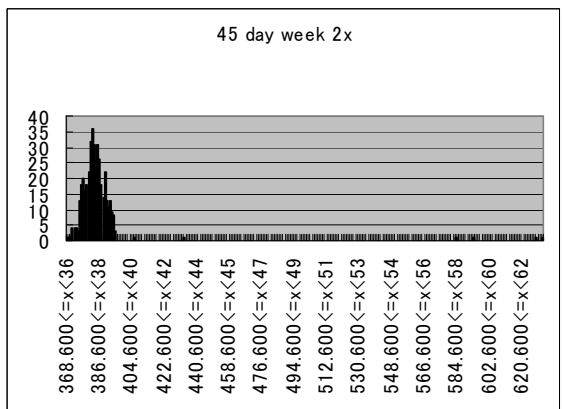
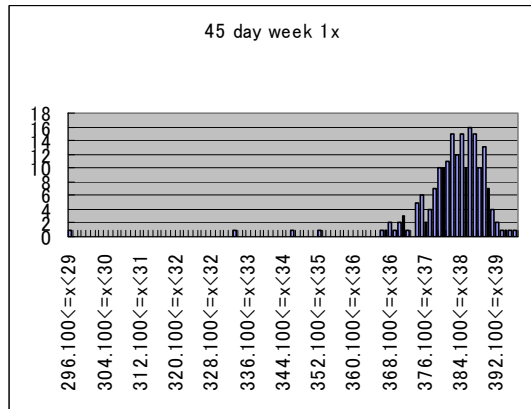
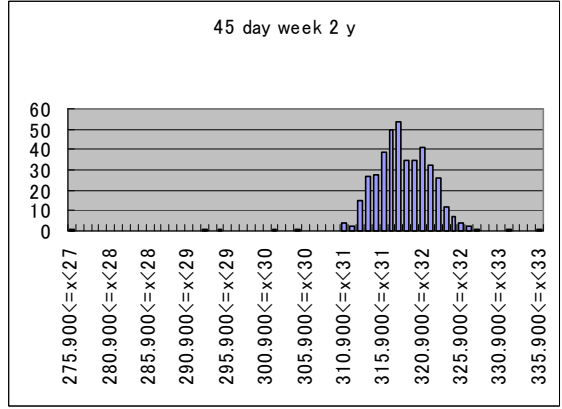
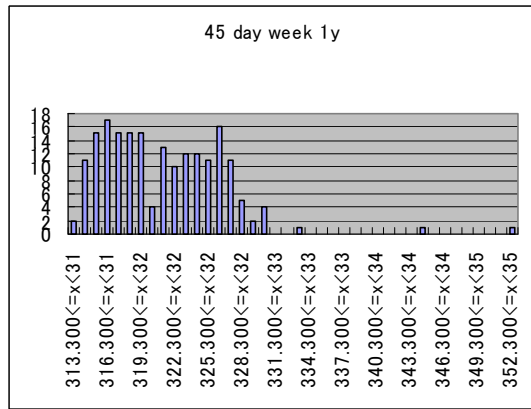
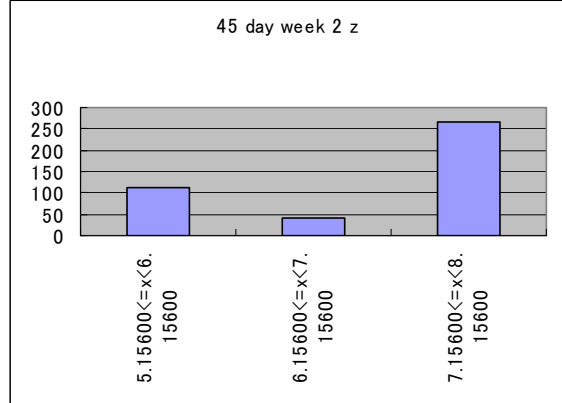
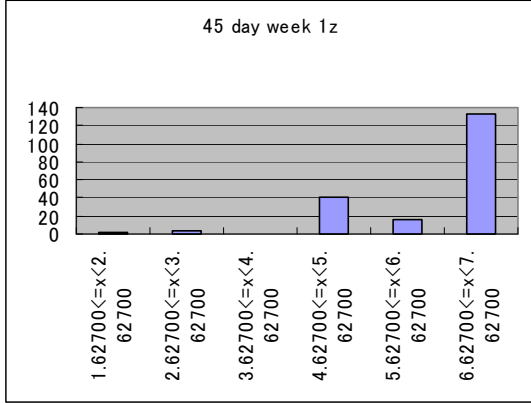
The following graphs show data from the first two weeks of five of the immature (3-4 kg) *E. dofleini* caught by SCUBA and box trap and tagged by Radio Acoustic Position transmitters. Presented here are frequency charts of the X, Y and Z reading from those tags, broken in to Dawn (3:00-7:00), Day (7:00-16:00), Dusk (16:00-21:00), Night (21:00-3:00).

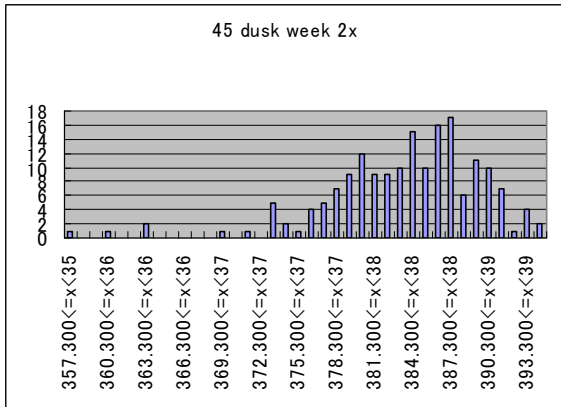
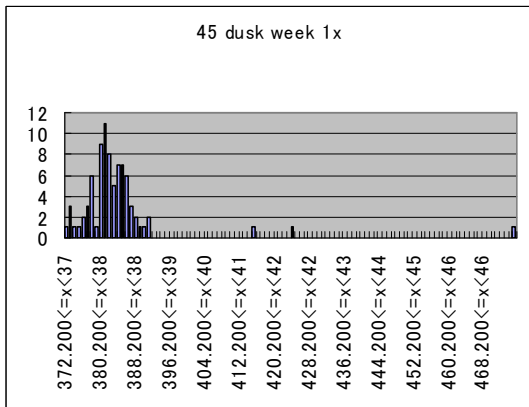
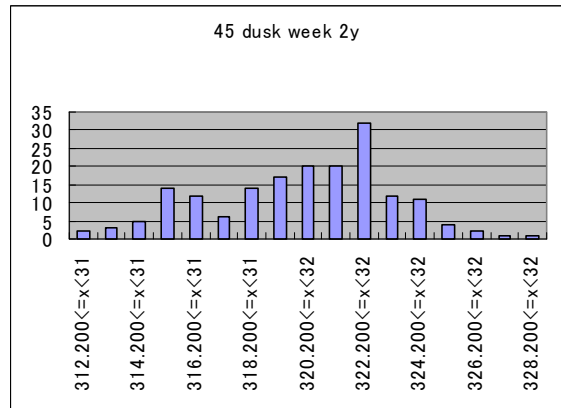
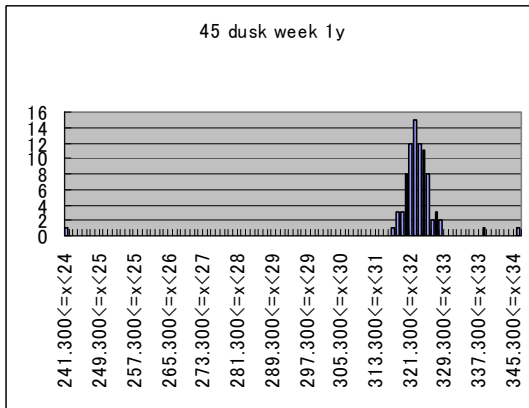
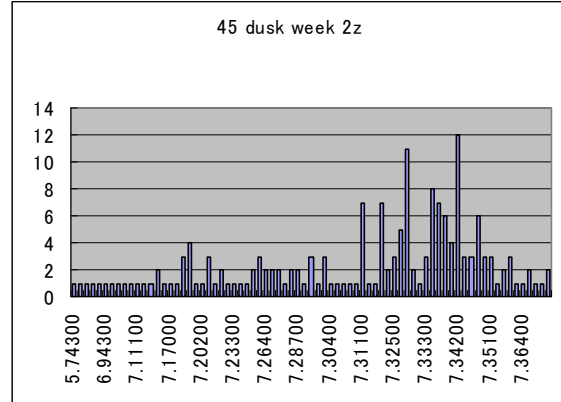
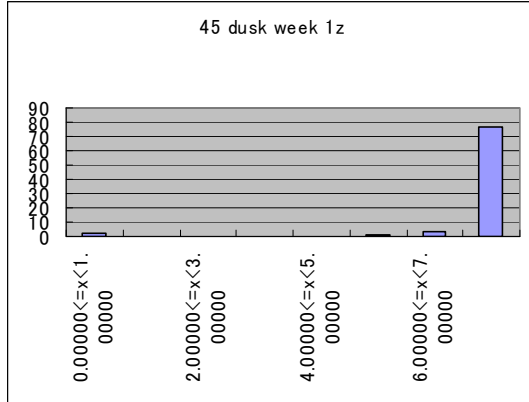


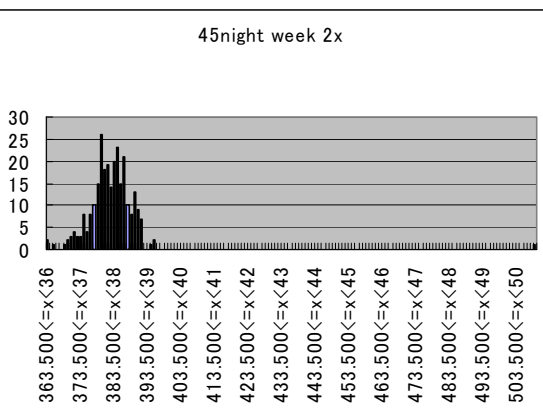
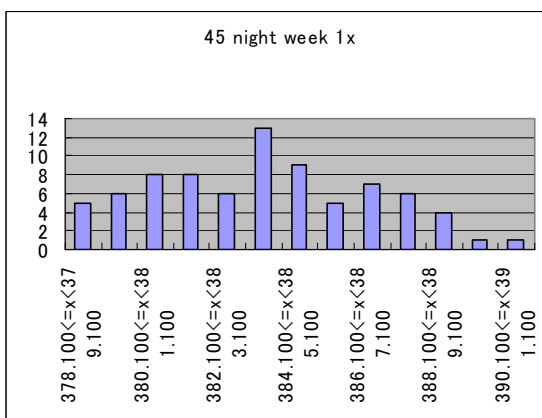
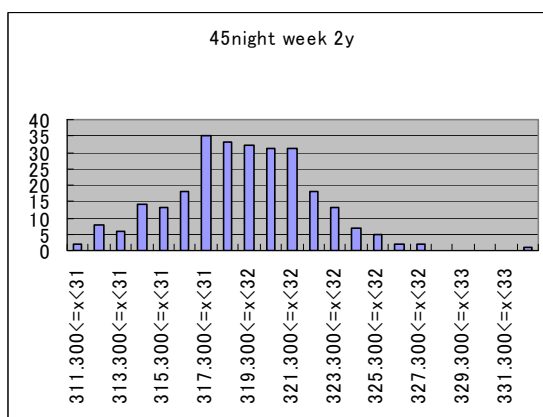
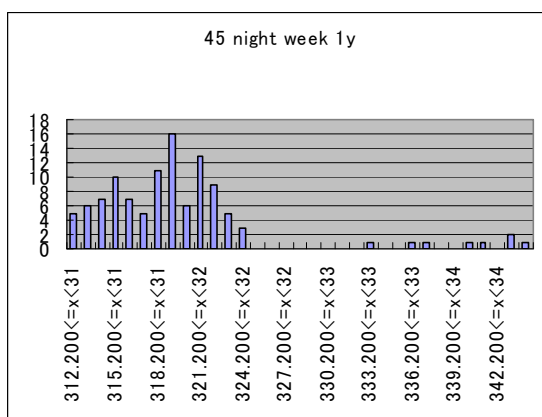
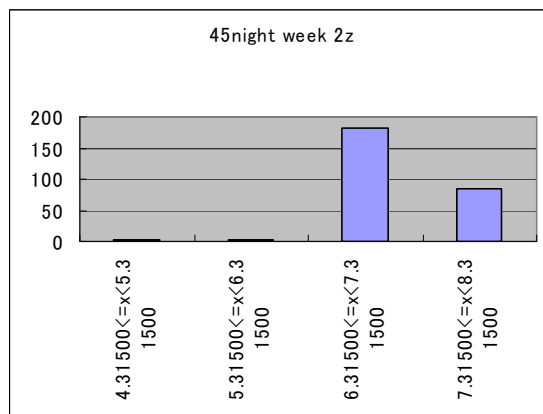
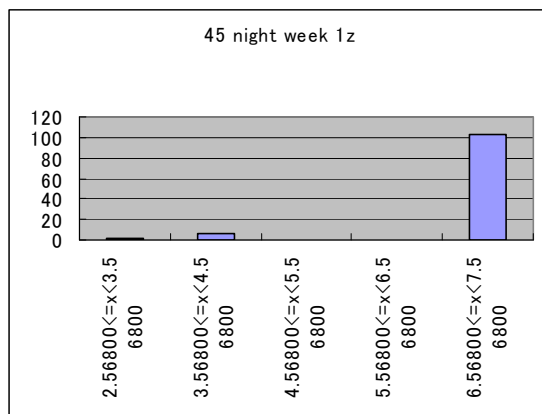


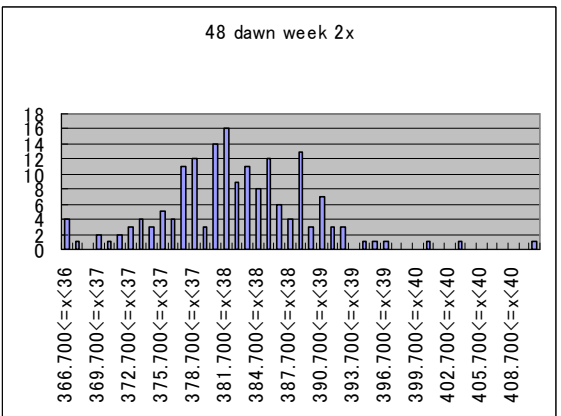
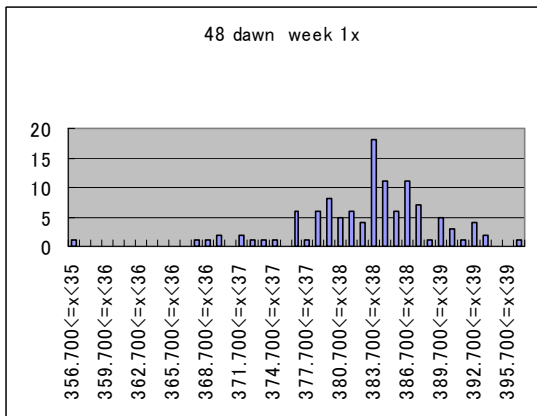
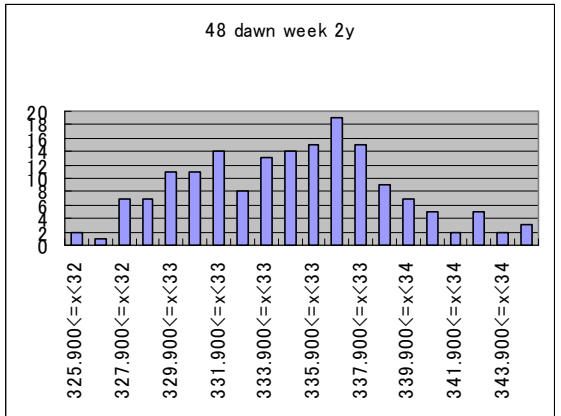
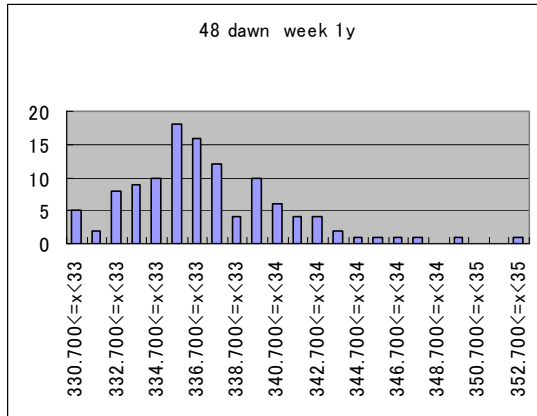
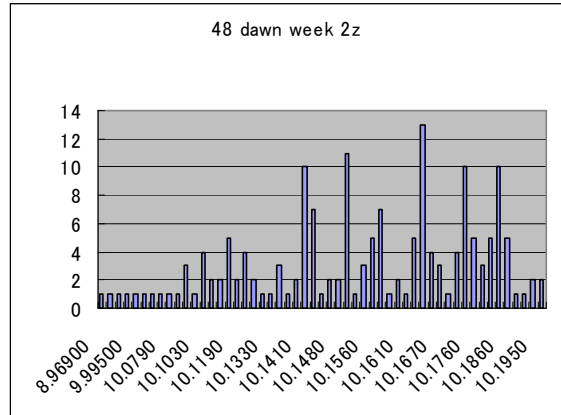
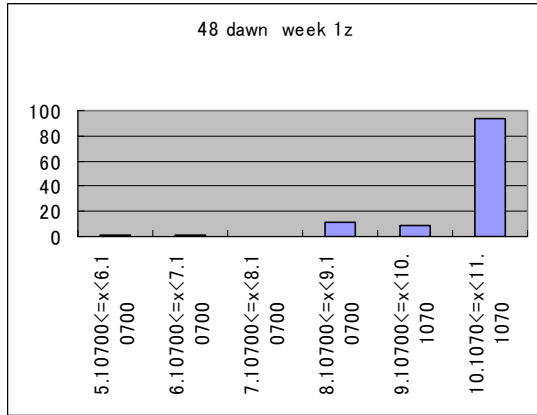


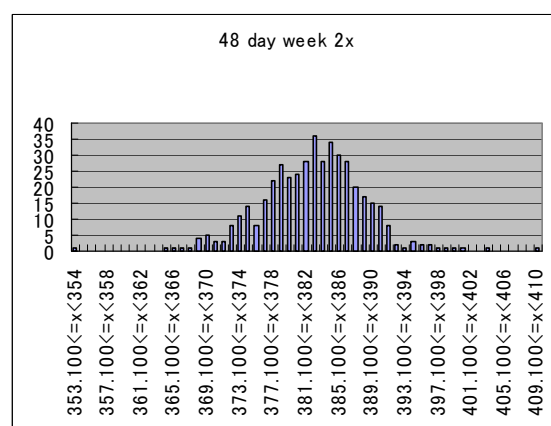
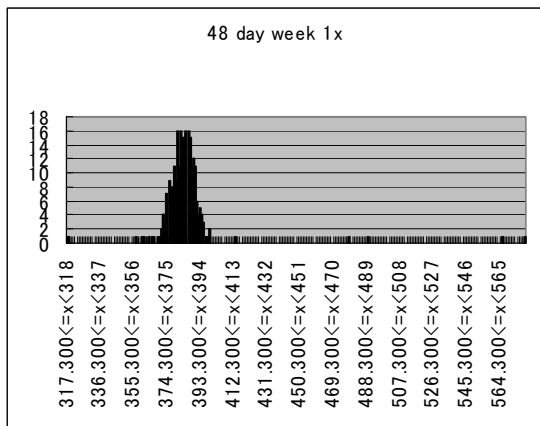
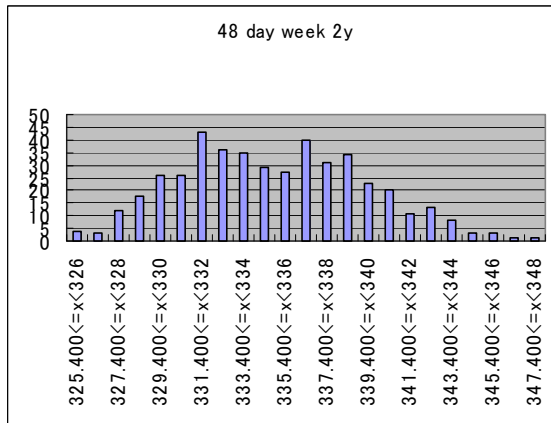
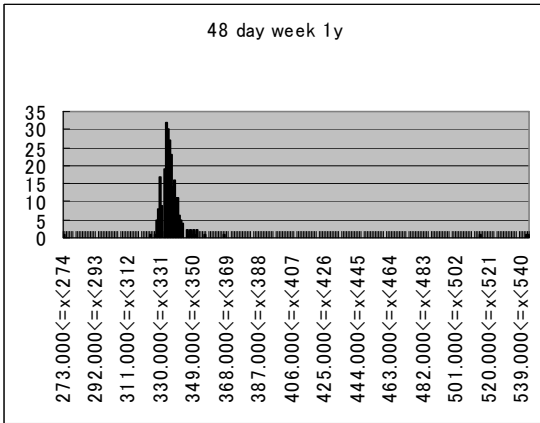
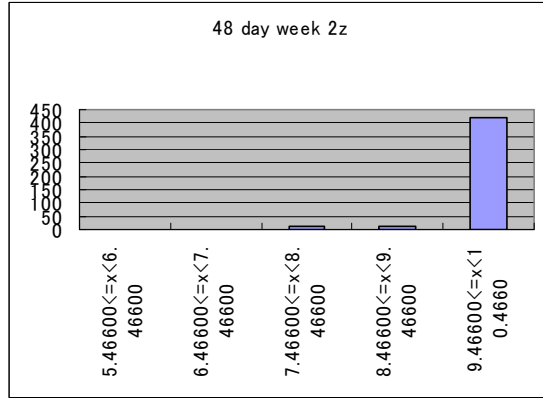
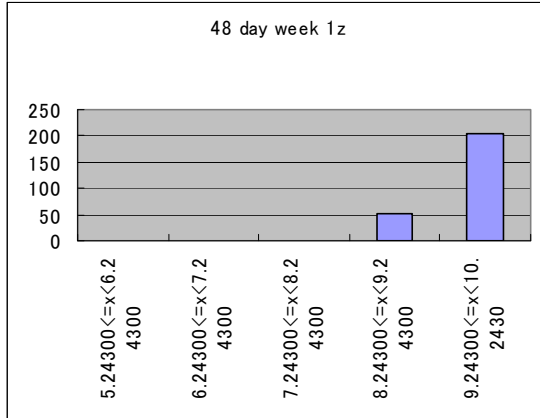


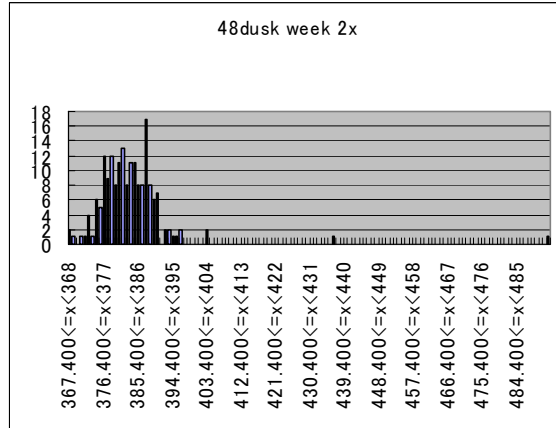
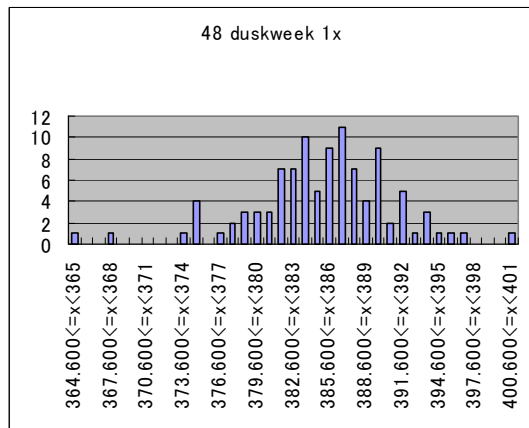
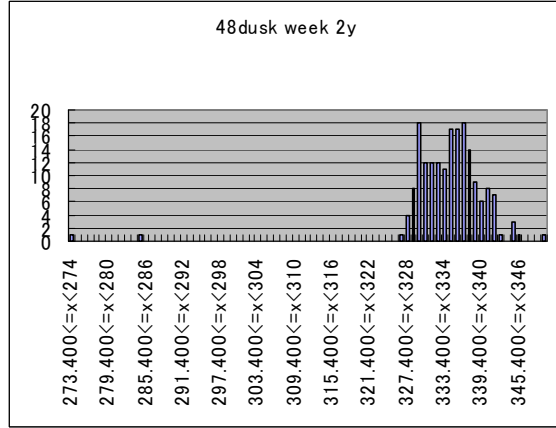
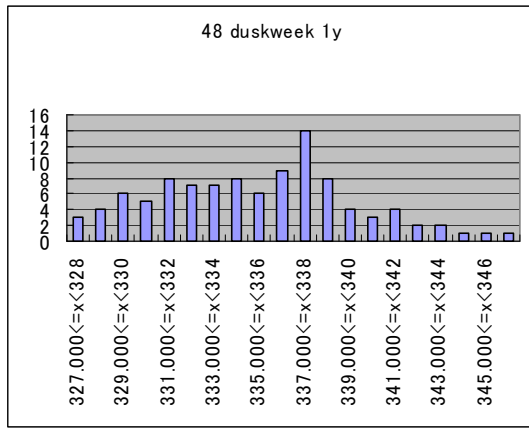
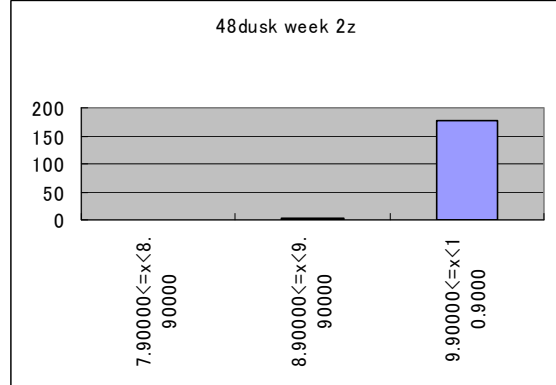
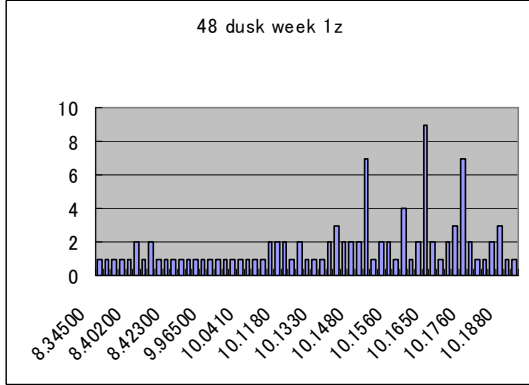


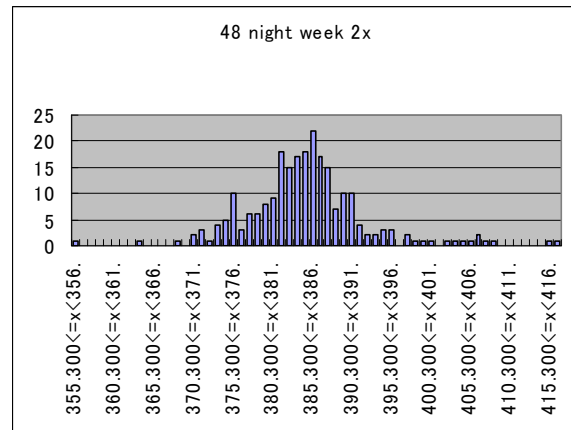
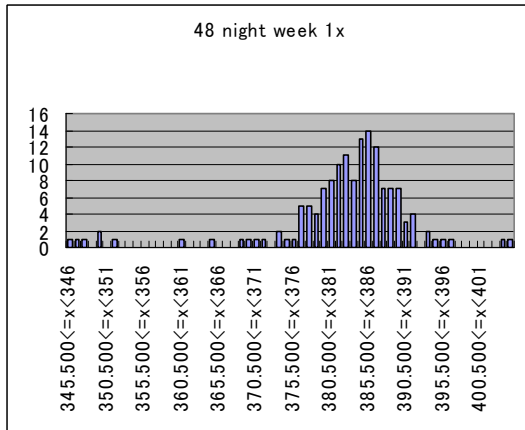
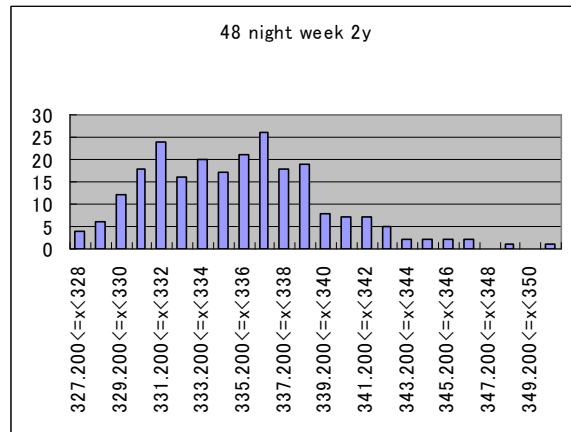
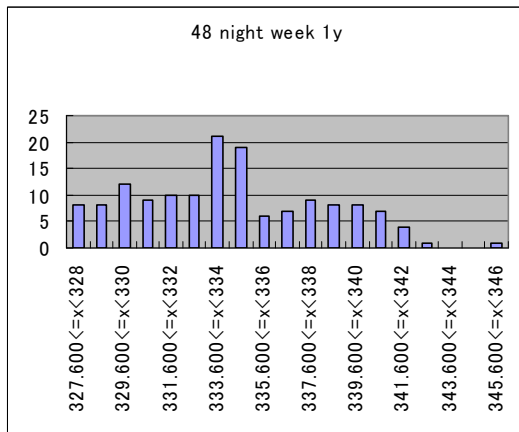
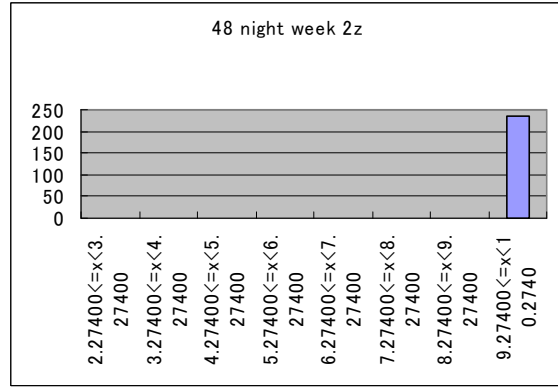
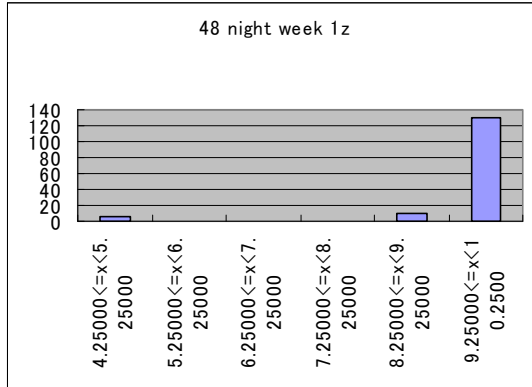


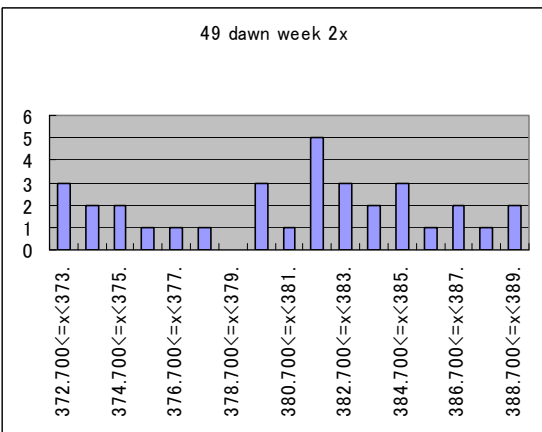
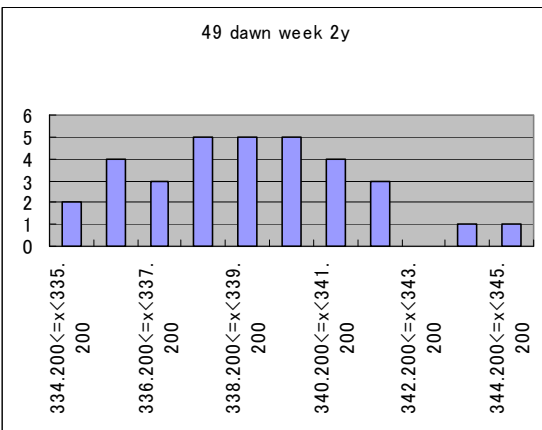
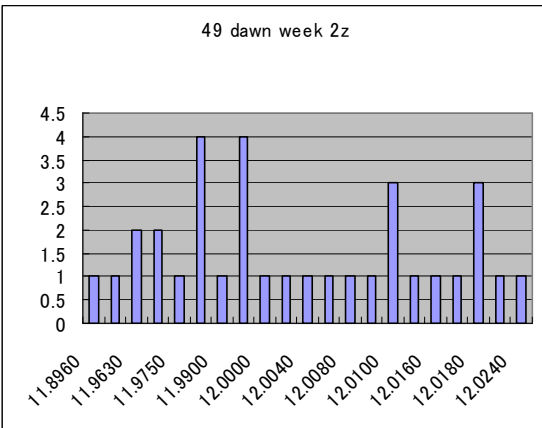


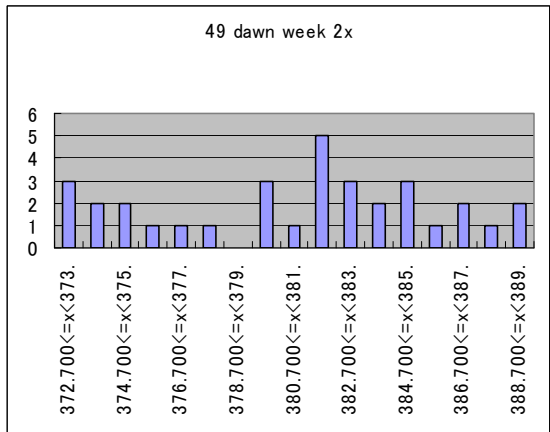
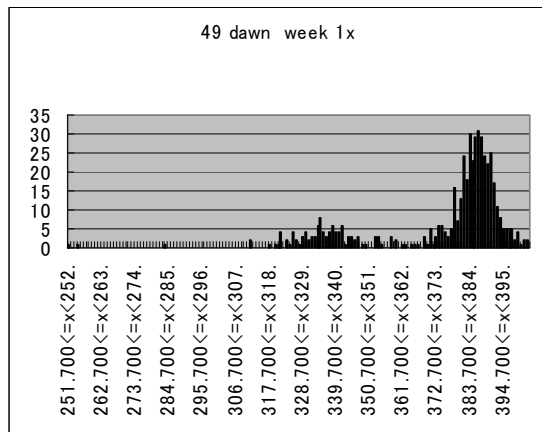
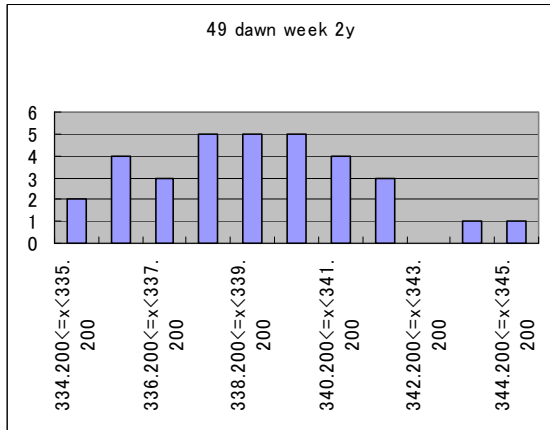
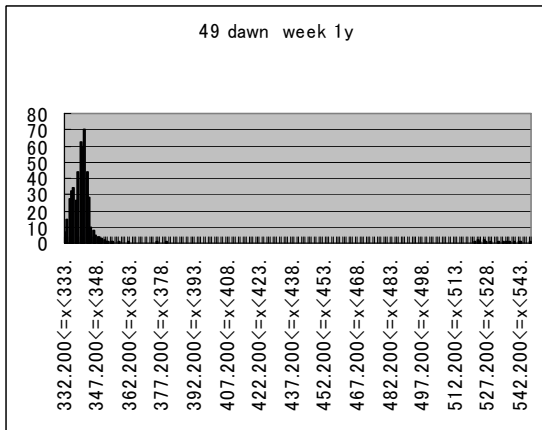
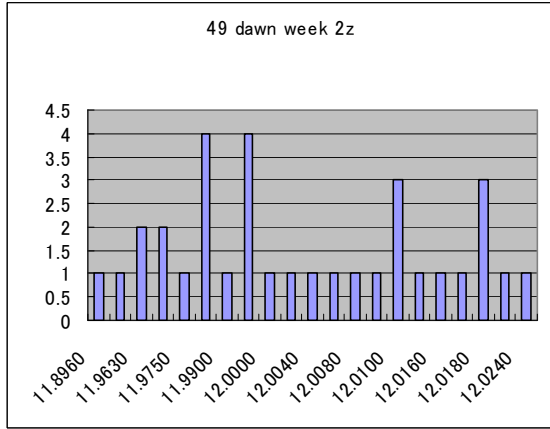
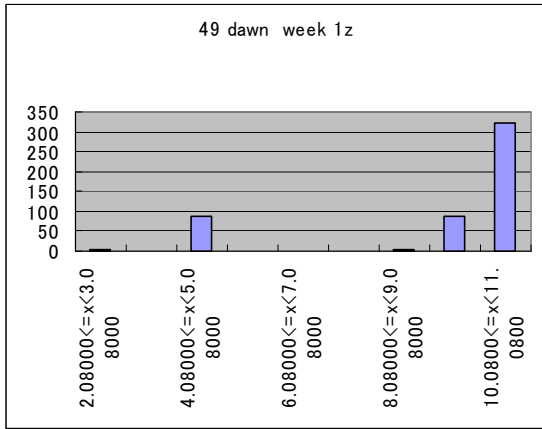


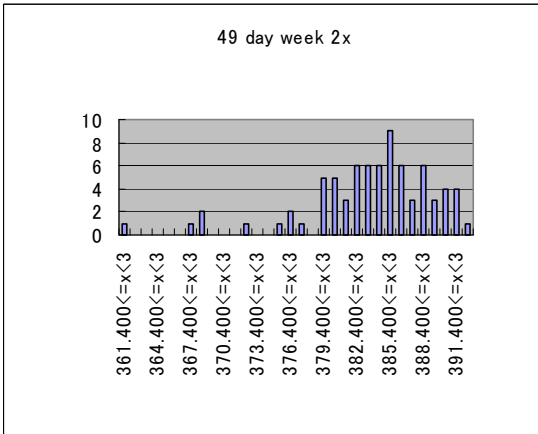
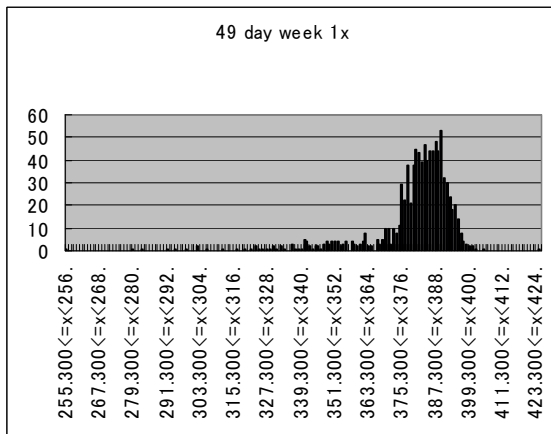
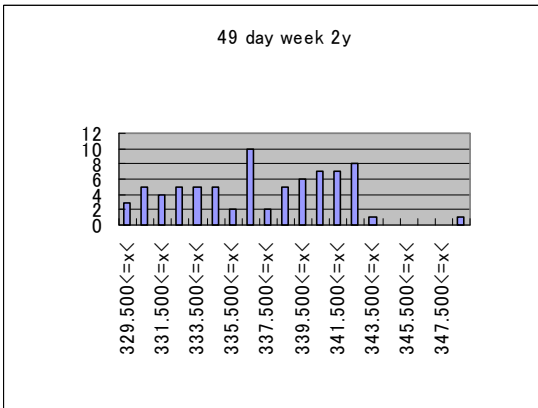
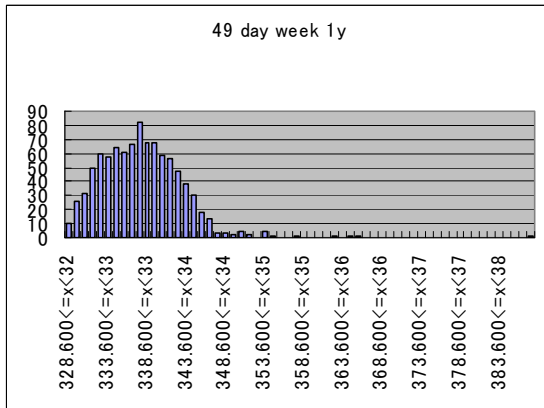
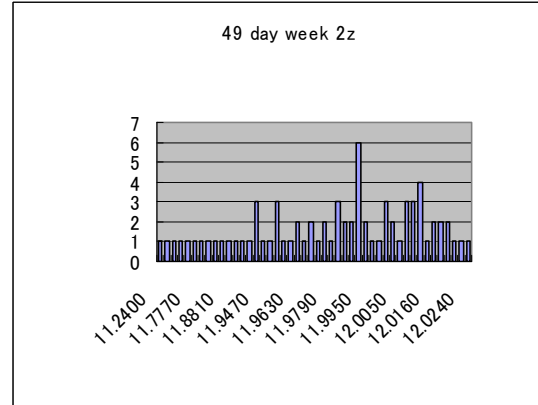
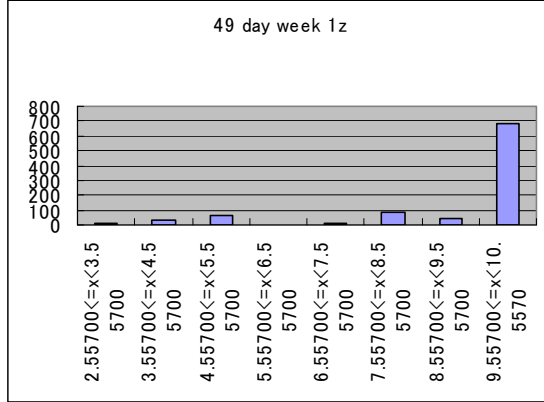


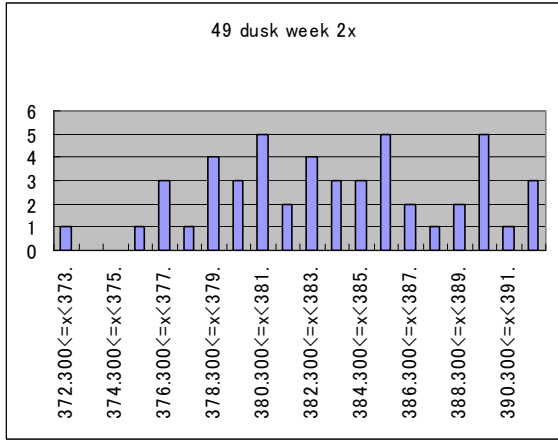
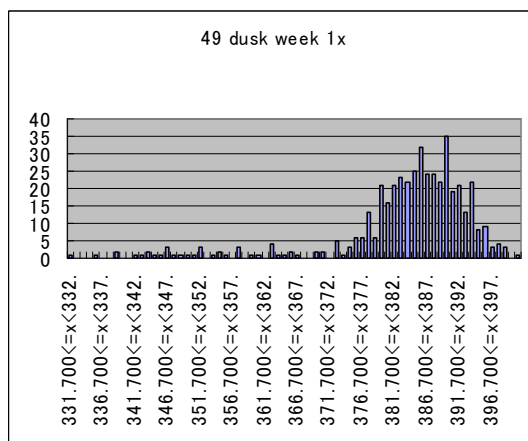
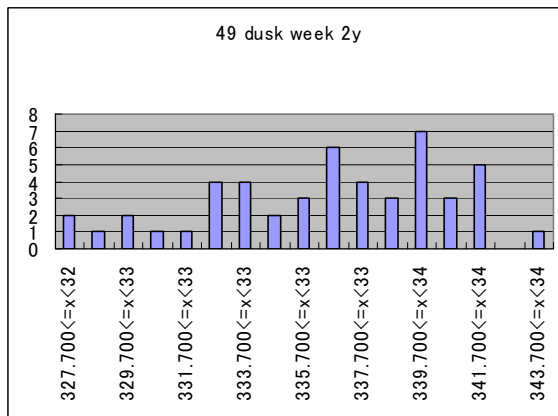
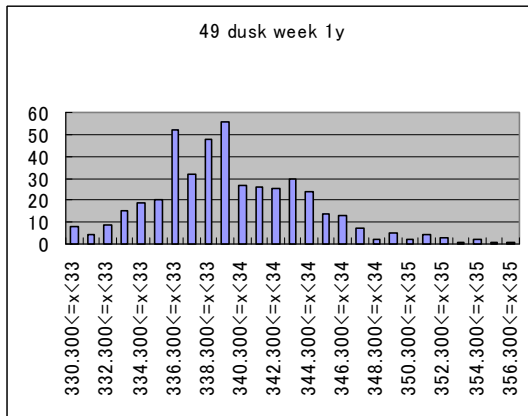
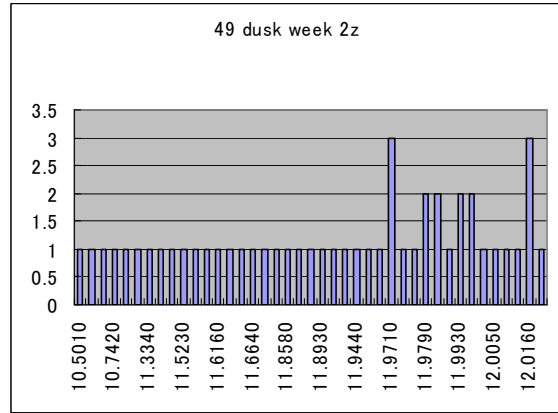
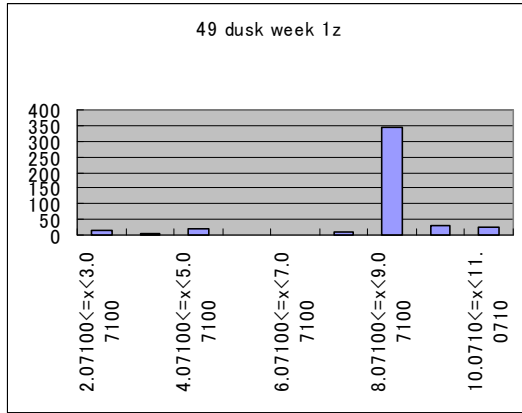


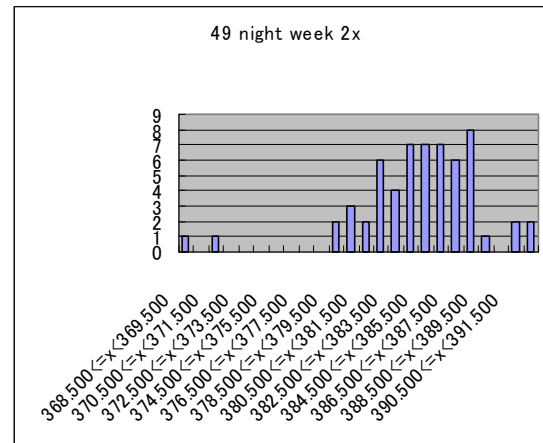
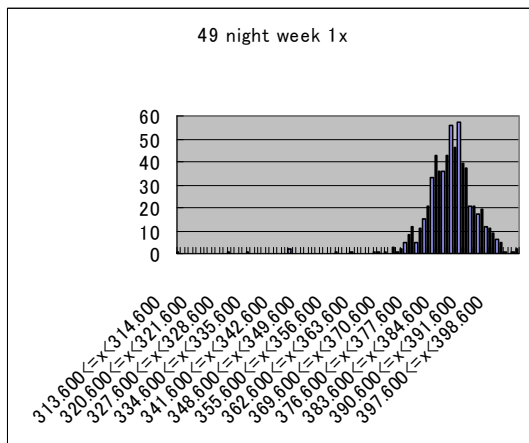
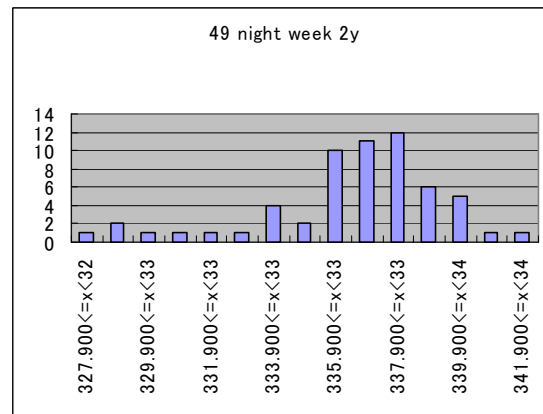
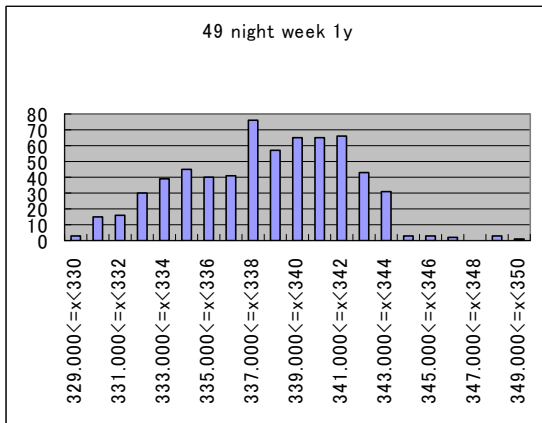
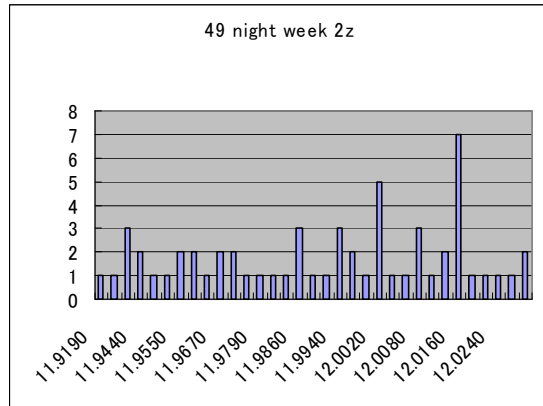
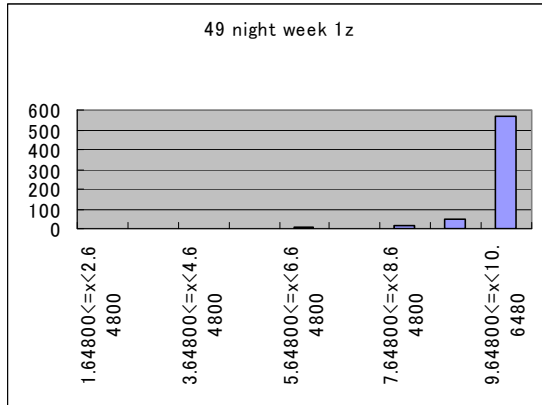


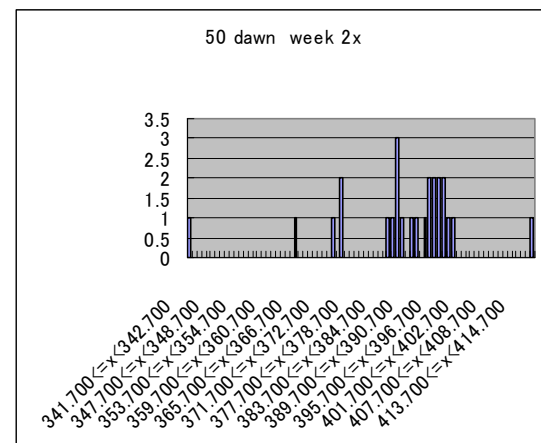
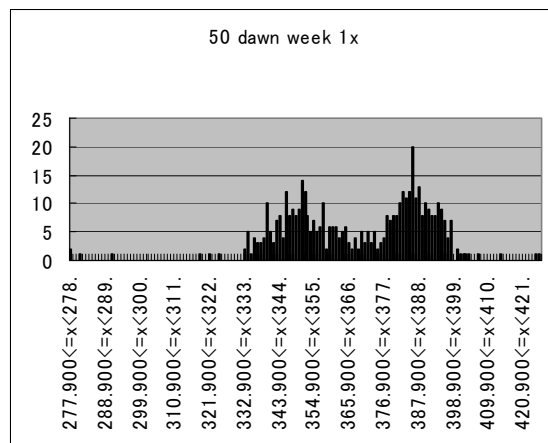
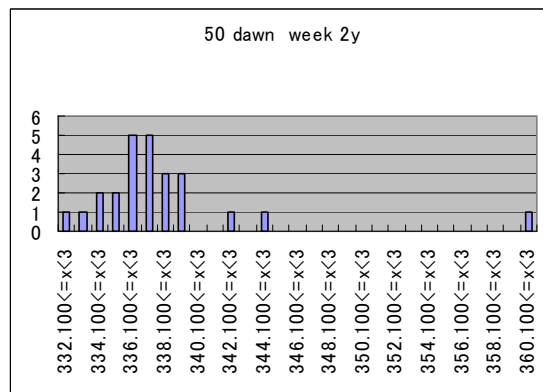
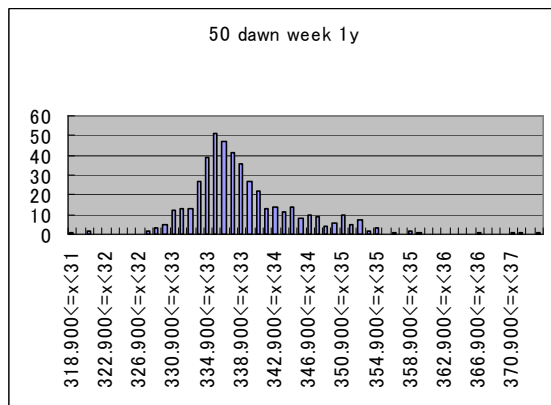
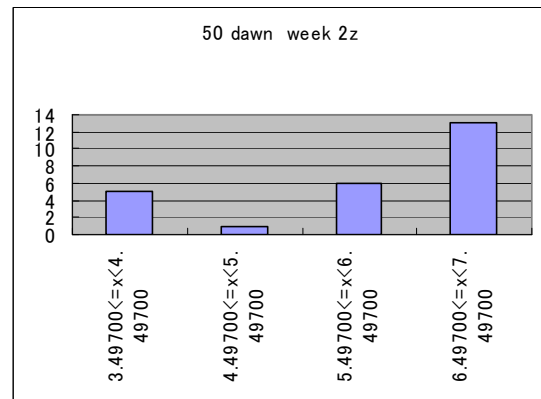
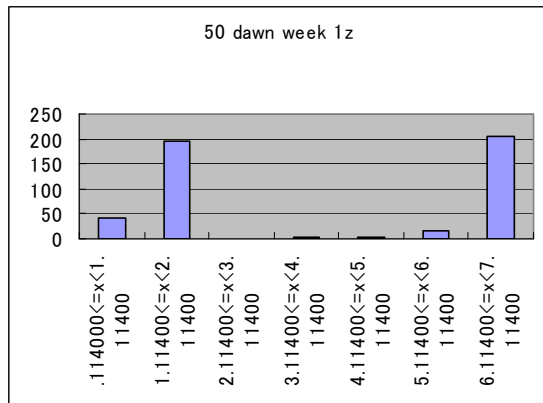


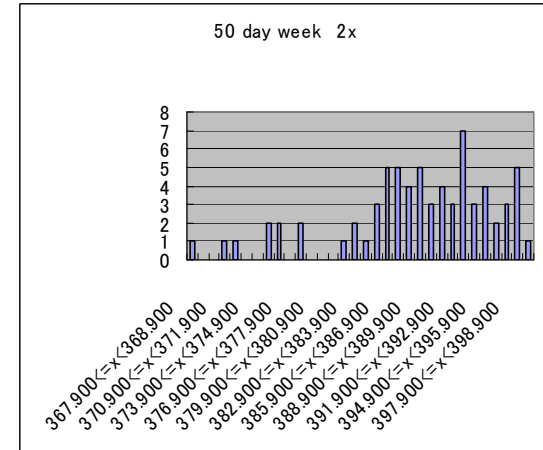
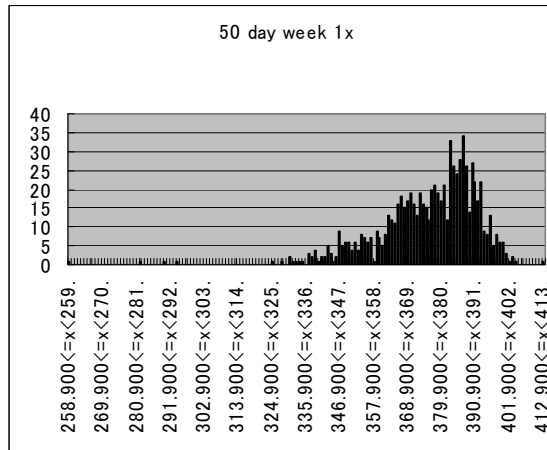
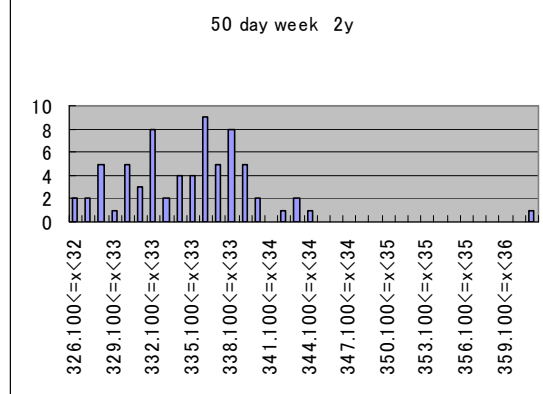
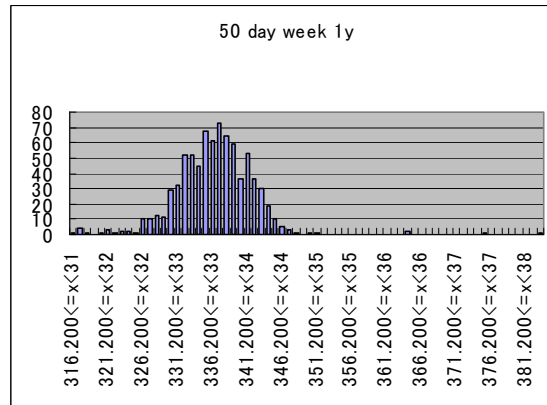
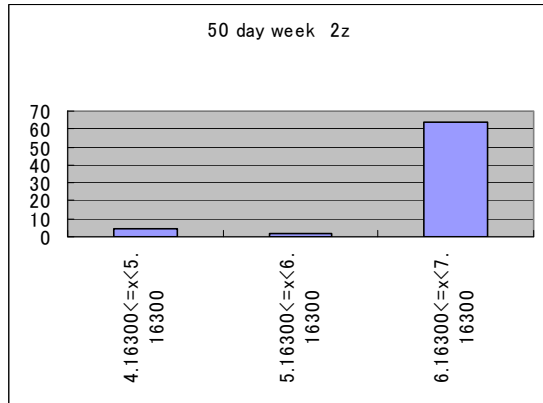
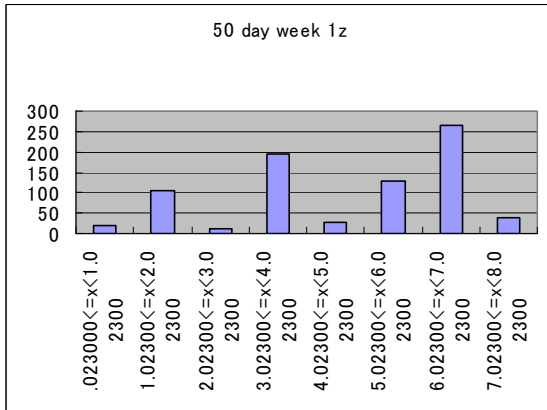


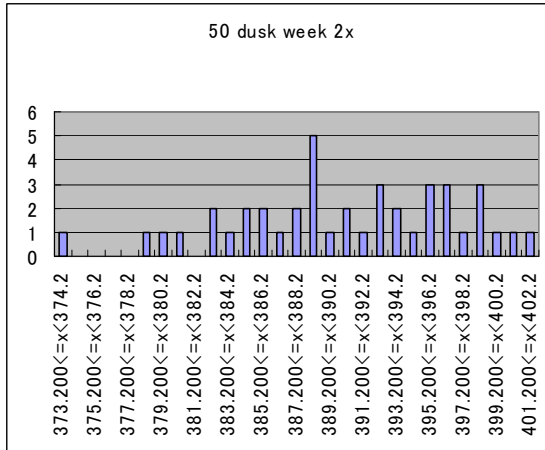
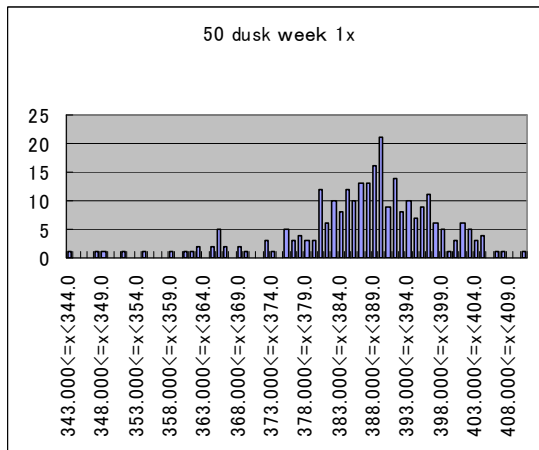
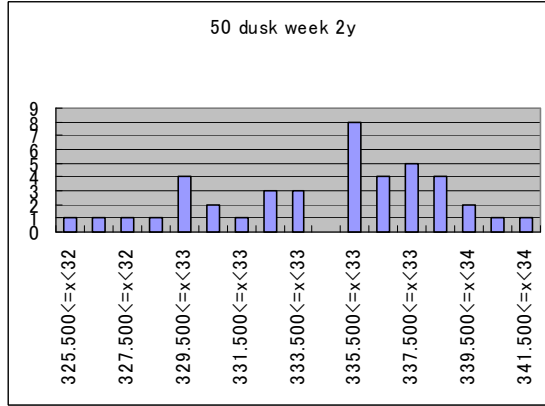
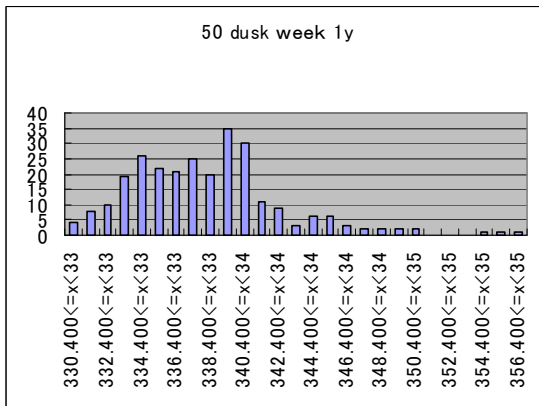
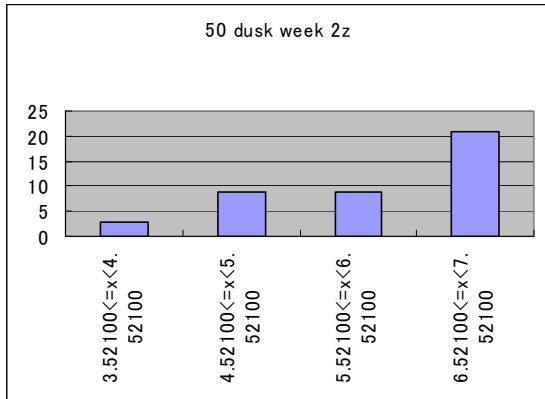
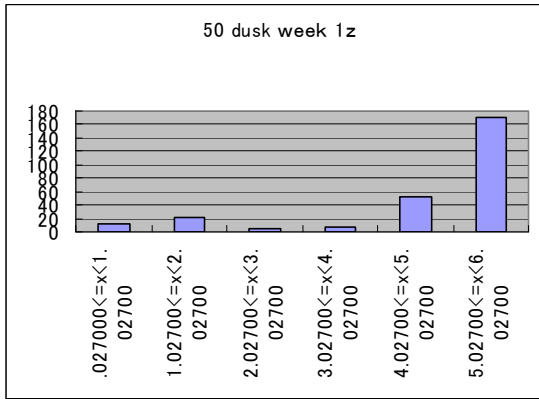


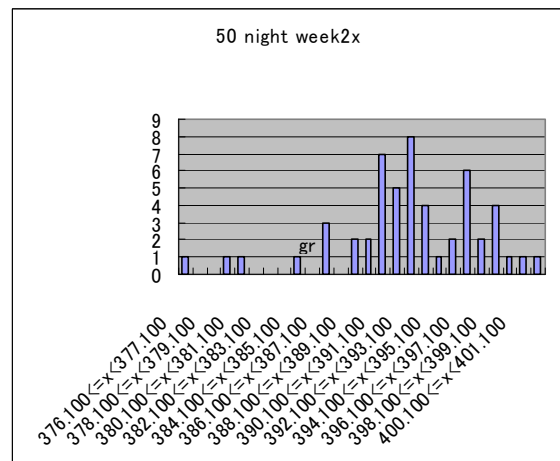
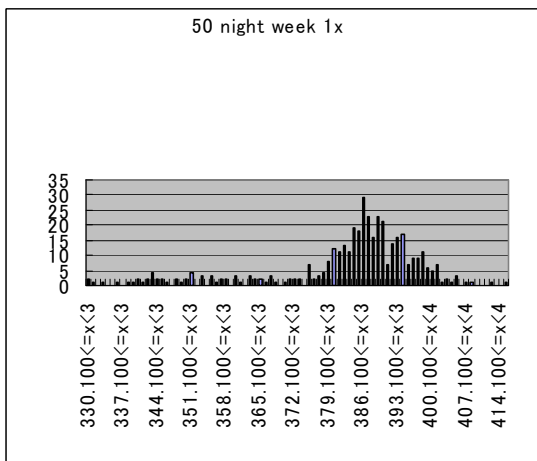
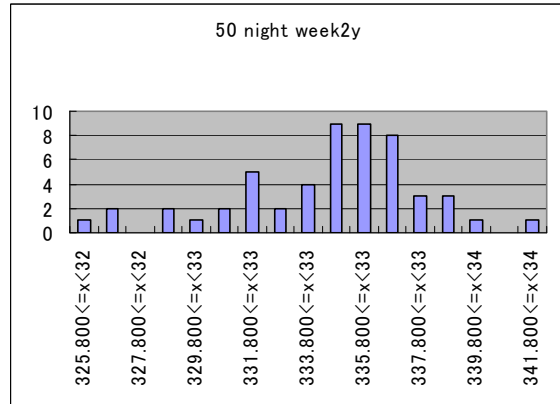
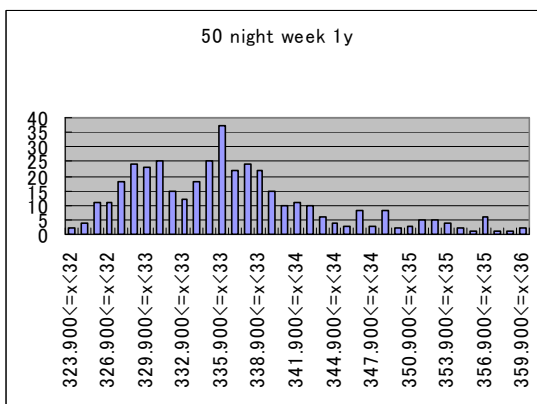
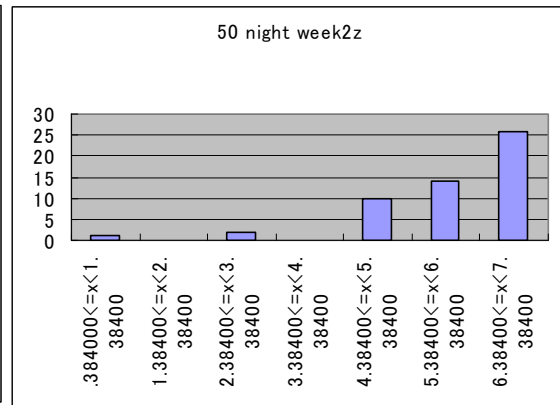
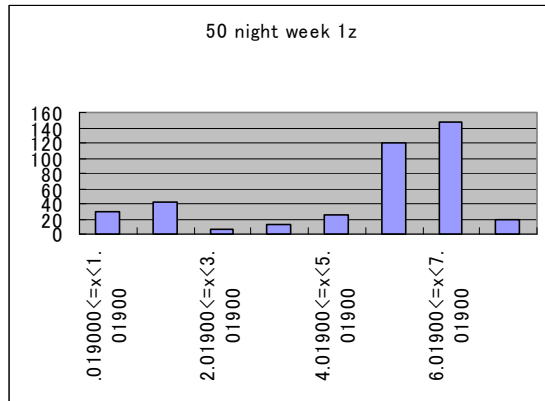












Appendix B

3D Graphs of Space Use

Data from the first two weeks of five of the immature (3-4 kg) *E. doffleini* caught by SCUBA and box trap that were successfully tagged with Radio Acoustic Transmitters. Presented here are the 3D graphs of space use broken in to Dawn (3:00-7:00), Day (7:00-16:00), Dusk (16:00-21:00), and Night (21:00-3:00).

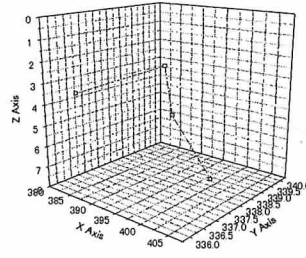
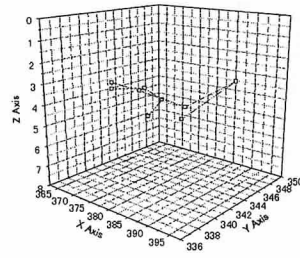
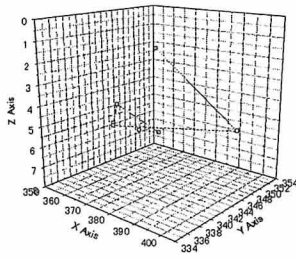
Dawn

Day

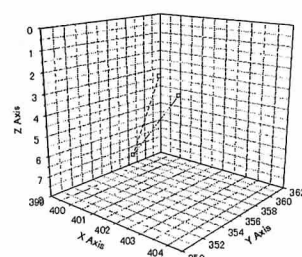
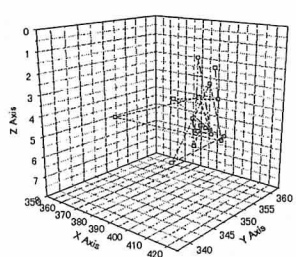
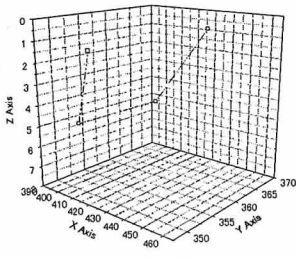
Dusk

Night

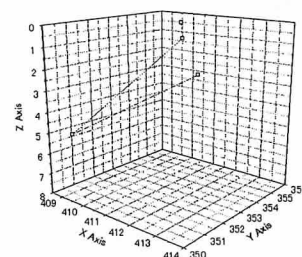
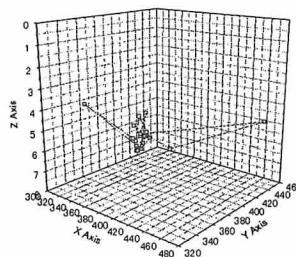
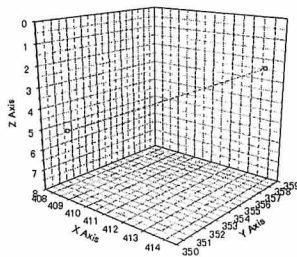
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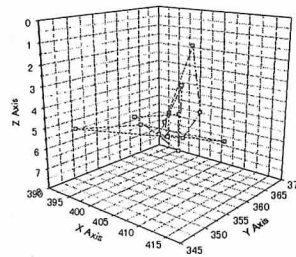
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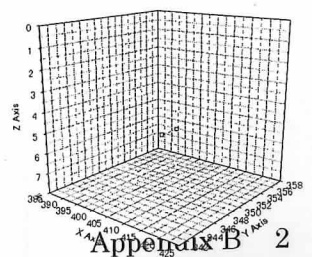
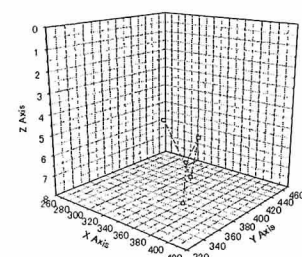
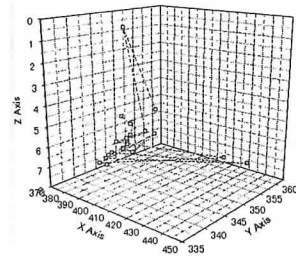
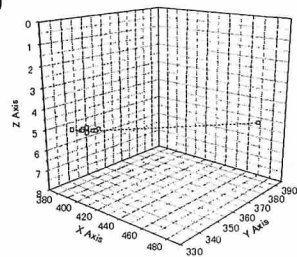
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6/9



6/10



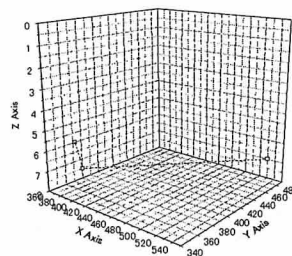
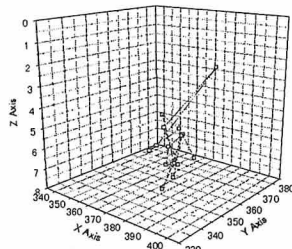
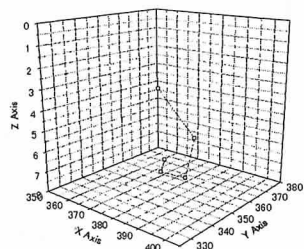
Dawn

Day

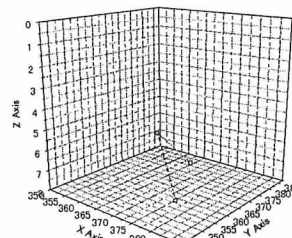
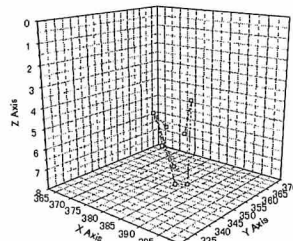
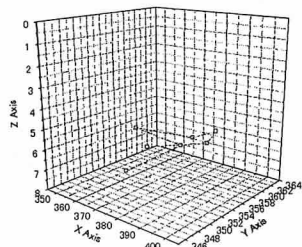
Dusk

Night

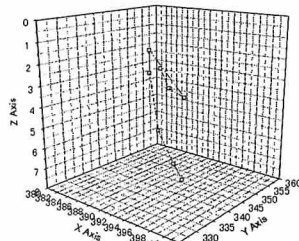
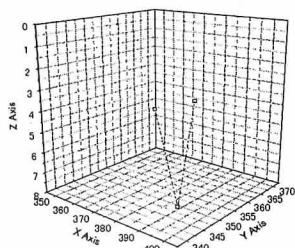
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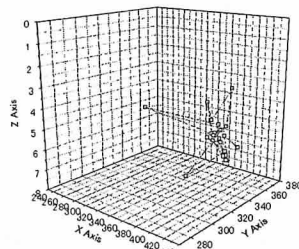
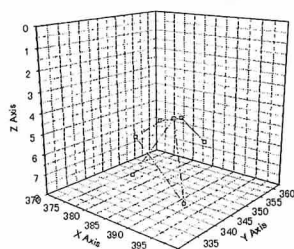
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6/13



6/14



Tag # ID 9341

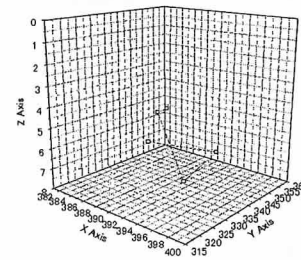
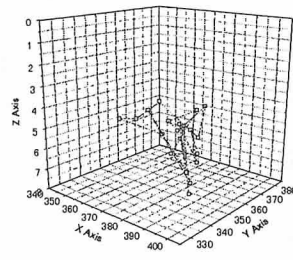
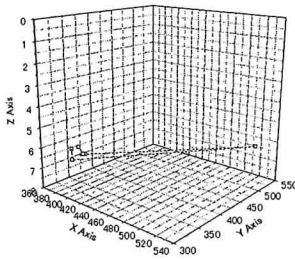
Dawn

Day

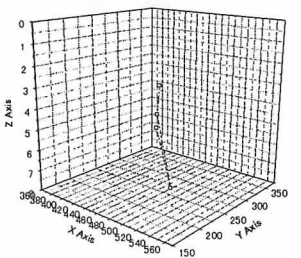
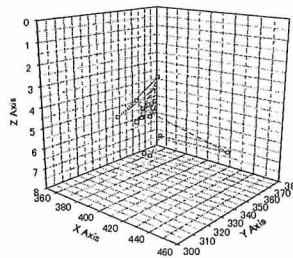
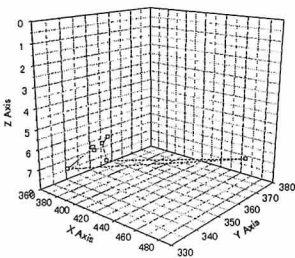
Dusk

Night

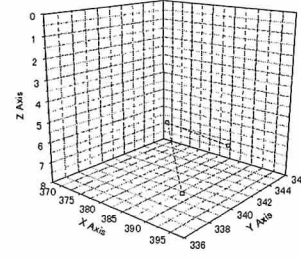
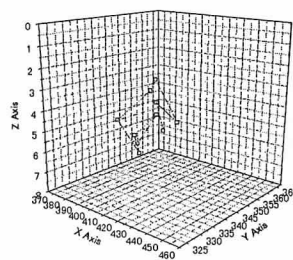
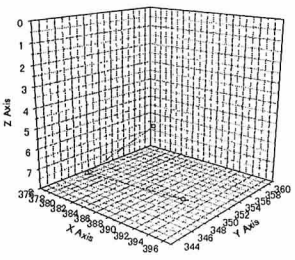
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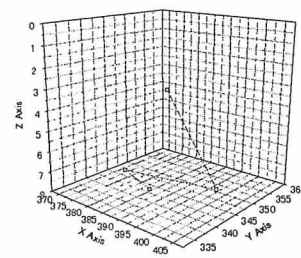
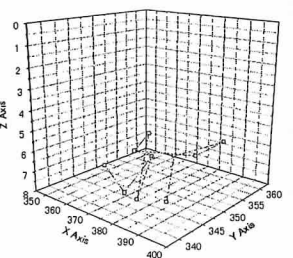
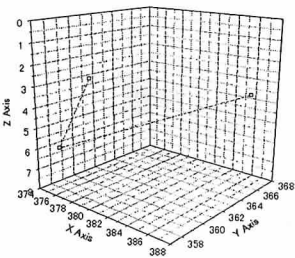
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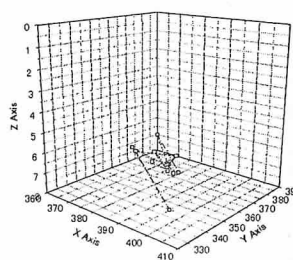
6/17



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6/19



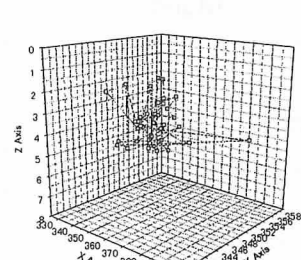
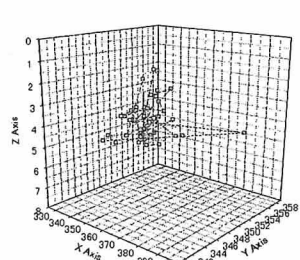
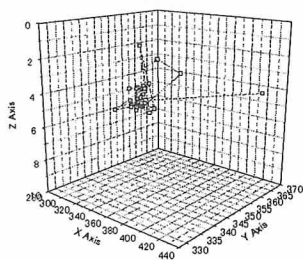
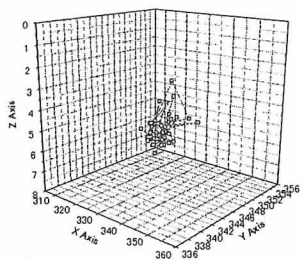
Dawn

Day

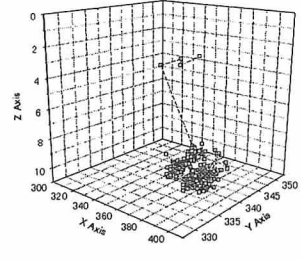
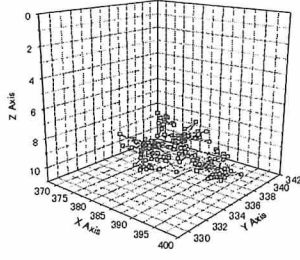
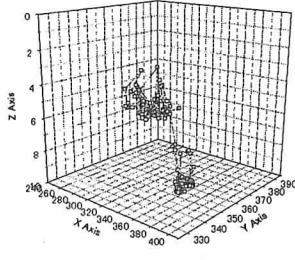
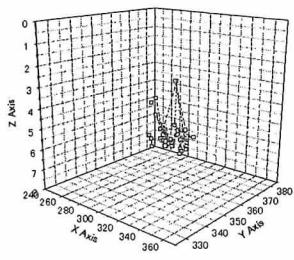
Dusk

Night

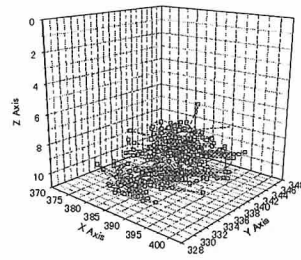
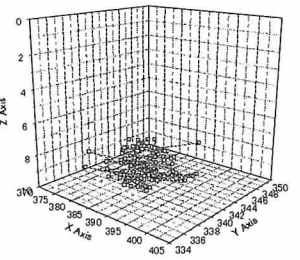
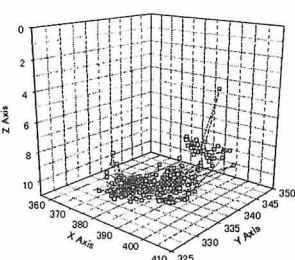
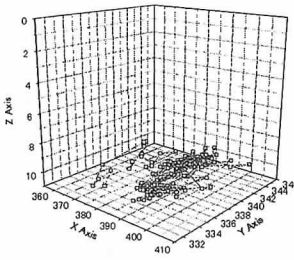
4/1 0



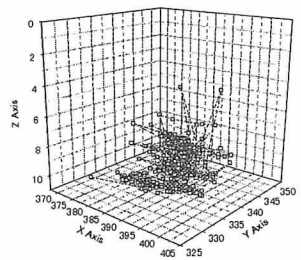
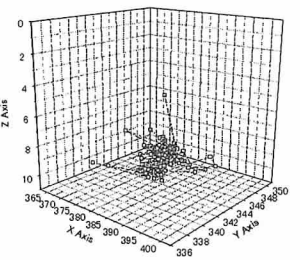
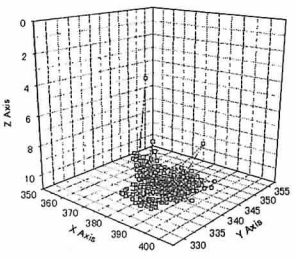
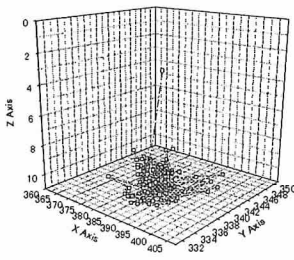
4/1 1



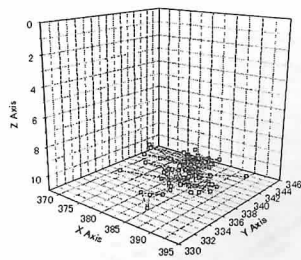
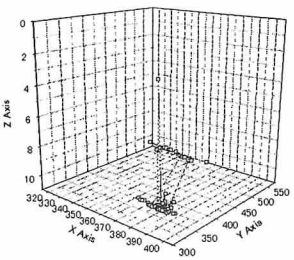
4/1 2



4/1 3



4/1 4



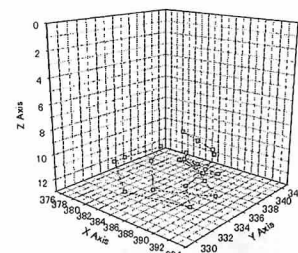
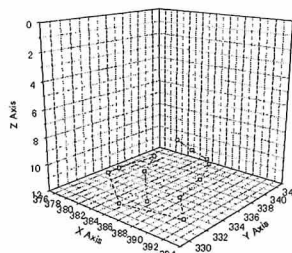
Dawn

Day

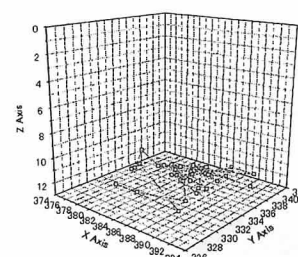
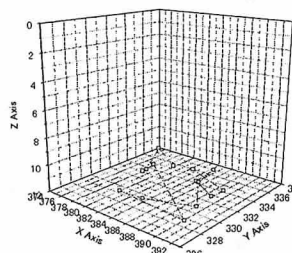
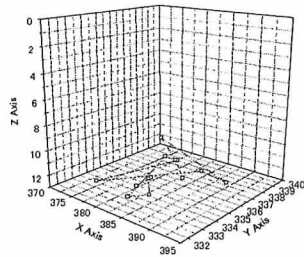
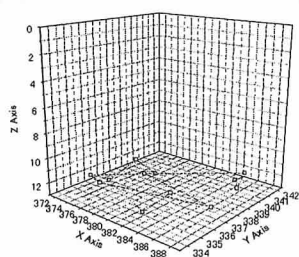
Dusk

Night

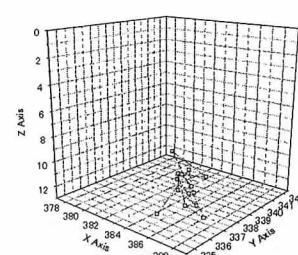
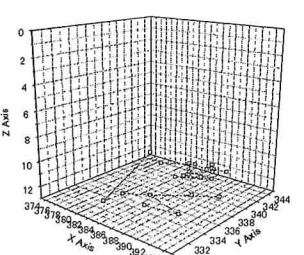
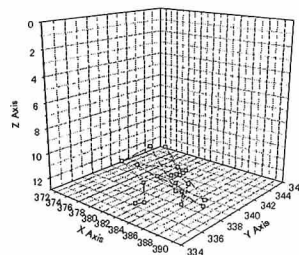
4/2 5



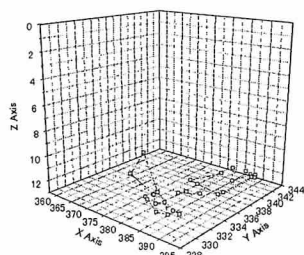
4/2 6



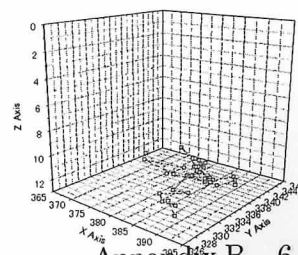
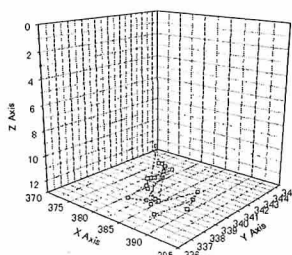
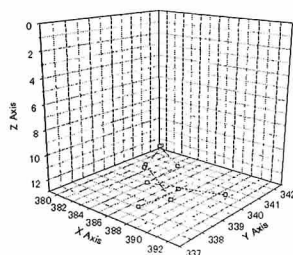
4/2 7



4/2 8



5 / 1



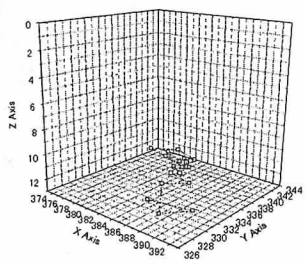
Dawn

Day

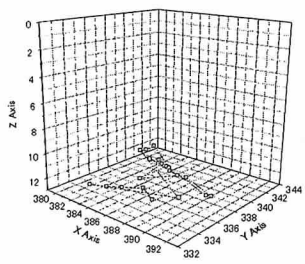
Dusk

Night

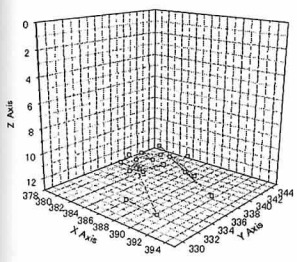
5 / 2



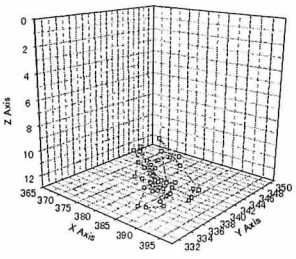
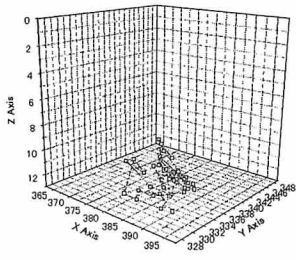
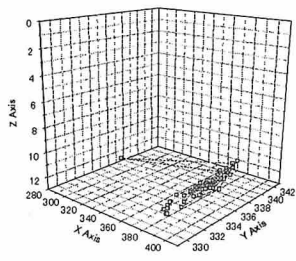
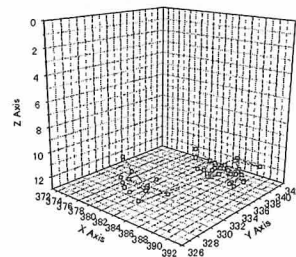
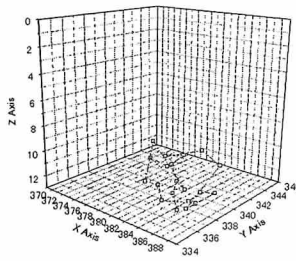
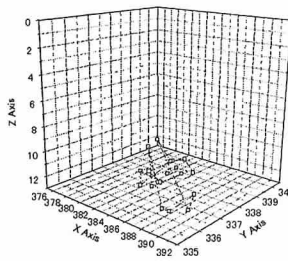
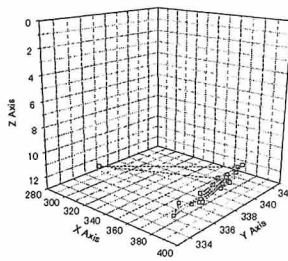
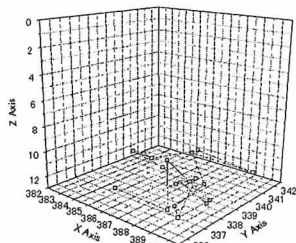
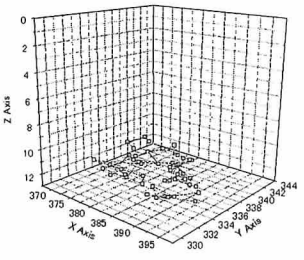
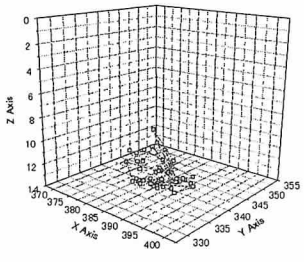
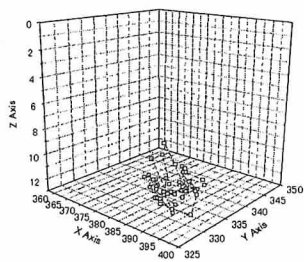
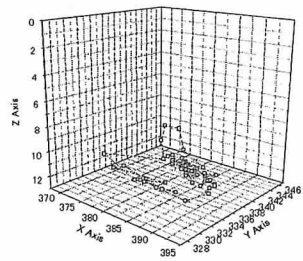
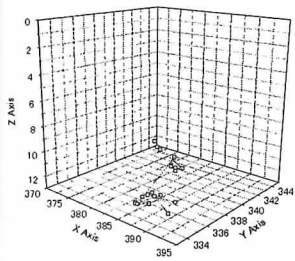
5 / 3



5 / 4



5 / 5



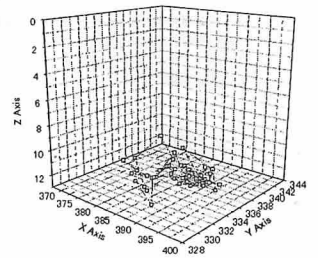
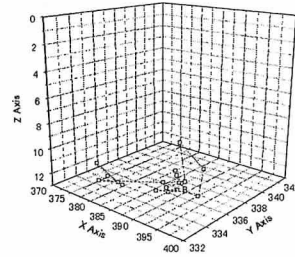
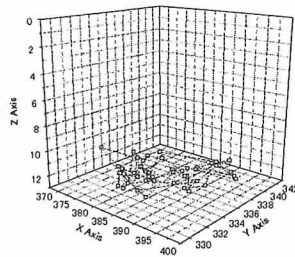
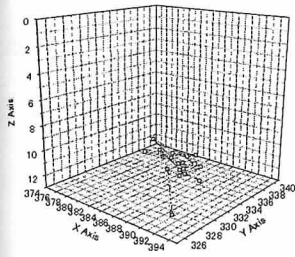
Dawn

Day

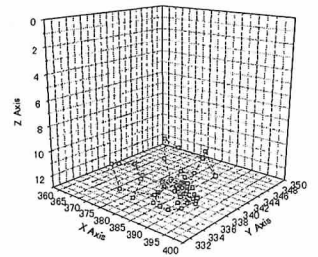
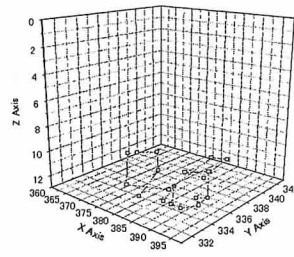
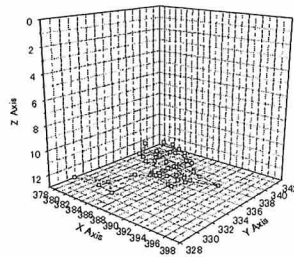
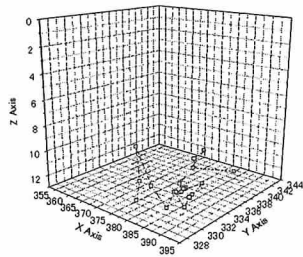
Dusk

Night

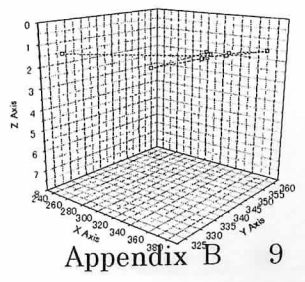
5/6



5 • 7



6/2 6



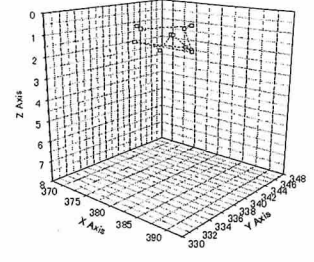
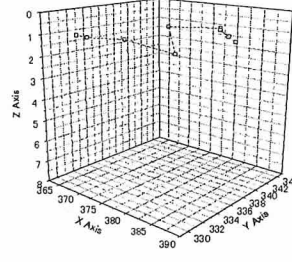
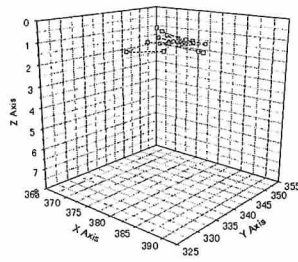
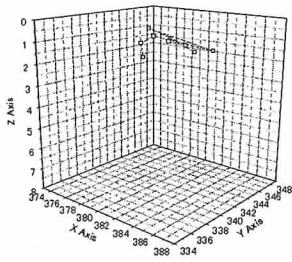
Dawn

Day

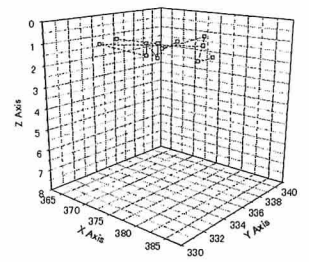
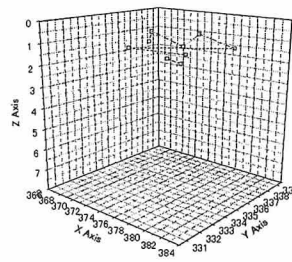
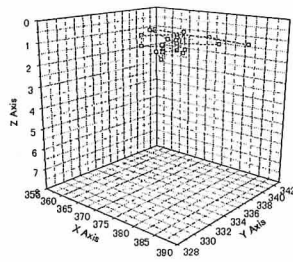
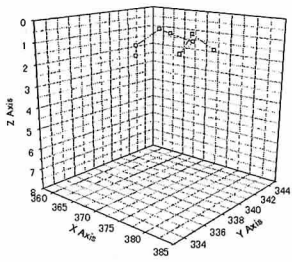
Dusk

Night

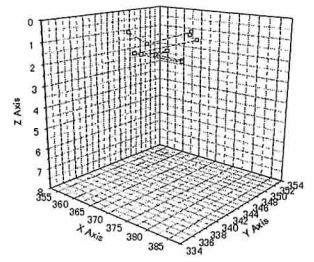
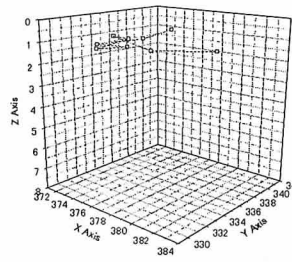
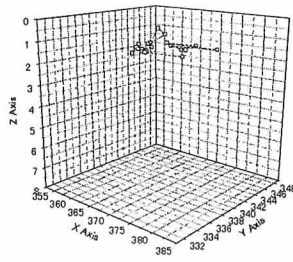
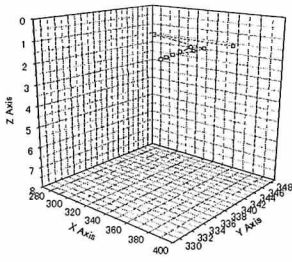
6/2 7



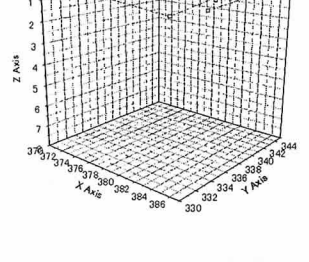
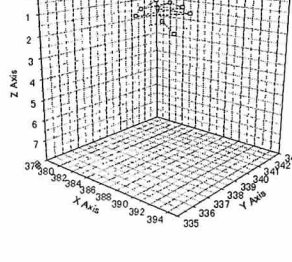
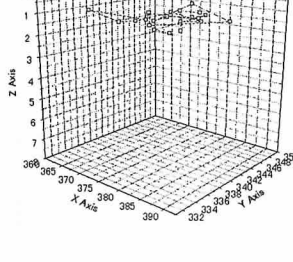
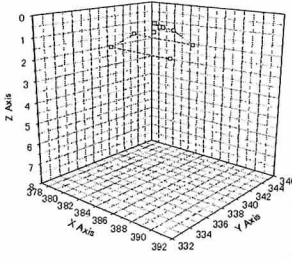
6/2 8



6/2 9



6/3 0

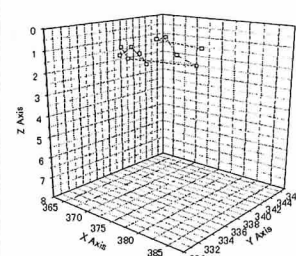
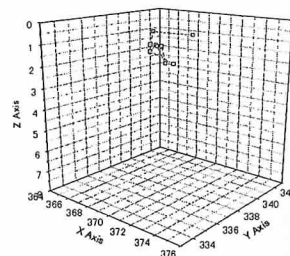
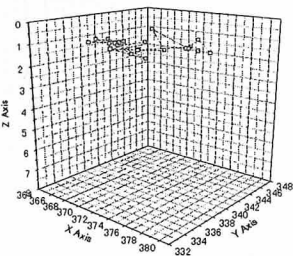
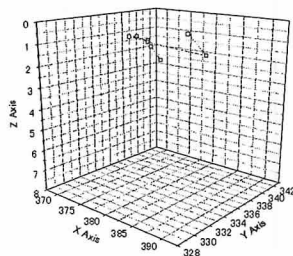


Day

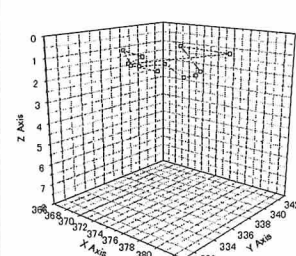
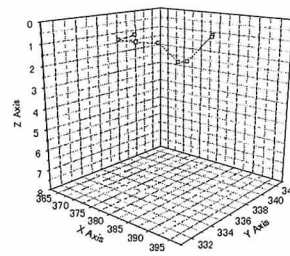
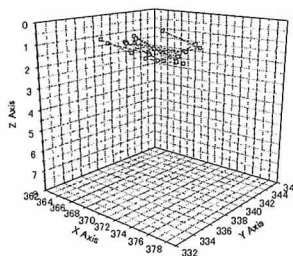
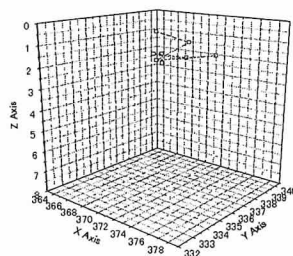
Dusk

Night

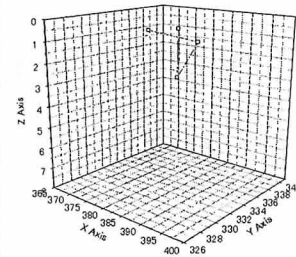
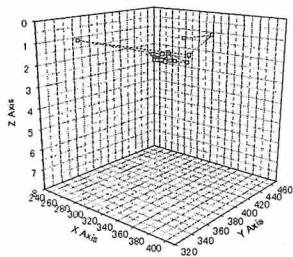
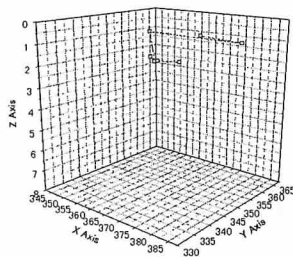
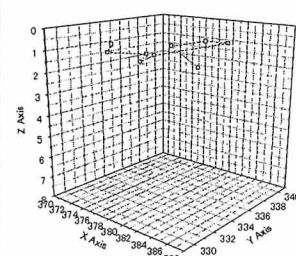
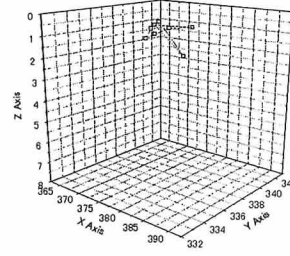
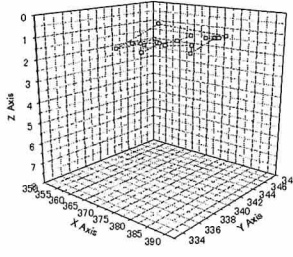
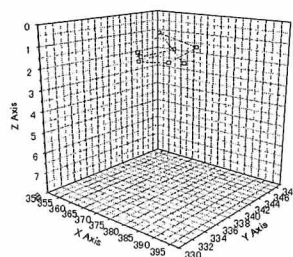
712



713



714



Dawn

Day

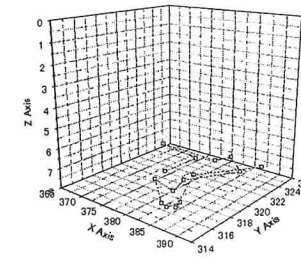
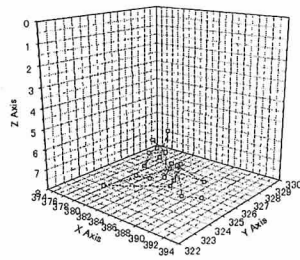
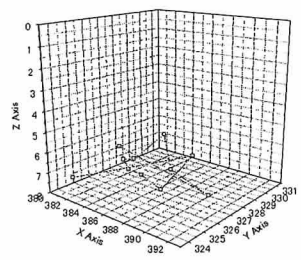
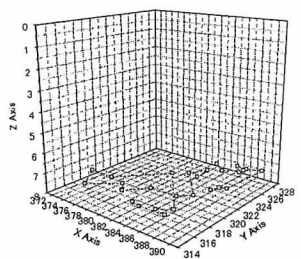
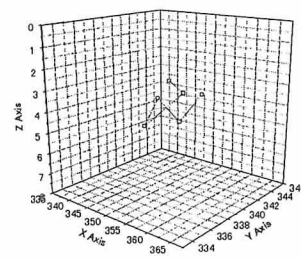
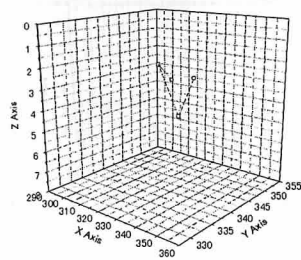
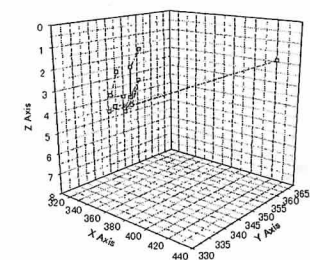
Dusk

Night

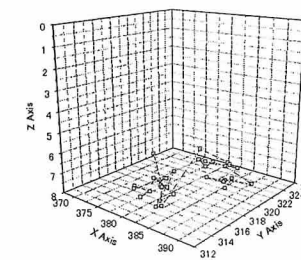
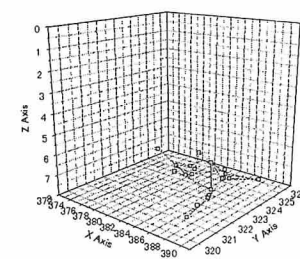
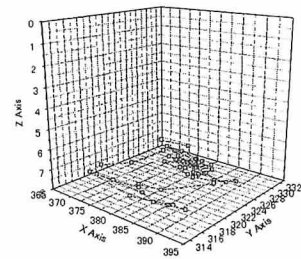
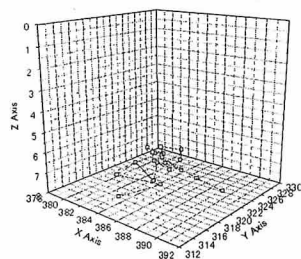
4/2 6

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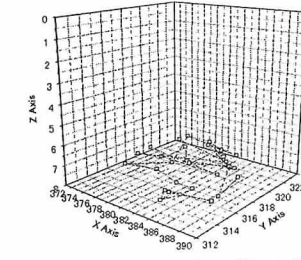
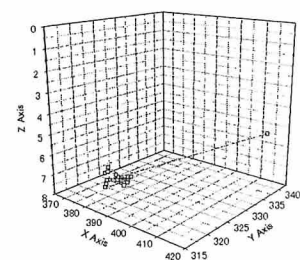
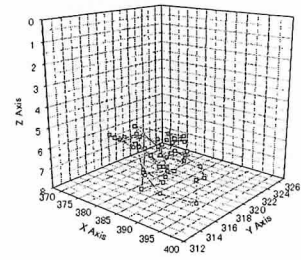
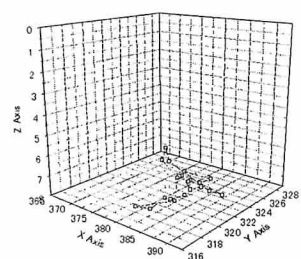
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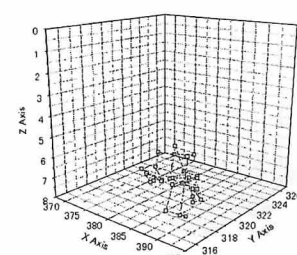
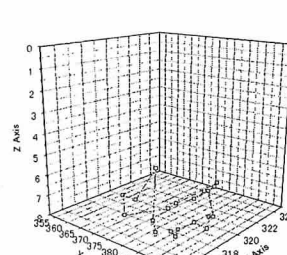
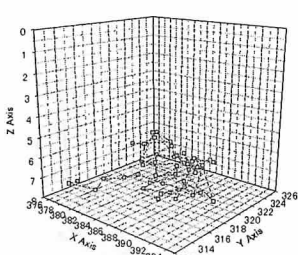
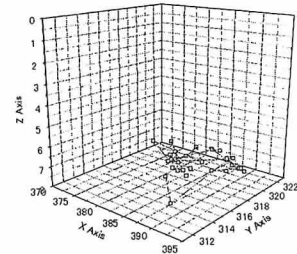
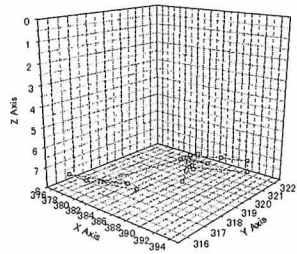
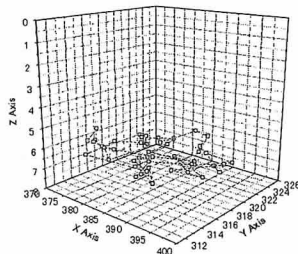
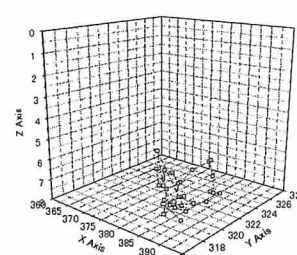
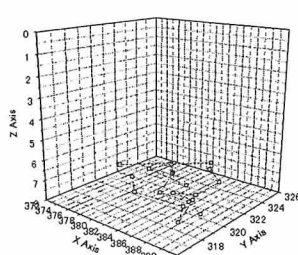
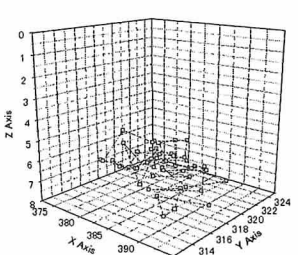
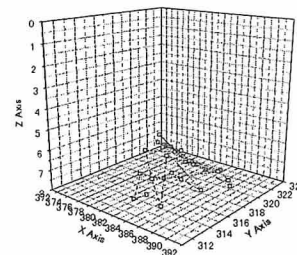
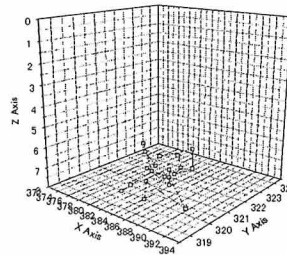
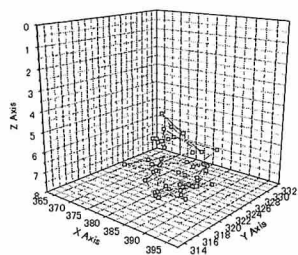


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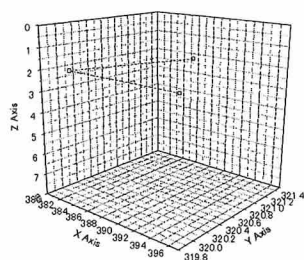
Dawn

Day

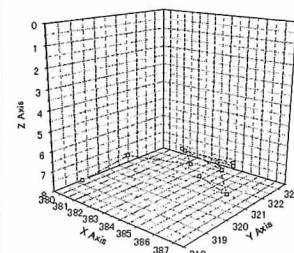
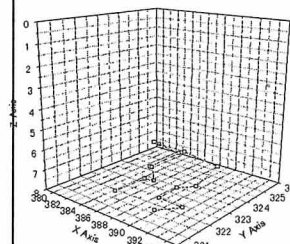
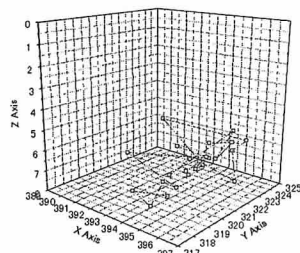
Dusk

Night

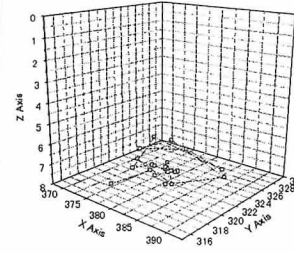
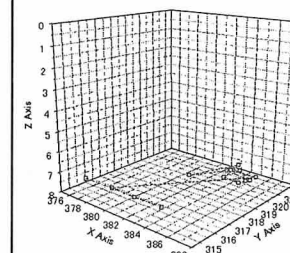
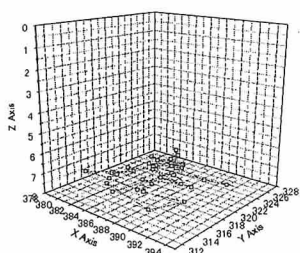
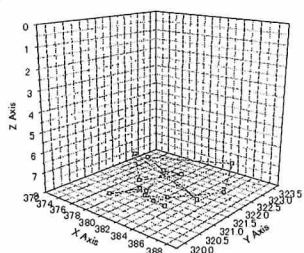
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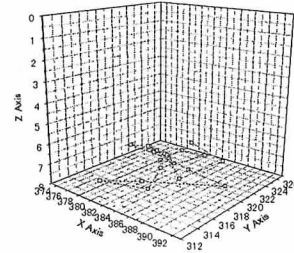
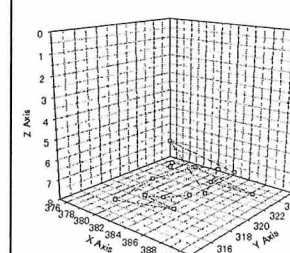
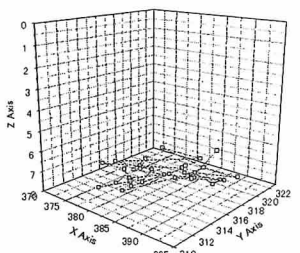
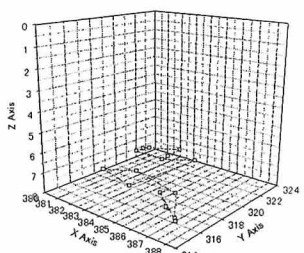
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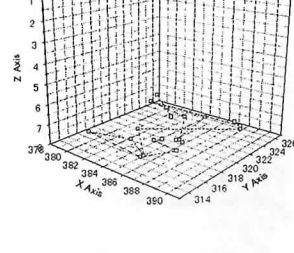
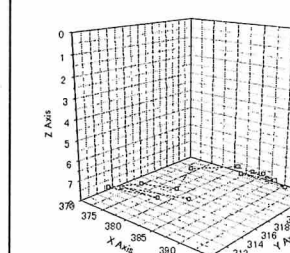
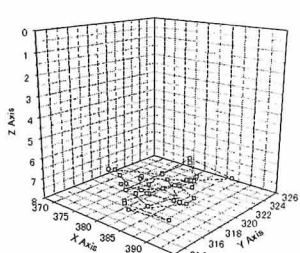
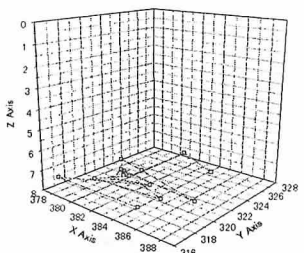
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5/11



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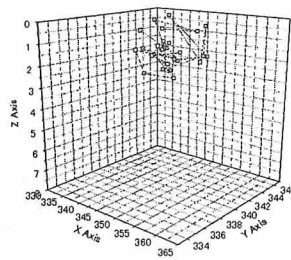
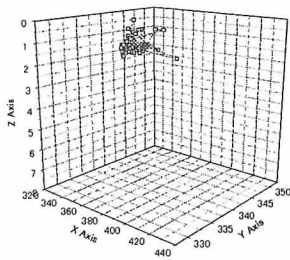
Dawn

Day

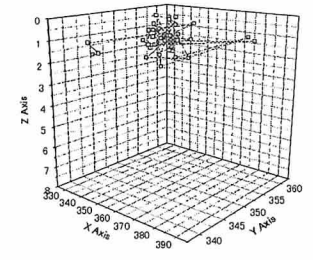
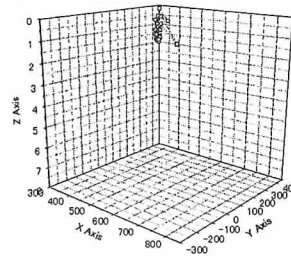
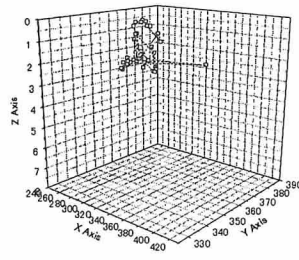
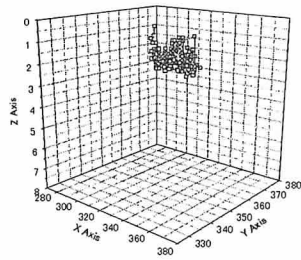
Dusk

Night

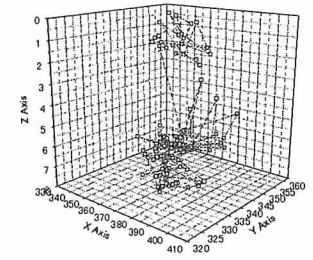
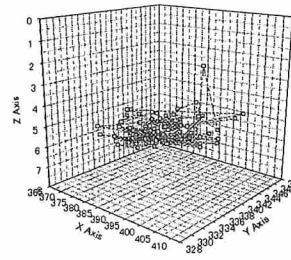
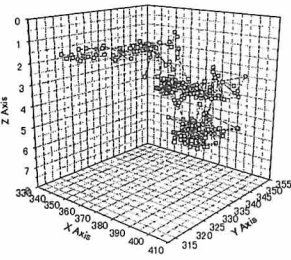
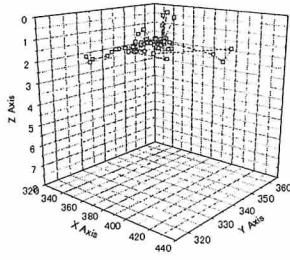
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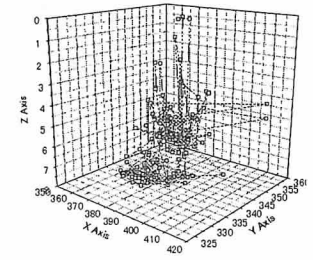
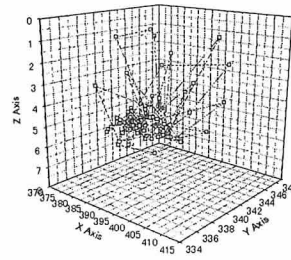
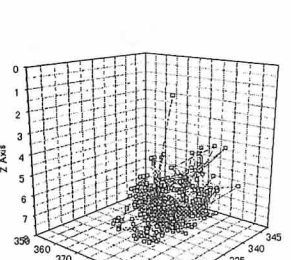
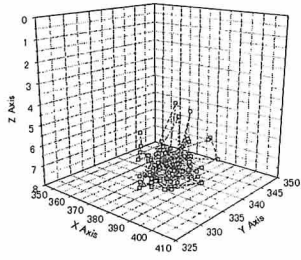
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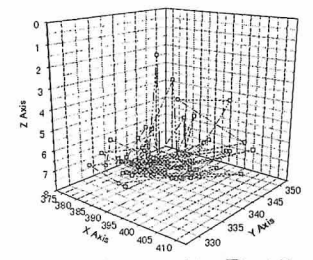
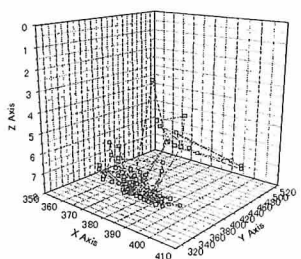
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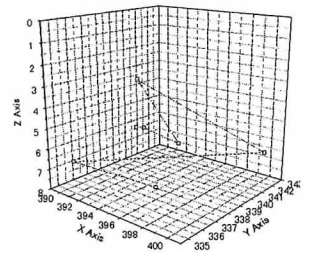
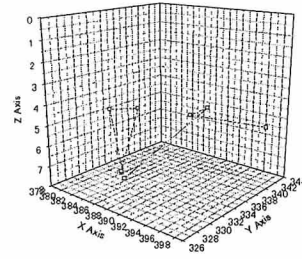
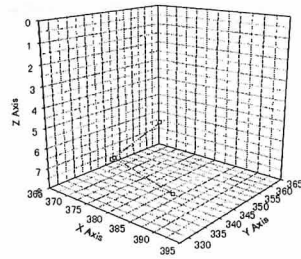
Dawn

Day

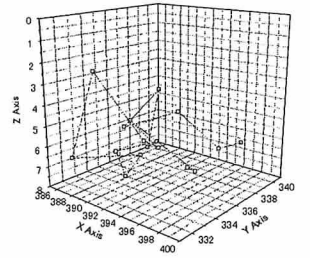
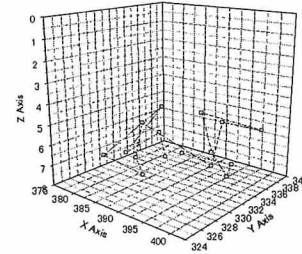
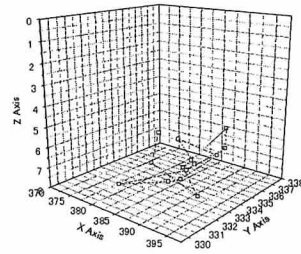
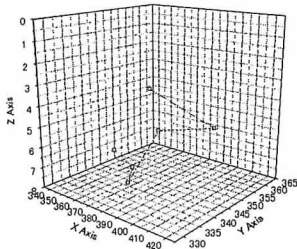
Dusk

Night

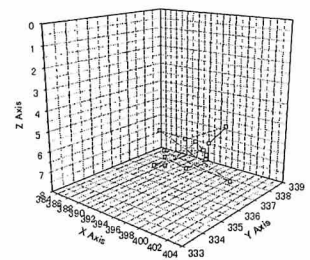
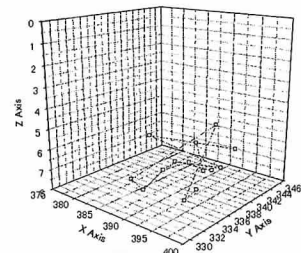
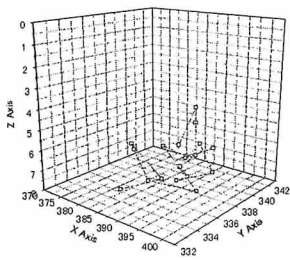
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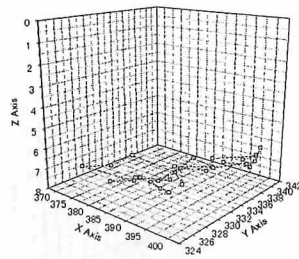
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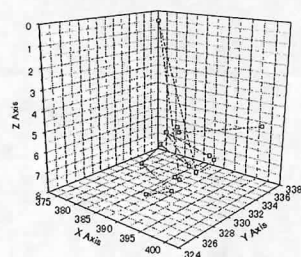
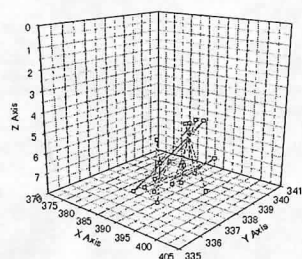
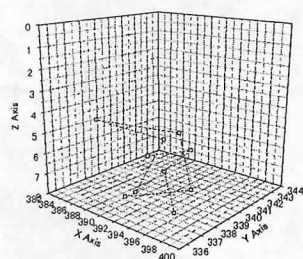
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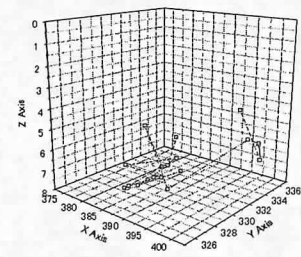
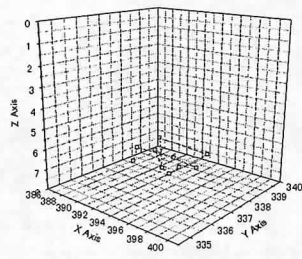
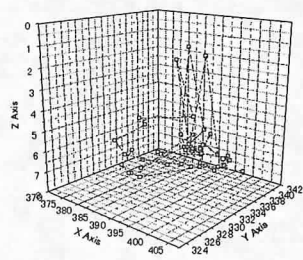
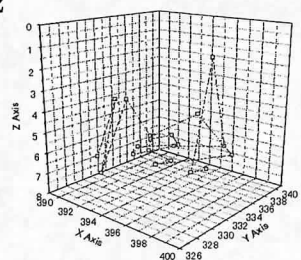
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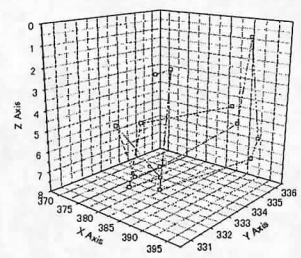
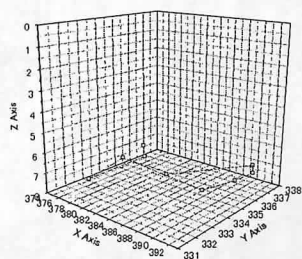
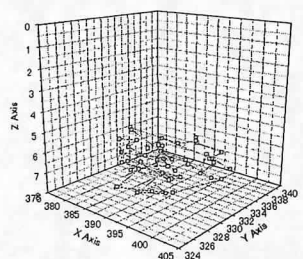
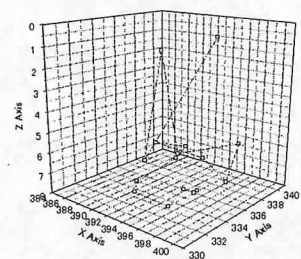
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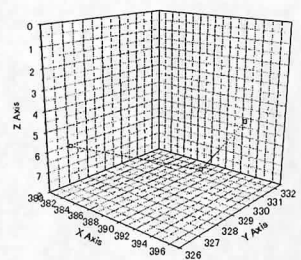
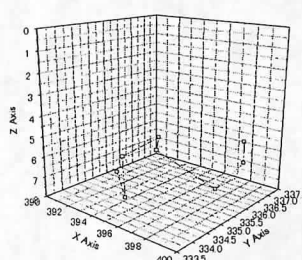
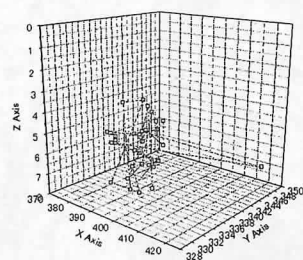
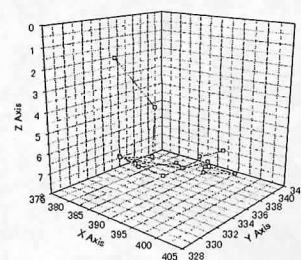
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