博士論文

論文題目　　Foraging habit and behavioural thermoregulation of ocean sunfish *Mola mola* in the Northwest Pacific

（北西太平洋域におけるマンボウ*Mola mola*の採餌生態と行動的体温調節）

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Chapter 1   General introduction

One of the long-standing ironies within marine science remains the lack of knowledge of many iconic and charismatic species, reflecting a lack of commercial interest in a particular species or an exclusion from the regional and international conservation strategies that focus research efforts upon certain target species (Pope et al. 2010).   These issues are inherent in many large pelagic fishes by the difficulties of gathering data because of their vast habitat.   The ocean sunfish (*Mola mola*) is one of the best adapted fish to pelagic habitat, and famous for the world's heaviest teleost species, growing up to 2,300 kg in mass and 2.7 m in length (Roach 2003).   The most obvious morphological characteristic of this species is the degeneration of the vertebral column resulting in the replacement of the caudal fin by a broad, stiff lobe, the *clavus* (Fraser-Brunner 1951).   This strange morphology, likened to a "swimming head" (Thys 1994).

Japanese people seem to have a lot of interest in this unique shaped fish from of old.   The first capture record of ocean sunfish in Japanese literature was in 1647, that one ocean sunfish caught at Fukagawa, Tokyo was presented to Edo bakufu (Isono 2005).   From that to 1856, ocean sunfish appeared in 28 records, at all around Japan including both Pacific side and Sea of Japan side, and the largest record was about 3.3 m in total length (Isono 2005).   The most detailed Japanese antique document specialized to ocean sunfish, "Manbou-Kou" (Kurimoto 1825), showed much biological information about this species, for example, they are rarely caught from winter to spring, but frequently captured from summer to autumn in Pacific side of east Japan, they were extremely sluggish and well observed 'sleeping' at the surface enabled fishermen to catch them easily, how to cook and eat them, and so on.   The documents also referred to foraging habits of this species, that ocean sunfish preferred to eat jellyfish and never ate other things.   Existence of these literatures suggests that old Japanese people was

interested in and tried to know about this impressive species.

Ocean sunfish are also popular to common people of recent years and several aquariums keep them for exhibition. Until recently, however, it has been difficult to keep them under captive condition because of lack of information about their ecology. Recently, keeping techniques for ocean sunfish has been developed and the longest record to maintain the ocean sunfish under captive condition was 2993 days (Nakatsubo et al. 2007). It is difficult to know age of sunfish because they don't have otolith, resulting no studies have been conducted on age, growth or reproductive biology of ocean sunfish in the wild, though information about growing of ocean sunfish has been inferred from the individuals under captive conditions. A growth curve derived from repeated measurements of captive specimens estimated an individual with a total length of 3 m would be about 20 years old (Nakatsubo 2008). Highly dependent on the starting size of the captive fish, ocean sunfish grow on average 0.1 cm day^{-1} in length (Nakatsubo and Hirose 2007). In addition, reproductive biology of ocean sunfish has been partly revealed from the captive individuals, that the spawning period of ocean sunfish in Japanese water is estimated as between August and October and the same study also identified asynchronous egg development, suggesting they are multiple spawners (Nakatsubo et al. 2007).

Recent study of the phylogenetic relationship of species *Mola mola* in Japanese water found there to be at least two genetically isolated clades (Groups A and B) (Yoshita et al. 2009, Yamanoue et al. 2010). Group A consisting of southern Pacific coast specimens, and group B containing specimens from various locations around Japan (Yoshita et al. 2009). These two groups also have morphological differences, however, it is still difficult to distinguish these two groups by appearance because ocean sunfish change its shape according to growth stage. Large individuals of group A sunfish have obvious characteristic (large head, smooth skin, round clavus) (Yoshita et al. 2009, Sawai et al. 2011). These characteristics are similar to southern sunfish *Mola*

ramsayi (Yoshita et al. 2009), whose habitat was regarded as only distributing in the southern hemisphere but also reported from the northern hemisphere (Yasemi and Nazari Bejgan 2014), and group A sunfish were genetically close to *Mola ramsayi* (Yoshita et al. 2009). Bass et al. (2005) also inferred there were two genetically isolated groups in *Mola mola* caused by possibly misidentifying. Samples were field identified as *Mola mola* usually with unfettered access to the entire fish. The consistent identification of these animals as *Mola mola* suggests that the large degree of genetic divergence we uncovered between *Mola mola* and *Mola ramsayi* is not reflected strongly in the overall morphology (Bass et al. 2005). Group A sunfish appeared at higher temperature than group B sunfish (Sawai et al. 2011) in the Pacific side of northeast Japan, and there only appear large individuals (>2 m in total length) belong to group A (Sawai et al. 2011). Fishermen in that region distinguish group A sunfish from group B sunfish by their different appearance and call them 'makkabu' or 'mizumori' (Nakamura personal observation). In addition, there exist another species of Molidae: sharptail mola *Masturus lanceolatus*, in the Pacific side of northeast Japan. Sharptail mola is considered to distribute in tropical and subtropical (Liu et al. 2009).

Ocean sunfish are considered to be obligate feeder on gelatinous zooplankton, and they are commonly found singly or often associated with swarms of salps or jellyfishes at the surface (Norman and Fraser 1949, Fraser-Brunner 1951, MacGinitie and MacGinitie 1968, Hooper et al. 1973, Bass et al. 2005). Wide range of prey items including gelatinous plankton, such as scyphozoan, hydrozoan, salps and ctenophores, were reported from stomachs of ocean sunfish (Fraser-Brunner 1951). Additionally, various benthic organisms including algae, crustaceans, ophiuroids, molluscs, hydroids and fish have also been found in their stomachs (Fraser-Brunner 1951; Norman and Fraser 1949, Clemens and Wilby 1961, Hart 1973). Schmidt (1921) stated that ocean sunfish was known to feed on small forms of pelagic life, including large numbers of leptocephalus eel larvae.

Movements of ocean sunfish under natural conditions were mysterious until recent years because of difficulty of observation. Most existing information on natural history suggests that ocean sunfish are planktonic species and moves primarily by passive transport via ocean currents (Norman and Fraser 1949). Recent electrical tagging studies have given a lot of information about their behaviour under natural conditions. Studies deploying accelerometers on ocean sunfish suggested that ocean sunfish were active swimmer because they continued to swim during deployment time for several hours (Watanabe and Sato 2008, Houghton et al. 2009). A study using acoustic tracking found that ocean sunfish spent the majority of their time near the surface and swam back and forth between the surface and over 100 m, thought to related to foraging (Cartamil and Lowe 2004). An obvious diel vertical distribution pattern was also found, with nocturnal vertical movements confined to the surface mixed layer and thermocline, while diurnal vertical movements included repeated dives below the thermocline (Cartamil and Lowe 2004). Other studies using satellite tracking also suggested that ocean sunfish occupied significant greater depth during the day (Sims et al. 2009a). This behaviour matches the strategy of normal diel vertical migration and has been attributed to a strategy of near continual feeding on vertically migrating prey (Sims et al. 2009a, Hays et al. 2009). However, concurrent data on the distribution of potential prey have not yet been analyzed, furthermore, there is currently no evidence of when, what and how they forage under natural condition. Despite showing normal diel vertical migration when it occurs, ocean sunfish repeatedly return to the surface during the day and do not simply remain at depth (Cartamil and Lowe 2004, Hays et al. 2009). The studies demonstrated a positive relationship between maximum dive depth and duration of post-dive period in the surface water, suggesting a mechanism of behavioural thermoregulation, gaining heat to aid in digestion, or recovery from lower dissolved oxygen at depth (Cartamil and Lowe 2004). However, there is currently no physiological evidence from ocean sunfish to support this. The surfacing behaviour

may also benefit as parasite elimination.   Ocean sunfish is known to harbor at least 54 different species of parasites (Love and Moser 1983).   Albatrosses have been observed picking parasites from the bodies of ocean sunfish during surfacing (Abe et al. 2012).

The objective of the present study was to clarify behavioural ecology of ocean sunfish especially on foraging in relation to food and temperature environments. Ocean sunfish behave according to food and temperature environments during foraging. To reveal their behaviour relation to temperature environment, fishing survey to clarify their thermal preference and behavioural study to investigate their usage of temperature environment were conducted.   To reveal their behaviour relation to food environment, dietary analyses to clarify their diets and behavioural study using animal-borne devices to investigate their foraging behaviour under natural condition were conducted.   All ocean sunfish used for the present study were distinguished by morphological characteristic as Sawai et al. (2011) and all ocean sunfish belong to group B (Yoshita et al. 2009).

Chapter 2   Thermal preference from catch survey

1. Introduction

The ocean sunfish is a typical pelagic species, so little is known about migration pattern of this species.   Recent tracking studies using satellite transmitters have revealed some of their seasonal migrations; moving to north during summer and coming back to south during winter in northern hemisphere (Sims et al. 2009a, 2009b, Dewar et al. 2010, Potter et al. 2011).   This migration pattern could be well explained by their foraging area or thermal preference.   They might be moving with seasonal change of frontal areas where their preys might be abundant.   However, these tracking studies showed only a few individuals movements, and little is known about relationship between temperature and ocean sunfish migration.

Seasonal catch rate of fishes at a certain region should reflect seasonal migration of the species into the region.   Unfortunately, there is no fishery that mainly targets on ocean sunfish in Japan, because of their quite low commercial value.   Main catch of ocean sunfish depends on occasional by-catch by offshore long-lines or coastal set-nets.   Ocean sunfish caught by coastal set-nets are consumed as local food in several coastal regions of Japan.   Sanriku region (Fig. 2-1 left), the Pacific side of northern Japan, is famous for one of the best fishing area of Japan and various size of ocean sunfish are caught by coastal set-nets from summer to autumn when water temperature becomes high (Sagara and Ozawa 2002, Sawai et al. 2011).   The catch rate of ocean sunfish in Sanriku region seems to reflect their temperature preference. In this chapter, the thermal preference of ocean sunfish was quantified by counting the number of sunfish caught by set-nets.   To investigate catch rate of ocean sunfish, there are several difficulties.   First, small ocean sunfish which are not enough large to sell are thrown away on the sea and are not landed onto the fish market.   In addition, large ocean sunfish are disassembled on the fishemen's boat and landed only their meats,

livers and intestines onto fish market.   Thus, it is necessary to investigate the number of ocean sunfish caught by set-nets on site.   Therefore, the daily number of sunfish caught by set-nets was counted on the fishermen's boat in the present study.

2. Materials and methods

From 2009 to 2013, the seasonal migration of ocean sunfish to Sanriku coast was quantified by counting the daily number of sunfish (group B).   The terms of investigation were from June to July in 2009, from June to November in 2010, from June to July and November in 2012, and from June to July in 2013 (Figs. 2-2 and 2-3). Tsunami caused by the 2011 earthquake off the Pacific coast of Tohoku damaged the set-nets in the study site unfortunately, so the survey was impossible in 2011.   Three set-nets, managed by Otsuchi fisheries cooperative, placed around Funakoshi bay and Otsuchi bay, Iwate, Japan (39°21'N, 141°58'E - 39°23'N, 141°59'E) were monitored in 2009 and 2010, and three set-nets, managed by Funakoshi bay fisheries cooperative, placed around Funakoshi bay, Iwate, Japan (39°24'N, 141°59'E - 39°25'N, 142°02'E) (Fig. 2-1 right) were monitored in 2012 and 2013.   Sunfish caught by the set-nets were divided into four size class by visual estimation of approximate total length (TL): less than 50 cm, from 50 to 100 cm, from 100 to 150 cm and over 150 cm, because it is difficult to directly measure TL of all sunfish.   Regional seawater temperature was obtained via the webpage managed by Iwate Fisheries Information Report System (Iwate Tairyo Navi: http://www.suigi.pref.iwate.jp/).   The temperature data measured by a temperature sensor deployed at 3 m depth in Funakoshi bay (39°24'N, 141°58'E) was used in 2009 and 2010.   However, this sensor was destroyed by the tsunami and still unrecovered, therefore temperature data measured by a sensor deployed at 5 m depth in Yamada bay was used in 2012, and temperature data measured by a sensor deployed at 3 m depth in Kamaishi bay was used in 2013.   In addition to the capture number survey, daily total catch of Molidae (mainly consisting by group B sunfish but

including group A ocean sunfish (Yoshita et al. 2009, Yamanoue et al. 2010) and sharptail mola: *Masturus lanceolatus*) by the kilogram was obtained from Iwate Fisheries Information Report System (Iwate Tairyo Navi: http://www.suigi.pref.iwate.jp/). Daily fishing catch reports of Otsuchi fish market were used in 2009 and 2010, and daily fishing catch reports of Funakoshi fish market were used in 2012 and 2013. The fishing reports show catch of Molidae only from June to August because fish markets in the study region don't deal in sunfish in autumn.

3. Results

Various sizes of ocean sunfish were caught by all set-nets (Fig. 2-1). The smallest sunfish was 27 cm TL and the largest sunfish was over 250 cm TL. From June to July, the number of sunfish increased with raising water temperature, and peaked from mid to late July when seawater temperature was approximately 17°C (Fig. 2-2). In 2010, no sunfish were caught during August and September when seawater temperature exceeded 20°C, and catches resumed in October when water temperature decreased below 20°C (Figs. 2-2 and 2-3). Total catch from the fishing reports peaked from mid to late July in 2009, 2010 and 2012, and peaked in late June in 2013 (Fig. 2-4). The number of sunfish per day peaked at 11-17℃, and especially catch of sunfish larger than 100 cm peaked between 15-17℃ (Fig. 2-5). Total catch by the kilogram per day peaked at 13-17℃ (Fig. 2-5).

Many sunfish of less than 50 cm TL were caught in July and early October 2010, and a large number of sunfish of 50-100 cm TL were caught during November 2012 (Figs. 2-2 and 2-3). Almost of all sunfish captured from October to November were in the same body size category (Figs. 2-3 and 2-6). From June to July, proportion of sunfish of less than 50 cm TL was larger in 2009 and 2010 than in 2012 and 2013, and the number of sunfish larger than 150 cm TL was more in 2012 and 2013 than in 2009 and 2010 (Figs. 2-2 and 2-6). In 2013, larger sunfish was well caught than the

other years (Fig. 2-6). Sunfish larger than 150 cm TL tended to be caught by the set-nets placed near the mouth of the bays (Fig. 2-1).

4. Discussion

Seasonal catch of ocean sunfish in the present study was similar to fishery data in Miyagi prefecture reported by Sagara and Ozawa (2002), placed south next to the present study region, Iwate,, where catch rates peak during June and July, decrease in August and increase again from October to November (Sagara and Ozawa 2002). Several previous satellite tracking studies also show ocean sunfish undertake seasonal north-south migrations in the Northern hemisphere (Sims et al. 2009a, b; Dewar et al. 2010, Potter et al. 2011). Sunfish migrations, along with those of other highly-migratory species such as whale sharks *Rhincodon typus* and swordfish *Xiphias gladius* (Eckert and Stewart 2001; Sedberry and Loefer 2001), appear to be related to sea surface temperature, with sunfish seeking out thermal fronts where high productivity results in abundant prey (Dewar et al. 2010; Potter et al. 2011). Sunfish catch rates peaked when water temperatures were between 11-17°C, especially between 15-17℃, and declined sharply when temperatures exceeded 20°C, suggesting ocean sunfish prefer these temperature range. Larger sunfish were well caught by the set-nets placed near the mouth of the bay, and barely caught by the set-net placed in the bay. The tendency that larger sunfish were captured by the set-nets facing the open ocean might be related to their pelagic habitat.

The sunfish catch is suspected from fishing reports of fish markets, however, it is impossible to know size variation and the number of sunfish because only enough large sunfish are disassembled on the fishermen's boat and landed onto the fish markets. In addition, during autumn season, ocean sunfish were not sold in the fish market because of their low commercial value in autumn. The present study showed high catch rate in 2012 and 2013 than in 2009 and 2010. The set-nets used in 2012 and

2013 placed near the mouth of the bay where facing the open ocean. This might be related to the high catch in the fishing reports in 2012 and 2013.

In conclusion, the catch of ocean sunfish was considered to reflect their thermal preference. The peak of catch at the study site suggested that sunfish preferred temperature about 15-17ºC. The catch of ocean sunfish at the study site peaked twice in a year; throughout the months of June until July and throughout the months of October until November, and few sunfish were caught in the August-September period suggested that ocean sunfish had narrow temperature preference and migrate according to seasonal change of water temperature.
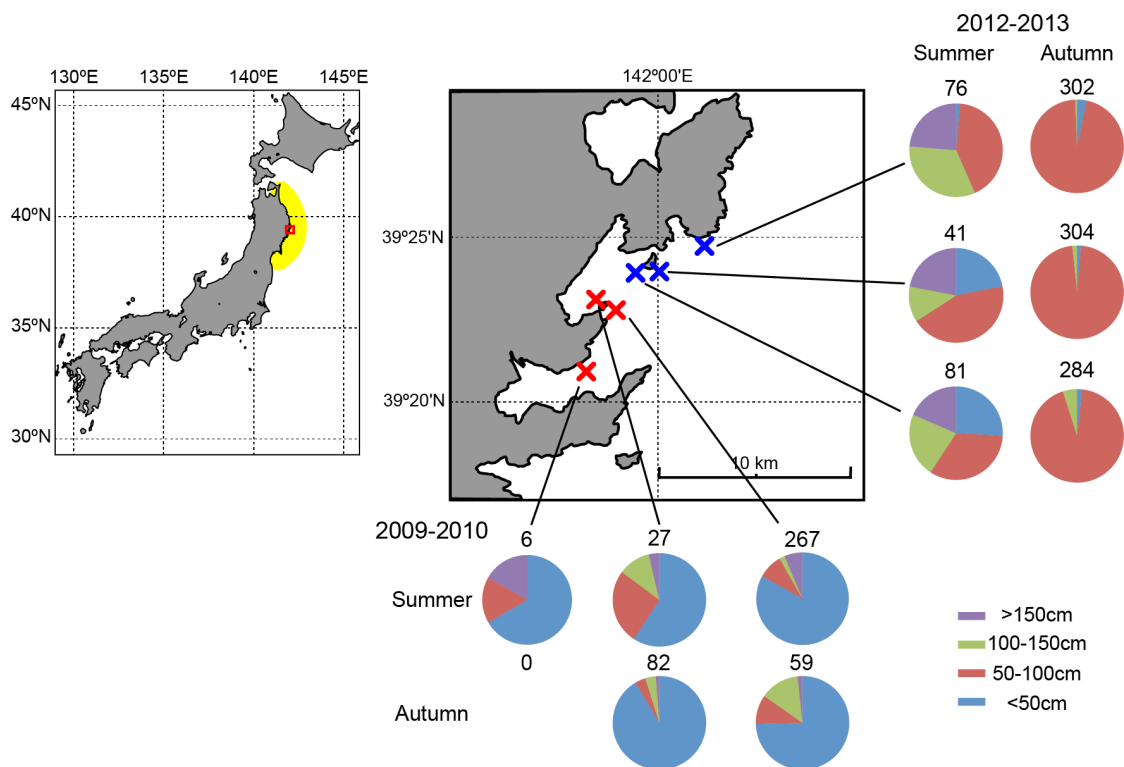
Fig. 2-1 The location of the study site (left). A red square in the map indicates the study site and a yellow coloured area indicates "Sanriku region". Enlarged map of the study site (right). Red and blue crosses in the right panel show the locations of the set-nets managed by Otsuchi fisheries cooperative and Funakoshi bay fisheries cooperative, respectively. Circular charts indicate the proportion of body size of sunfish caught by each set-net. The numbers above the charts indicate total number of sunfish caught by each set-net.
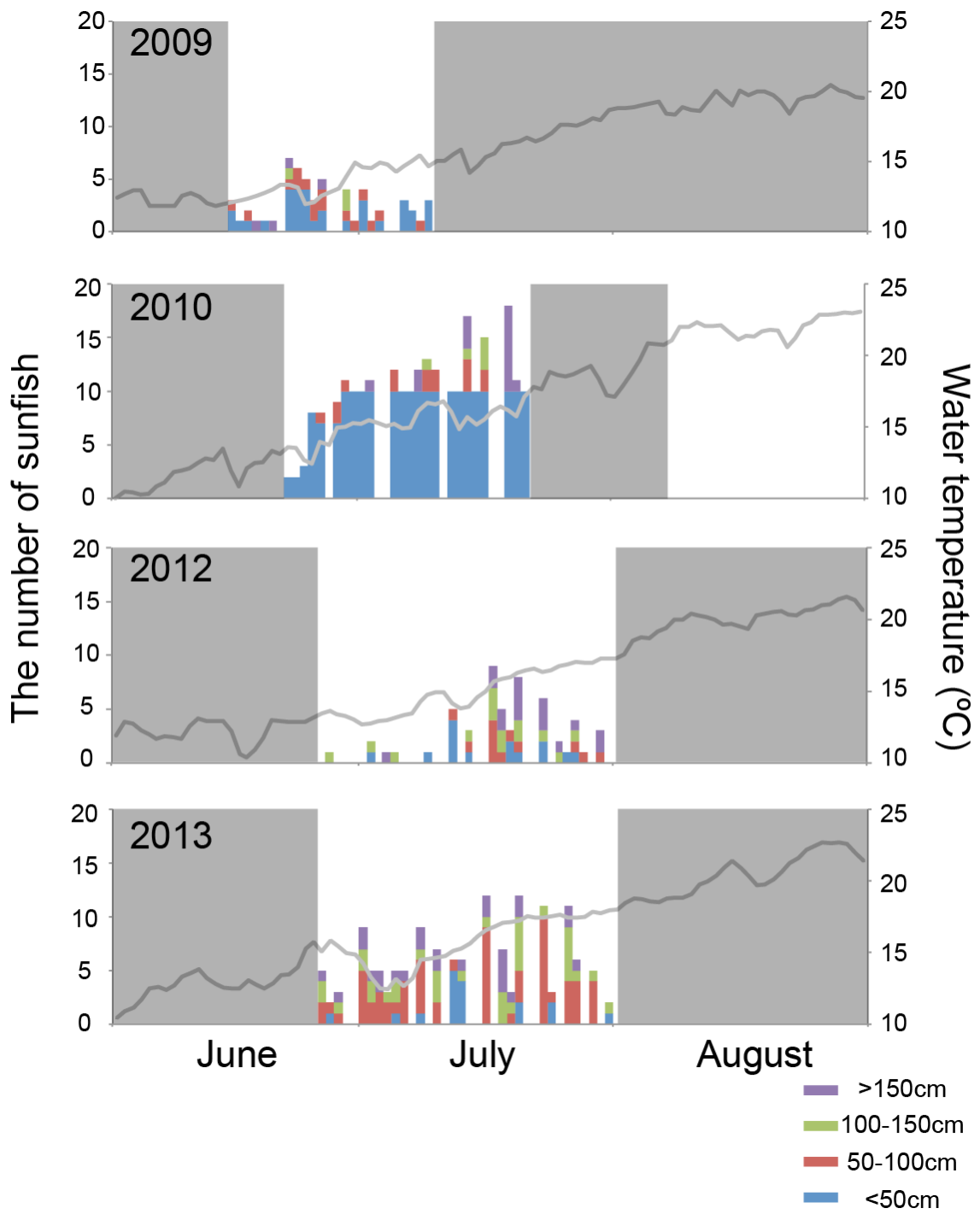
Fig. 2-2 The daily catch number of ocean sunfish from June to August. Lines indicate daily water temperature via web site (Iwate Tairyo Navi). Shadow areas in each graph indicate out of investigating terms.
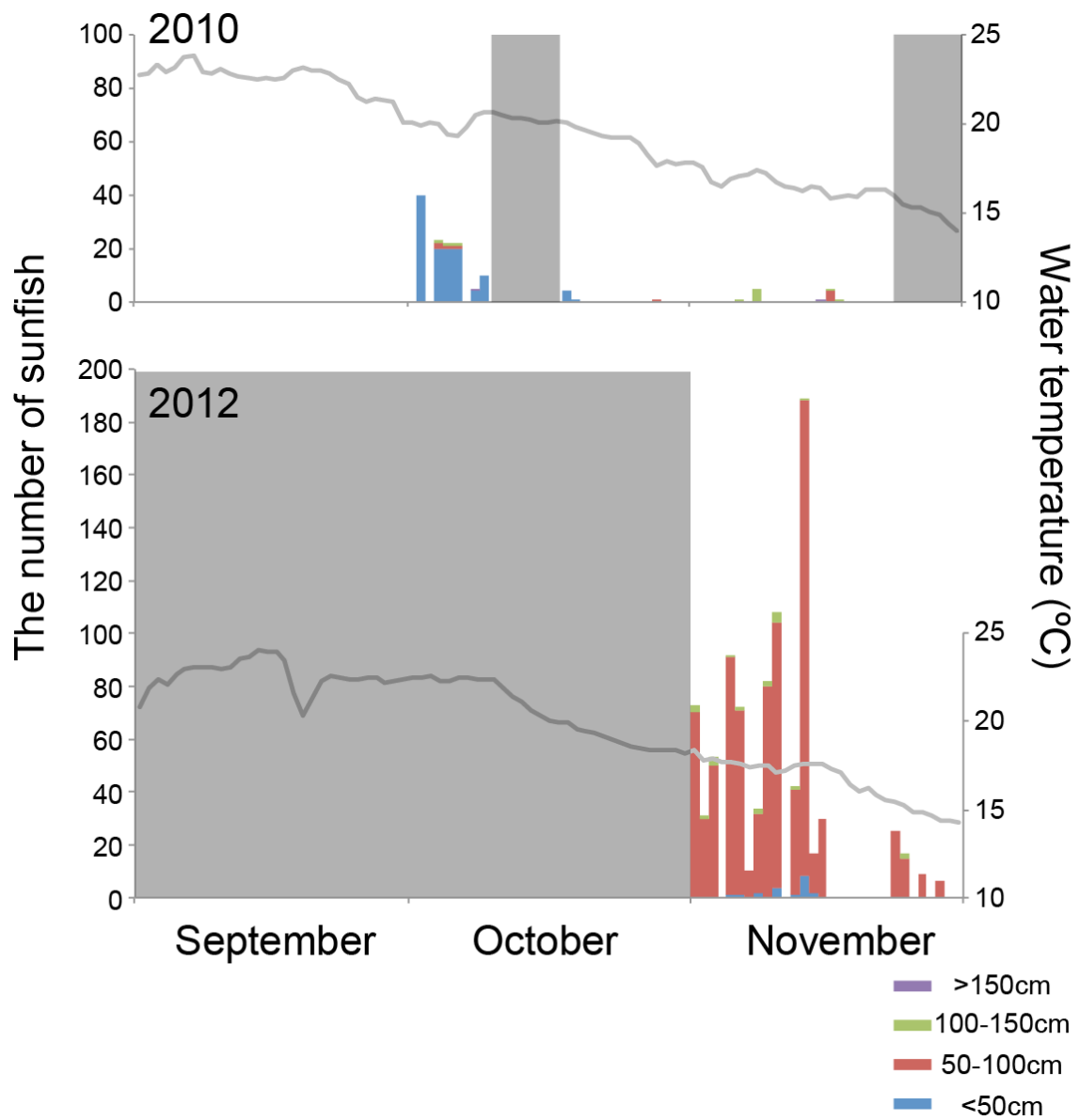
Fig. 2-3 The daily catch number of ocean sunfish from September to November. Lines indicate daily water temperature via web site (Iwate Tairyo Navi). Shadow areas in each graph indicate out of investigating terms.
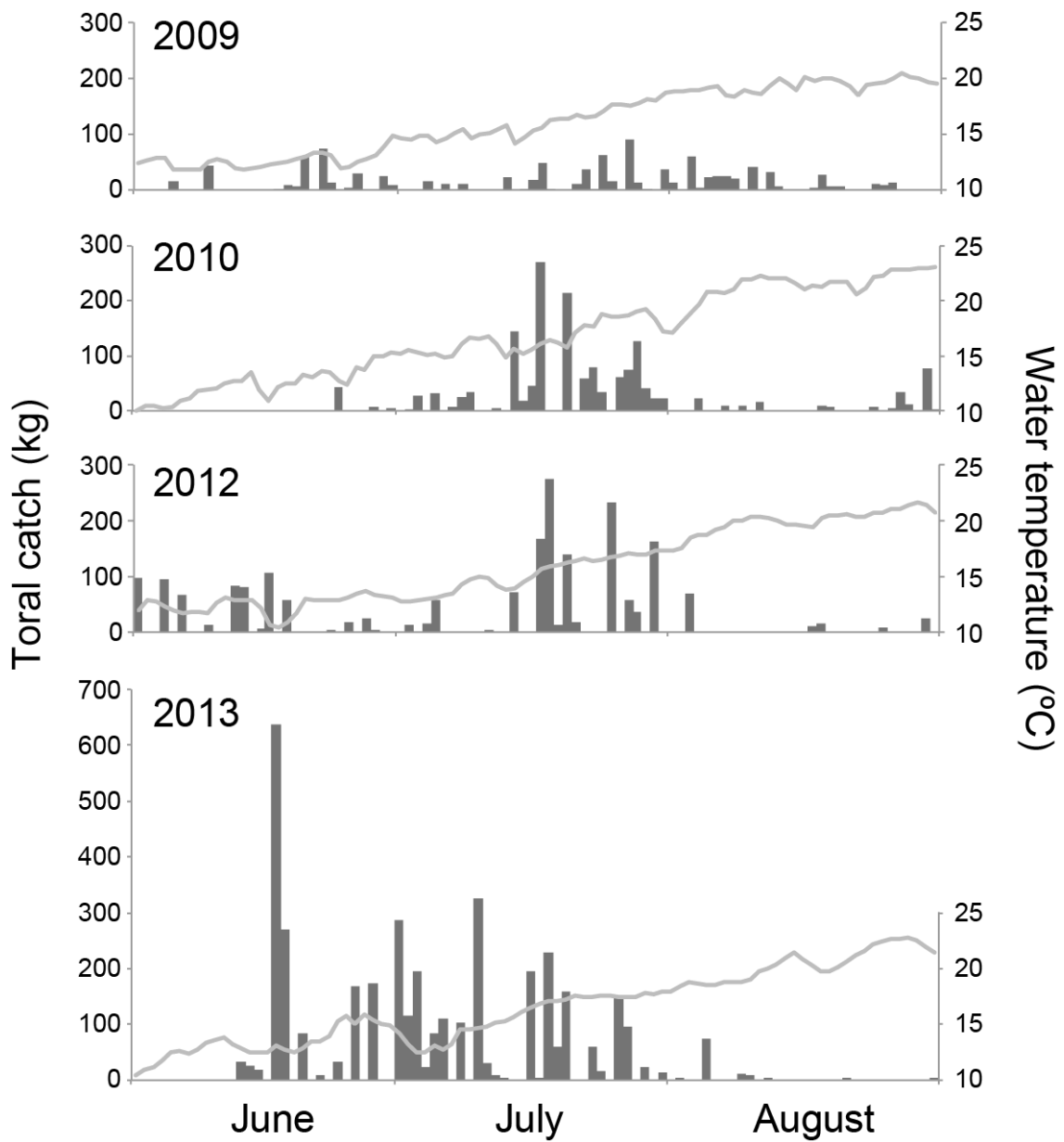
Fig. 2-4 The daily total catch of Molidae in the kilograms by fishing reports (via Iwate Tairyo Navi) from June to August.　Lines indicate daily water temperature via web site (Iwate Tairyo Navi).
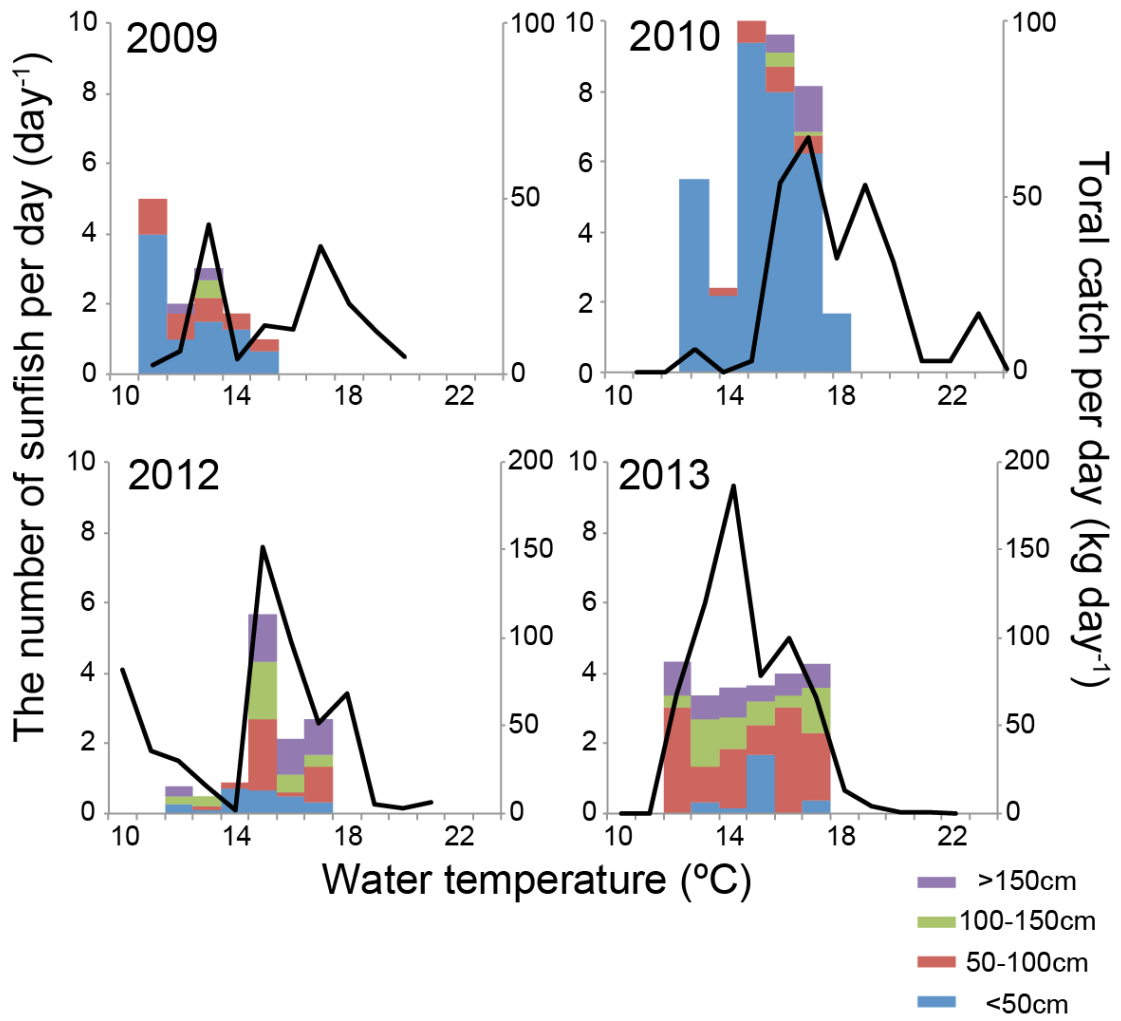
Fig. 2-5 The number of ocean sunfish per day from June to August in each water temperature. Lines indicate daily total catch of Molidae per day in each water temeperature.
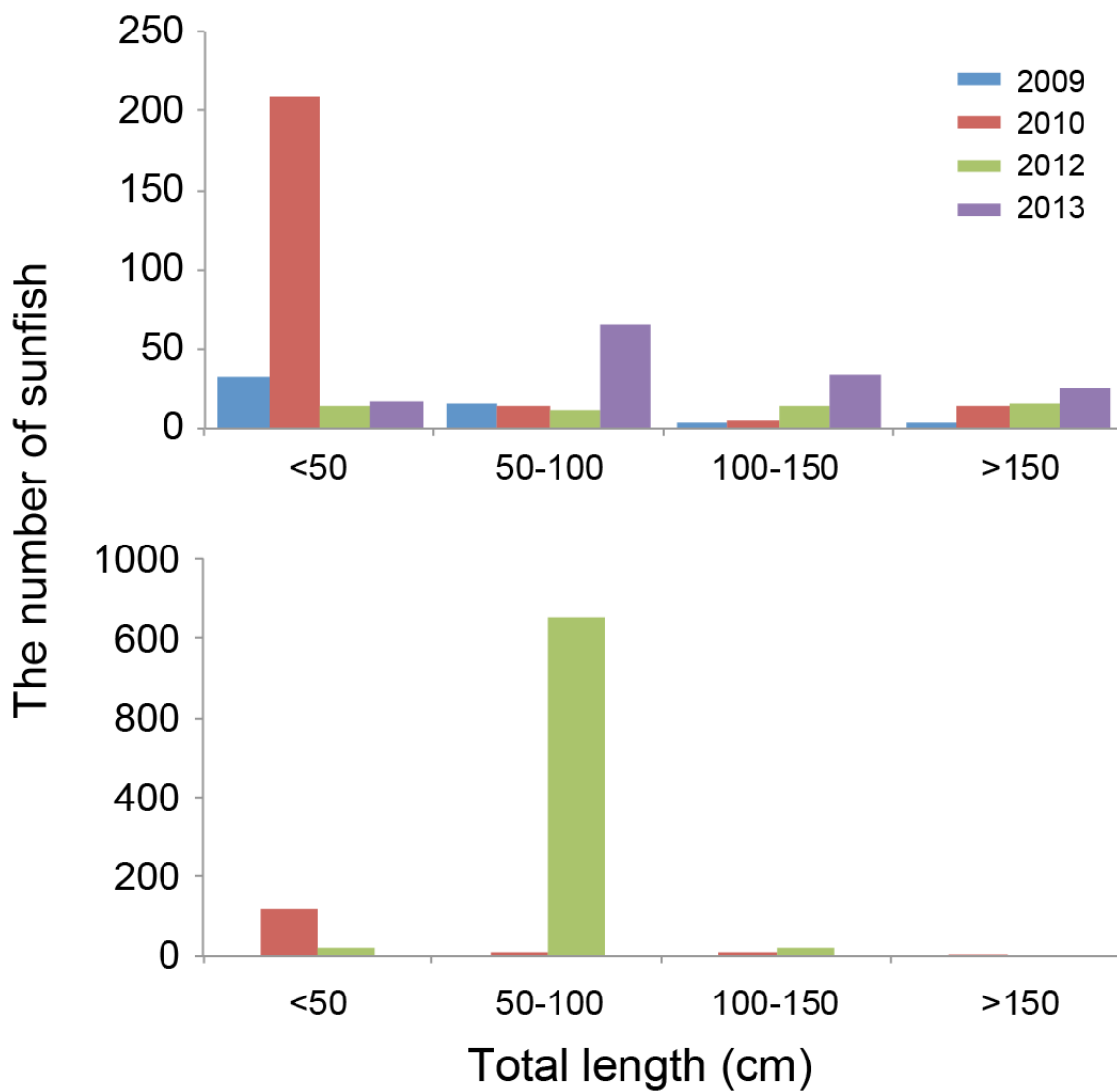
Fig. 2-6 Size distribution of ocean sunfish from June to August (above) and from September to November (below).

Chapter 3 Dietary analyses

1. Introduction

The ocean sunfish are generally considered to be planktonic fish because of their surface 'basking' behavior, and diet consisting primarily of gelatinous zooplankton like jellyfish (Hoar 1953).   However, various benthic organisms including crustaceans, brittle stars and flounders have also been found in their stomachs (Fraser-Brunner 1951; Norman and Fraser 1949).   Traditional stomach content analysis of diet (e.g. Lowe et al. 1996; Wetherbee et al. 1996) may produce biased results if the diet consists partly of fragile, difficult to detect, items like jellyfish

Stable isotope ratio analyses of animal tissues provide a robust, alternative method for investigating diet (Menzies et al. 1961, Parker et al. 1964).   Recent application of stable isotope techniques on ocean sunfish also suggests that half of assimilated diet of juvenile sunfish was likely benthic origin (Syväranta et al. 2012, Harrod et al. 2013).   One possible explanation for varied diet is that ocean sunfish undergo an ontogenetic shift in feeding habits.   In this chapter, combination of stomach contents analyses and stable isotope ratios is used to characterize the ontogenetic shift in diets of ocean sunfish.

Bulk $\delta^{15}N$ of the animal tissue reflect the $\delta^{15}N$ of their preys.   However, the bulk $\delta^{15}N$ method requires the isotopic composition of either their preys.   Moreover, the magnitude of ^{15}N enrichment can vary among organisms, leading to further uncertainty about the estimated trophic level.   Recent developed techniques of $\delta^{15}N$ of amino acid of animal tissues were used to estimate trophic position of the animal (Chikaraishi et al. 2007, Miller et al. 2012).   In addition above, trophic position of ocean sunfish is estimated using stable isotope ratios of amino acids that composed of tissues of ocean sunfish.

2. Materials and methods

Sixteen sunfish captured by the set-nets managed by Otsuchi fisheries cooperative and one sunfish harpooned by research vessel 'Yayoi' from International Coastal Research Center, Atmosphere and Ocean Research Institute, the University of Tokyo from June to October 2010 were used for dietary analyses. The total length (cm) of each sunfish was recorded, stomach contents were collected and preserved in 99% ethanol, and individual prey items were identified to the lowest possible taxon. In addition, carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively) of muscle tissue from the sunfish were measured. Two samples from white dorsal-fin muscle of each individual were used for bulk stable isotope ratio analyses. The stable isotope composition of possible prey for ocean sunfish (five species of medusa: *Aurelia sp.*, *Aurelia limbata*, *Aequorea coerulescens*, *Chrysaora melanaster*, *Cyanea capillata* were collected from the set-nets along with ocean sunfish) were measured. Only the bells of these jellyfishes were used for stable isotope analyses. All samples for stable isotope analyses were individually into labeled zip-lock bags and preserved in frozen at -20ºC for 1-3 months, then freeze-dried and ground into a powder before stable isotope analyses.

All samples were without acid washing to remove carbonates. The $\delta^{13}C$ and $\delta^{15}N$ values for each sample were obtained using continuous flow stable isotope-ratio mass spectrometry at Atmosphere and Ocean Research Institute, the University of Tokyo. Samples were combusted in an elemental analyzer (FLASH EA, Thermo Fisher Scientific, Massachusetts, USA) interfaced via a continuous flow interface (Conflo III, Thermo Fisher Scientific) to a mass spectrometer (Delta plus XP, Thermo Fisher Scientific). Stable isotope ratios were reported in standard δ notation, as parts per thousand (‰) relative to Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen, using the equation $\delta X = [R_{sample}/R_{standard}-1] \times 1000$, where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$. Standard samples (alanine, histidine) whose stable

isotope ratios were known and obtained analytical errors were measured. Analytical errors of $\delta^{13}C$ and $\delta^{15}N$ were <0.26‰ and <0.52‰ respectively. To correct effects of lipid on $\delta^{13}C$, a correction methods using C:N values according to Kiljunen et al. (2006) was applied. To test whether $\delta^{13}C$ and $\delta^{15}N$ values were affected by body size, generalized linear mixed effects models (GLMM) and Akaike Information Criteria (AIC) to select the most parsimonious model were used (Wada and Kashiwagi 1990). A function *glmm* contained in *repeated* package of R ver.3.0.2 was used to calculate AIC. $\delta^{13}C$ and $\delta^{15}N$ were set as response variables in the models, body size was set as explanatory variables, and individual was set as a random effect. LOESS smoothing was used to look for breakpoints in the relationship between isotope ratios and body size. A function *loess.as* contained in *fANCOVA* package of R ver.3.0.2 (R project) was used to determine the smoothing parameters for LOESS smoothing.

In 2012, two sunfish caught in July (TL 95 cm and 200 cm) and one sunfish caught in November (TL 94 cm) were analyzed for their nitrogen isotopic composition of phenylalanine ($\delta^{15}N_{Phe}$) and glutamic acid ($\delta^{15}N_{Glu}$) to estimate their trophic position. Samples were hydrolyzed, hydrophobic constituents such as lipids were removed and the amino acids were extracted. Nitrogen isotopic composition of the two amino acids was determined by gas chromatography/combustion/isotope-ratio mass spectrometry at JAMSTEC (Japan Agency for Marine-Earth Science and Technology). The trophic level of each specimen was calculated, using the previously established formula: Trophic level = $(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4)/7.6 + 1$ (Chikaraishi et al. 2007). Only $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ were used in the analysis, because this combination of amino acids appears to provide the most precise estimate of the trophic level of marine organisms.

3. Results

All stomachs with food items of sunfish smaller than 50 cm TL contained crustaceans (Gammaridea, Corophiidea and Brachyuran megalops (Fig. 3-1)), and

bivalve shells were found in one of these individuals. Scyphozoa were found in all stomachs of sunfish larger than 200 cm TL (Table 3-1). No solid items were found in stomachs of sunfish between 50 and 200 cm TL. In addition, a kind of gut parasites (Cestoda sp.), were found in intestine of sunfish larger than 40 cm TL.

The models including body size showed the lowest AIC in both $\delta^{13}C$ and $\delta^{15}N$, indicating that the muscle tissues from larger sunfish had higher values of both $\delta^{13}C$ and $\delta^{15}N$ than those of smaller sunfish. The $\delta^{15}N$ values for all jellyfishes were higher than those of smaller sunfish, and $\delta^{13}C$ values for all jellyfishes were higher than those of smaller sunfish. Jellyfishes had lower both $\delta^{13}C$ and $\delta^{15}N$ values than those of larger sunfish (Fig. 3-2). LOESS smoothing between isotope values and body sizes found breakpoints at around 100 cm TL in $\delta^{13}C$ and around 80 cm TL in $\delta^{15}N$ (Fig. 3-3).

$\delta^{15}N_{Glu}$ of ocean sunfish of 200 cm in July, 95 cm in July, and 94 cm in November were 25.5‰, 22.3‰ and 22.2‰, respectively. While, $\delta^{15}N_{Phe}$ of these sunfish were 0.6‰, -3.2‰ and -3.1‰, respectively. Thus, the trophic levels of these sunfish were, 3.8, 3.9 and 3.9, respectively.

4. Discussion

The presence of crustaceans such as megalops and amphipoda in stomach contents, suggests small (at least <50 cm) sunfish are predominantly benthic foragers. In contrast, large sunfish (at least >200 cm) were obviously gelatinous plankton feeder. From the literature, the diet of larger sunfish appears to consist of both gelatinous plankton like jellyfish and salps (Fraser-Brunner 1951; MacGinitie and MacGinitie 1968; Hooper, Paradis and Ackman 1973; Bass et al. 2005), and benthic items like crustaceans, brittle stars, mollusks and hydrozoans (Norman and Fraser 1949; Fraser-Brunner 1951; Clemens and Wilby 1961; Hart 1973). These suggesting an ontogenetic shift in sunfish diet from benthic crustaceans to gelatinous zooplankton. However, because no prey items were recovered from stomachs of intermediate sized

(50-200 cm) sunfish in the present study, the specific size at which sunfish changed their diets was not identified directly. An ontogenetic shift in sunfish diet is also suggested by higher carbon and nitrogen stable isotope values in larger individuals. Only large sunfish in the present study had higher $\delta^{15}N$ values than Scyphozoa jellyfish, suggesting only these large individual were able to consume these jellyfishes.

Stable isotope ratios of amino acid suggested that large (200 cm) and medium (about 100 cm) sunfish were similar trophic position at 3.8-3.9. The medium sunfish sampled in different season showed similar value in both isotope values, suggesting that similar size of sunfish might not change their prey among seasons. However, $\delta^{15}N_{Phe}$ of the medium sunfish showed about 3.7‰ lower value than the large sunfish, suggesting the medium sunfish might forage in warm water, where nitrogen fixation is high and leads to low $\delta^{15}N$, on the other hand the large sunfish might forage in cold water (Ohkouchi personal communication). Warm and cold water are able to co-exist vertically in the ocean, and $\delta^{15}N$ of particulate organic nitrogen was low in surface warm water and high in deep cold water (Saino and Hattori 1980). The difference of $\delta^{15}N_{Phe}$ among body sizes might reflect difference of foraging depth.

In conclusion, ocean sunfish might change their diet from benthic crustacean to gelatinous zooplankton as growing and large and medium ocean sunfish might forage in different water masses.

Table 3-1 Diet of ocean sunfish of four size classes from northern part of Japan in 2010. For each size class, n = number of stomachs containing the item and percent occurrence (%O) = number of stomachs containing the item in proportion to number of stomachs that contained food.
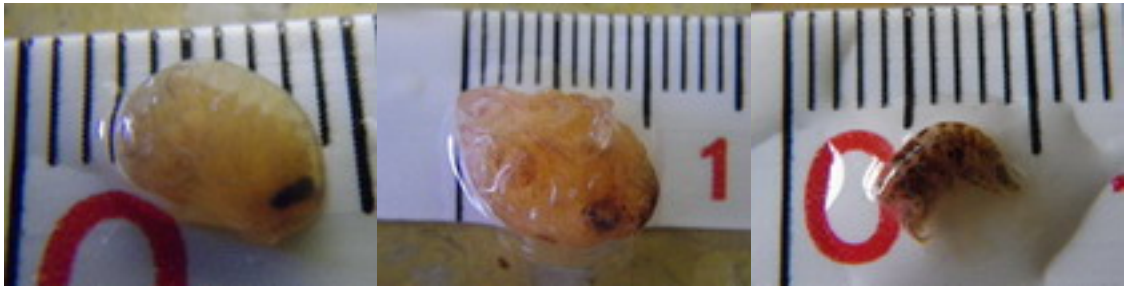
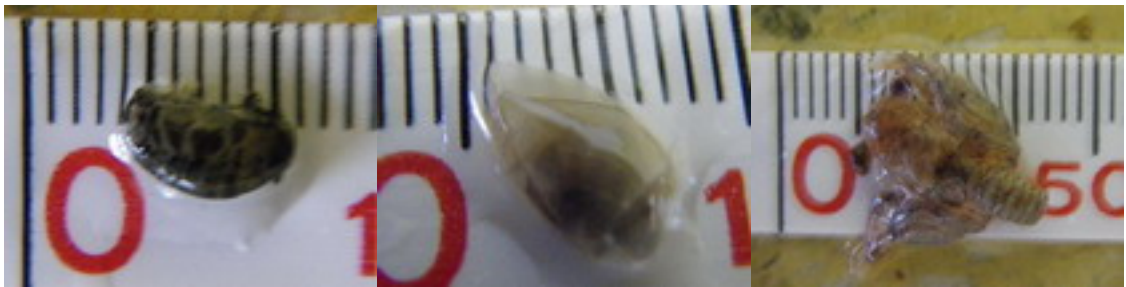| Total length (cm)	<50		50-100		100-200		>200	
Stomachs examined	12		2		1		2	
Stomachs with food	8		0		0		2	
Prey items	n	%O	n	%O	n	%O	n	%O
Crustacean	8	100	-	-	-	-	-	-
Gammaridae	5	62.5	-	-	-	-	-	-
Brachyra	2	25	-	-	-	-	-	-
Corophiidea	5	62.5	-	-	-	-	-	-
Argulidae	1	12.5	-	-	-	-	-	-
Bivalvia	1	12.5	-	-	-	-	-	-
Schyphozoa	-	-	-	-	-	-	2	100

A. Bivalvia B. Gravel C. Corophiidea

D. Gammaridae E. Gammaridae F. Gammaridae

G. Gammaridae H. Crustacean shell I. Megalop of Brachyra

J. Gelatinous tissue K. Arguidae

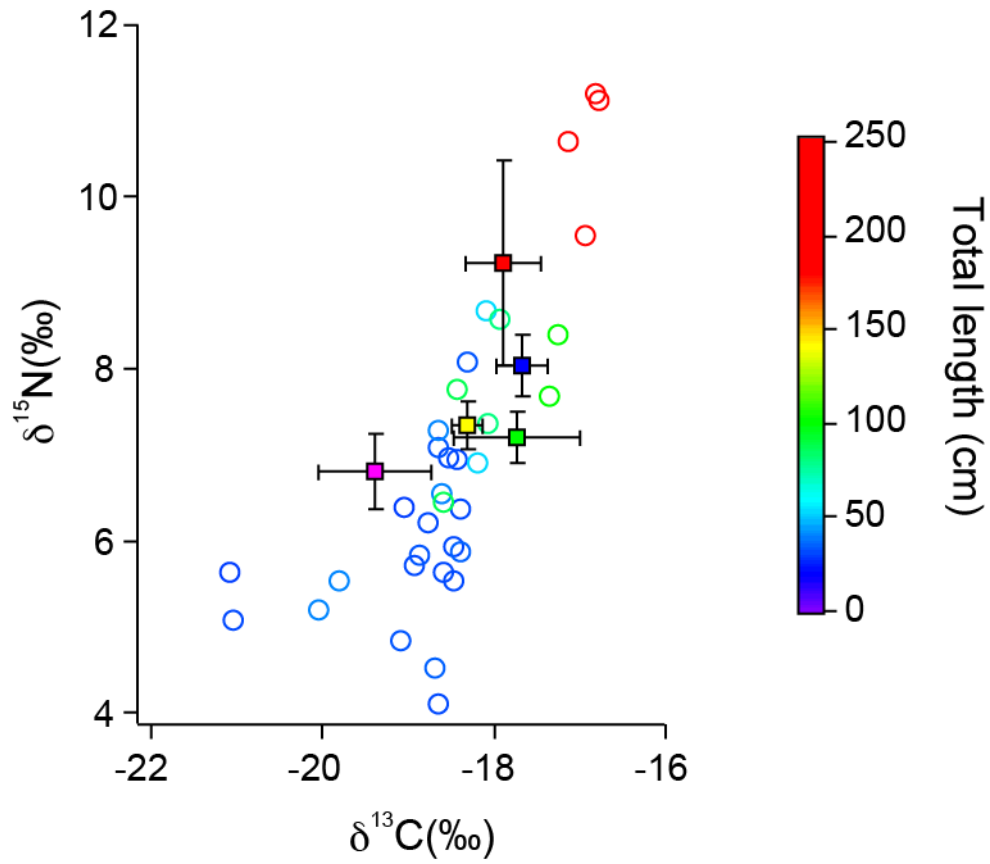**Fig. 3-1** Examples of the prey items from stomachs of sunfish.

Fig. 3-2 Carbon and nitrogen isotope ratios of the muscles of sunfish (circles) and the five jellyfish species (squares). The colours of the circles indicate total length of the sunfish. Error bars of jellyfishes indicate standard deviations among samples and colours indicate purple *Aurelia sp.*, blue *Aurelia limbata*, yellow *Chrysaora melanoster*, red *Cyanea capillata*, and green *Aequorea coerulescens*.
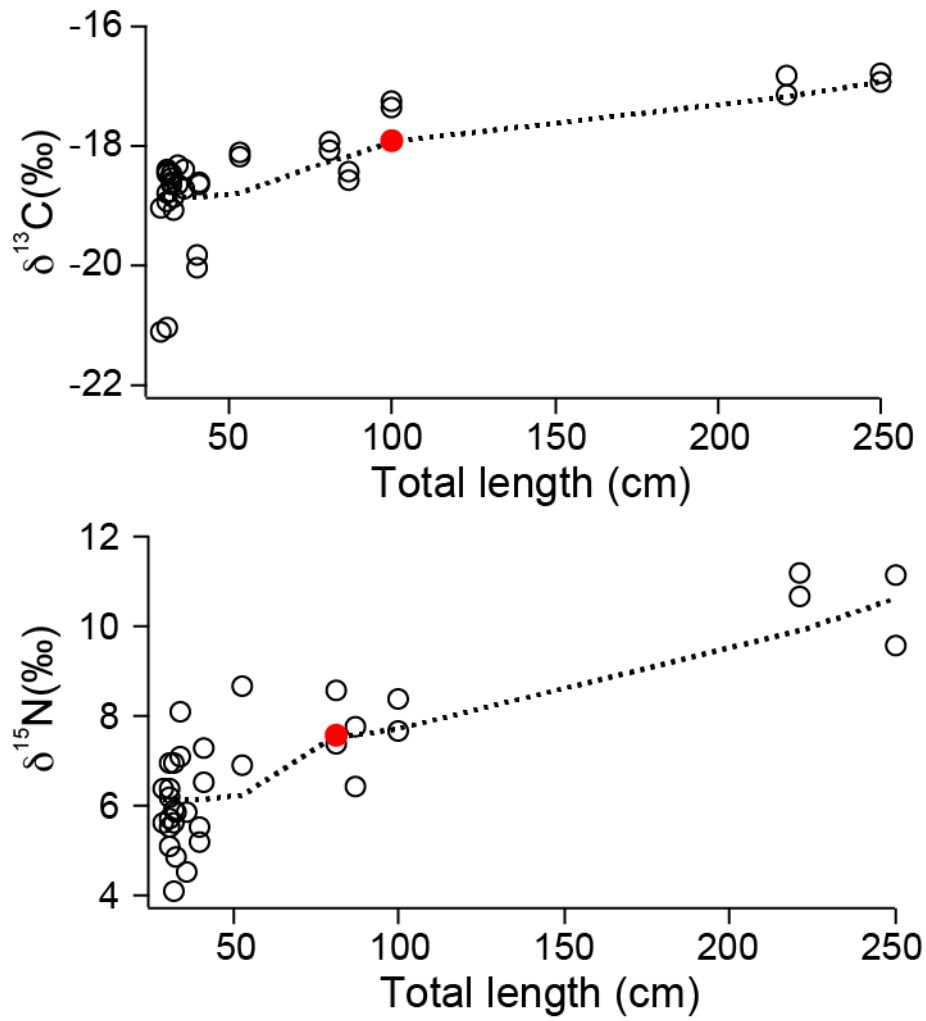
Fig. 3-3 Relationship between body size of the sunfish and $\delta^{13}C$ or $\delta^{15}N$. Lines were estimated using LOESS smoothing and red dots indicate breakpoints.

Chapter 4   Foraging behaviour

1. Introduction

The dietary analyses suggested ontogenetic dietary shift in ocean sunfish. Although techniques such as stomach content and stable isotope analyses can provide important insights into diet, they reveal little about actual feeding behaviours under natural conditions.

Recent electronic tagging studies have shown ocean sunfish are active swimmers that make frequently vertical migrations between the surface and depths of several hundred meters (Cartamil and Lowe 2004; Watanabe and Sato 2008, Houghton et al 2009). Satellite tracking has further revealed that ocean sunfish migrate large distances and dive to over 500 m (Dewar et al. 2010; Hays et al. 2009; Potter and Howell 2011; Sims et al. 2009a, 2009b). These observations suggest ocean sunfish may be deep foragers, but to date there has been no empirical evidence of them feeding in deep water. The recent development of small, animal-borne data-logging devices such as accelerometers and cameras has provided new tools for studying difficult to observe behaviors such as deep-water feeding (Nakamura et al. 2011; Heithaus et al. 2008).

To characterize their foraging habit under natural condition, animal-borne devices were used. Field experiments started from 2009 and the focuses of the experiments were developed every year. In 2009 and 2010, the study focus was on difference of feeding habit between small (TL<60 cm: body mass (BM) ≤10 kg estimated by the equation $BM = 52.96 \text{x} (TL/100)^{2.96}$ according to Watanabe and Sato 2008) and medium (TL 60-120 cm: BM 10-100 kg) to large (TL >120 cm: BM ≥100 kg) sunfish by short (<half day) deployment. In 2011, the field works were not conducted because tsunami on March 11 destroyed set-nets around coast. In 2012 and 2013, the device retrieving protocol was improved by using satellite transmitters and

long behavioural data (>3 days) were obtained.   In 2012, the study focus was on when, how deep and what larger sunfish forage, and in 2013, the study focus was on a detail of foraging events (e.g. how they approach and feed on their preys).

2. Materials and methods

Devices

i. Accelerometers

Four types of accelerometers were used to record behaviours of ocean sunfish;

(1) W1000-PD2GT (22 mm in diameter, 123 mm in length and 80 g in air, Little Leonardo, Tokyo, Japan), which recorded bi-axial (lateral and longitudinal axis of sunfish) accelerations at 32 Hz, swimming speed, depth and temperature at 1 Hz,

(2) W1000-PD3GT (22 mm in diameter, 123 mm in length and 80 g in air, Little Leonardo, Tokyo, Japan), which recorded tri-axial (lateral, longitudinal and dorso-ventral axis of sunfish) accelerations at 32 Hz, swimming speed, depth and temperature at 1 Hz,

(3) W380-PD3GT (21 mm in diameter, 123 mm in length and 63 g in air, Little Leonardo, Tokyo, Japan), which recorded tri-axial accelerations at 16 Hz, swimming speed, depth and temperature at 1 Hz,

(4) W1000-3MPD3GT (26 mm in diameter, 166 mm in length and 132 g in air, Little Leonardo), which recorded tri-axial accelerations at 16 or 32 Hz, and tri-axial magnetism, swimming speed, depth and temperature at 1 Hz.

The speed sensor was a propeller placed at a front of the syringe-type loggers, and speed data were recorded as the number of propeller rotations per second and were converted to meters per second using a regression equation derived from empirical calibration tests.

During the calibration tests, the accelerometer was towed from 40 m depth to the surface (0 m) at 5 times at each different speed using an electric reel on a vessel, and

the duration of each tow was measured using a stopwatch.   Then, the mean number of propeller rotations per tow and calculated speed were compared (40 m per towing time). The linear regression line was obtained with $R^2 > 0.99$ (n = 5).

Acceleration data were recorded as a 12-bit number (0-4095) and converted to meter per square second on the basis of acceleration due to gravity (9.8 m s^{-2}).   During calibration, the accelerometer was systematically rotated and held to record the value of gravity acceleration in all three axes.   Magnetisms and depth recorded by the accelerometers were affected by ambient temperature.   The accelerometers were put into different temperature of water and got relationships between temperature and magnetisms or depth.   The quadratic regression line was obtained with $R^2 > 0.99$. Then, adjusted magnetism data in a 12-bit number (0-4095) were converted to magnetic unit nT on the basis of magnetism due to vertical component of total magnetic field using the same method as calibration of acceleration.

ii. Animal-borne cameras

To record their feeding events visually, video logger (DVL400, 22 mm in diameter, 50 mm in length and 40 g in air, Little Leonardo), which recorded 720 pixel footage for total 70 min and started recording 2 days after release and recording cycle was set as 10 min recording and 80 min resting, or an animal-borne camera (DSL400-VDTII, 22 mm in diameter, 132 mm in length and 82 g in air, Little Leonardo) with LED flashlight (LET400, 22 mm in diameter, 110 mm in length and 80 g in air, Little Leonardo) was used in addition to the accelerometers.   The DSL400 can record average 9,000 still images depending on the data size of images and set to record at every 4 sec in 2009, 30 sec in 2012, and 4 sec intervals at depth deeper than 50 m in 2013.

iii. Design of instrument packages including retrieval system

The devices were combined into instrument packages, which also included a retrieval system consisting of a timed-release mechanism, and a syntactic foam buoy. Instrument packages automatically detached from each sunfish after deployment times according to auto-release timers, floated to the surface, and were located using the VHF transmitter (MM130B, 20 g in air, Advanced Telemetry System, USA). In 2012 and 2013, the Argos transmitter (SPOT5, 30 g in air, Wildlife Computers, USA) was also used to detect floating locations of the packages by Argos system (CLS, France).

The combination of the devices was different among years (Table 4-1). In 2009, two types of package were used, one included only the accelerometer W1000-PD2GT (Package I), and the other included the accelerometer W1000-PD3GT and the digital still camera DSL400-VDTII (Package II). In 2010, one type of package included only the accelero-magnetometer W1000-3MPD3GT (Package III) was used. In 2012, two types of package were used, one included only the accelero-magnetometer W1000-3MPD3GT (same as Package III), and the other included W1000-3MPD3GT and the digital still camera DSL400-VDTII with the flashlight LET400 (Package IV). In 2013, two types of package were used, one included the accelerometer W380-PD3GT, the digital video camera DVL400 (Package V), and the other included the accelero-magnetometer W1000-3MPD3GT, and the digital still camera DSL400-VDTII with the flashlight LET400 (same as Package IV).

Fieldwork

Fieldwork took place from 2009 to 2013 around Otsuchi bay, Iwate (39°25'N, 142°00'E). The instrument packages were attached onto total twenty-three ocean sunfish. The instrumented sunfish were caught by three set-nets, managed by Otsuchi fisheries cooperative, placed around Funakoshi bay and Otsuchi bay, Iwate, Japan (39°22'N, 141°58'E) in 2009 and 2010, and by three set-nets, managed by Funakoshi

bay fisheries cooperative, placed around Funakoshi bay, Iwate, Japan (39°25'N, 142°00'E). The sunfish were captured by the set-nets, lifted with a crane, brought onto the deck of the fishing vessel, measured (TL in cm) and equipped with instrument packages via cable ties (used plastic cable ties in 2009 and 2010, and stainless metal cable ties in 2012 and 2013) threaded through a small hole pierced in back of the fish, anterior to their dorsal fin, and secured around the package float (see details in Watanabe and Sato 2008) (Fig. 4-1). During attachment procedure, sunfish gills were irrigated with fresh seawater. The sunfish were released into the ocean just out of the set-nets where the sunfish were captured. In 2009, Package I was deployed on two sunfish (TL 49 and 51 cm) and Package II was deployed on three sunfish (TL 51, 54 and 58 cm) in July (Table 4-1). In 2010, Package III was deployed on four sunfish (TL 84, 87, 130 and 164 cm) in July, and on three sunfish (all were TL 105 cm) in November (Table 4-2). In 2012, Package III was deployed on two sunfish (TL 95 and 148 cm) and Package IV was deployed on three sunfish (TL 125, 136 and 200 cm) in July, and Package III was deployed on two sunfish (TL 67 and 74 cm) and Package IV was deployed on one sunfish (TL 94 cm). In 2013, Package V was deployed on one sunfish (TL 125 cm) and Package IV was deployed on two sunfish (TL 105 and 191 cm) (Table 4-1).

The length of deployment was short (6-8 hours) in 2009 and 2010, and long (3-6 days) in 2012 and 2013. The instrument packages automatically detached from each sunfish after deployment times according to auto-release timers, and floated to the surface. For short deployments, the positions of the instrument packages were located using a radio receiver with Yagi antenna and detected the direction of the package from land. For long deployments, the positions of the packages were located via Argos system using the satellite transmitter. Then, the packages were retrieved by detecting the signals from the VHF transmitter by the radio receiver with Yagi antenna on a research vessel 'Yayoi' from International Coastal Research Center, Atmosphere and

Ocean Research Institute, the University of Tokyo in short deployments, or by a fishermen's boat 'Ko-yo maru' in long deployments.

Analyses

i. Behavioural data

Igor Pro Ver. 6.32A (WaveMetrics, Lake Oswego, OR, USA) was used to analyze the behavioral data. Time series acceleration data can be separated into components related to the orientation of the animal with respect to gravity (static components) and specific accelerations imposed by fish movements (specific components). Lower-frequency gravity-based accelerations were used to calculate posture of animals (Sato et al., 2003; Watanabe et al., 2006). Static components of acceleration were extracted from the raw acceleration data using the low-pass filter (contained within Igor Pro). To confirm the slowest frequency of movements of sunfish, I calculated the power spectral density (PSD) of the entire acceleration data set from each individual by fast Fourier transformation, and I regarded the peaks of the PSD as the frequency of the movements of sunfish (Sato et al. 2007, Shiomi et al. 2010) (Fig. 4-2). Cut-off frequency was set at 0.1 Hz because the slowest frequency of movements of sunfish was estimated 0.2 Hz from PSD. Then the gravity components of acceleration were used to calculate data logger orientation (a proxy for the physical orientation of the sunfish body) at 1 sec intervals. As data loggers were not attached exactly parallel to the body axis of each sunfish, an adjustment angle (i.e. difference between pitch and logger angles) was also estimated (see Sato et al. 2003 for additional details on pitch adjustment protocol). 'Ethographer ver.2.0.1' software for the Igor Pro (Sakamoto et al. 2009) was used to generate spectrograms for frequency spectrum analysis by wavelet transformation. A spectrogram was generated from lateral acceleration based on the continuous wavelet transformation, and then grouped data into 1 s intervals. 'Peak tracer' function in Ethographer was used to determine stroke

frequency of the sunfish.   In addition, the three dimensional movement path of each sunfish equipped with accelero-magnetometers was reconstructed, using a dead-reckoning method calculated from tri-axial magnetometer data (i.e. compass heading), data logger orientation, pitch and speed (Shiomi et al. 2010).

ii. Images from animal-borne cameras

Whether possible prey objects were seen in the images recorded by the digital still logger (DSL) or not was checked, and the prey items were identified to the lowest possible taxon.   Whether possible feeding events were seen in the footages recorded by digital the video logger (DVL) or not was also checked.   After feeding events were determined from the visual data, the characteristic of these feeding events was identified in the behavioural data.   Then, the feeding events were extracted using the characteristics from all of the behavioural data and when and how deep they occurred.

3. Results

All instruments except one package on an individual (MA123) were retrieved, yielding a total of 53 days of behavioral data from twenty-two sunfish (Table 4-1).   A sunfish (M092) swam into another set-net 2 hours after release was found because the package floated within the set-net and inside of the net was seen in still images from this individual, and another sunfish (MA122) died and sank to 800 m depth 2 days after release according to the behavioural data (see Fig. 4-7).   The package pop-up locations of short deployments in 2009 and 2010 occurred further offshore in large than small sunfish (Fig. 4-3).   The package pop-up locations of long deployments in July 2012 and 2013 occurred around 38ºN line where sea surface temperature reported by the website (Japan Meteorological Agency: http://www.data.kishou.go.jp/kaiyou/db/kaikyo/daily/sst_jp.html) was around 20ºC (Fig. 4-4 upper), while in November 2012 occurred around 39ºN line where sea surface

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temperature was around 17ºC (Fig. 4-4 lower).   Data loggers documented seasonal (July versus November) differences in vertical profiles of seawater temperature.   Sea surface temperatures was 17-18°C in July, and decreased with depth to <12ºC at depths below 100 m (Fig. 4-5 upper).   While, sea surface temperatures in November were 17-18°C with similar temperatures (>15ºC) recorded at 100 m (Fig. 4-5 lower).

Vertical movements

The vertical movement of the small sunfish (TL <60 cm) in short deployments in July varied among individuals (Fig. 4-6).   Three sunfish (MS091, MS092 and MS094) made frequent vertical movement, one sunfish (MS095) stayed near the surface during whole deployment time, and another sunfish (MS093) stayed near the surface and at depth of 30-40 m for several ten minutes (Fig. 4-6).   All individuals didn't swim below 100 m depth.   The large sunfish (TL >120 cm) in July swam back and forth between the surface and deep water (>100 m) both in short and long deployments (Figs. 4-7 and 4-8).   From long deployments, the large sunfish showed typical day-night behaviour, that they swam repeatedly to deep water during the day and stayed within the surface layer (<20 m) during the night (Fig. 4-8).   The medium sunfish (TL 60-120 cm) in July showed two different types in vertical movement (Figs. 4-7 and 4-9).   Two sunfish (MS101 and MS131) showed similar vertical movement to the large sunfish (Fig. 4-7 and 4-9), and the sunfish (MS131) dove to 670 m, which was the maximum depth of all deployed individuals (Fig. 4-9).   Two sunfish (MS103 and MS121) rarely swam into over 100 m (Figs 4-7 and 4-9).   The medium sunfish (TL 60-120 cm) in November swam within the mixed layer (0-100 m) (Figs. 4-7 and 4-9).   Mean of the depth distribution of the small and large sunfish in July peaked at <10 m, while mean of the depth distribution of the medium sunfish in July peaked at 0-30 m   (Fig. 4-10). Peak of depth distribution of the medium sunfish in November were between 40 and 110 m (Fig. 4-10).   Peak of temperature distribution were similar among each body

size and season at 13-16ºC (Fig. 4-11).

Foraging behaviours from image recorder

Small sunfish

It was impossible to identify obvious feeding events from still images recorded by the small individuals. However, one small sunfish (MS093) showed behaviour possibly associated with benthic foraging. The sunfish occasionally swam along the seabed, stopping periodically in a head down orientation (Fig. 4-12). Stop (swimming speed <0.1 m s^{-1}) with head down (pitch <-20º) during swimming near the seabed (identified from the still images) was regarded as possible benthic foraging behaviour.

Medium to large sunfish

It was successful to obtain images from all of the sunfish deployed the digital still logger except one individual (MS131) because of a problem in starting recording. It was hard to see anything in the footage in deep water recorded by the digital video logger on one individual (MS132) because of lack of light. The sill camera couldn't get images in shallow bright water (<40 m) because of high gain for deep dark. 57, 10, 6 and 112 prey items were distinguished from still images recorded by four sunfish (MS122, MS124, MS125 and MS133), respectively (Table 4-2). The images included obvious feeding events (Figs. 4-13, 4-14 and 4-15). The prey items were composed by siphonophore (at least two species of Prayidae), scyphozoa (lion's mane *Cyanea capillata* and non-identifiable species), ctenophore (non-identifiable species), and unknown items, and siphonophore was most observed in all individuals (Fig. 4-16). The peak of prey occurrence was around 50 m and 160 m depth, and mode of temperature was around 8ºC (Fig. 4-17).

The detail of feeding events was characterized by the still images taken at every 4 sec by a sunfish (MS133). There were several patterns in feeding events. In

case of feeding on scyphozoa (lion's mane *Cyanea capillata*), the sunfish approached to a jellyfish and ate, however, the sunfish only ate gonads and oral arms of the jellyfish (Fig. 4-13).   Characteristics of this feeding event in the behavioural data were that the sunfish approached to the jellyfish with deceleration and stopped swimming during eating (Fig. 4-18).   In case of feeding on a chain of siphonophore (Prayidae *sp*.), the sunfish approached to a chain of siphonophore, bit and ate, however, the sunfish didn't eat whole of the chain (Fig. 4-14).   Characteristics of this feeding event were similar to the feeding on scyphozoa, and the sunfish approached to the chain of siphonophore with deceleration and stopped swimming during eating (Fig. 4-19).   There was another pattern of feeding on a chain of siphonophore (species unknown), and the sunfish approached to a chain of siphonophore, bit and ate whole of the chain (Fig. 4-15). However, characteristics of this feeding behaviour were different from the previous one, and the sunfish approached to the chain of siphonophore with acceleration and continued swimming during eating (Fig. 4-20).

Thus, obvious feeding events detected by digital still camera occurred accompanied by decelerating or accelerating.   Therefore, swimming speed of sunfish was used to detect each feeding event.   Decelerating (swimming speed decreased more than 5% of average swimming speed per second) and slowing down to swimming speed less than half of average swimming speed was regarded as feeding events with deceleration, and accelerating (swimming speed increased more than 15% of average swimming speed per second) and speeding up to one and a half of average swimming speed was regarded as feeding events with acceleration.   These criteria were used to extract feeding events from behavioural data from long deployment in July.   In the behavioural data of the sunfish (MS133) (the still images were recorded at high frequency (4 sec interval)), 77 out of 112 true feeding events (prey items were observed in the still images) were detected by this method, thus the detection rate of feeding events was 69%, and 48 events with no prey items in the still images were extracted,

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thus the false detection rate was 62%. In other three sunfish (MS122, MS124 and MS125), 42 out of 57 (74%), 8 out of 10 (80%), and 3 out of 6 (50%) true feeding events were detected, respectively.

Foraging behaviour from the behavioural data

Small sunfish

23 of the possible feeding associated behaviour were extracted from behavioural data of the small sunfish (MS093). The mean depth was 45.2 m (31-64 m).

Medium to large sunfish

Total feeding events with deceleration and acceleration were extracted 5198 times and 354 times respectively from total 42 days behavioural data obtained from the eight sunfish by long deployments in July (Table 4-2). Feeding events with acceleration occurred more commonly in water shallower than 50 m in both medium and large sunfish (Fig. 4-21). While, feeding events with deceleration occurred more commonly in water shallower than 40 m in the sunfish which swam within surface layer (TL <100 cm), but occurred at 100-180 m in the sunfish which went on deep excursions (TL >100 cm) (Fig. 4-21). Feeding events with acceleration occurred more commonly during the day than during the night, while feeding events with deceleration occurred both during the day and night in the sunfish which swam within surface layer (Fig. 4-22). Feeding events with acceleration occurred both during the day and night, while feeding events with deceleration occurred mostly during the day in the sunfish which went on deep excursions (Fig. 4-22).

4. Discussion

Historically, ocean sunfish have been classified as large plankton, drifting

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passively with the currents (McCann 1961, Holt 1965, Lee 1986). However, most of tracking studies on ocean sunfish concluded they were active swimmer because of high travel speed and movements against general current patterns (average rate of 10-20 km day^{-1}, reaching 31.9 km day^{-1}) (Cartamil and Lowe 2004, Sims et al., 2009, Hays et al., 2009, Potter et al. 2011). A recent satellite tracking of ocean sunfish revealed the seasonal migration pattern off Japan, suggesting their movements to higher latitudes as temperatures increase in the summer (Dewar et al. 2010). This pattern of moving progressively northward as the temperatures getting warm is also supported by catch rates of ocean sunfish in fish markets along the Japanese coast (Sagara and Ozawa, 2002). In the present study, however, all of the ocean sunfish released in July swam to south over 120 km away from the release point, where sea surface temperature were around 20℃, at highly migration speed similar to other reports (>20 km day^{-1}). In the study area, general ocean current in coastal area is in a south direction at 0.5-1 knot per hour (≒ 22-44 km day^{-1}) (Japan Meteorological Agency web site: http://www.data.kishou.go.jp/db/SN/daily/current_SN.html). The travel distance calculated from behavioural data recorded by the magneto-accelerometer using dead-reckoning method covered only less than 50% of the distance between the release and the package pop-up locations. Major part of migration of ocean sunfish might depend on ocean current. The recent satellite tracking suggested that ocean sunfish seek out thermal fronts where high productivity results in abundant prey (Dewar et al. 2010; Potter et al. 2011). The area where ocean sunfish moved in the present study is Kuroshio-Oyashio transition region, where biological productivity is high because of complication of warm and cold waters (Odate 1994).

Sunfish swimming depth was influenced by season. In July, water temperature decreased with depth and sunfish spent most time in warm surface waters with periodic deep excursions into colder water. Larger sunfish utilized a wider range of depths than smaller sunfish, possibly because larger individuals had slower core

cooling at depth due to size-related thermal inertia. Vertical temperature profiles also differed between July, when the ocean was highly-stratified with a narrow surface mixed layer, and November, when the water was thoroughly mixed with similar water temperatures from the surface to 100 m depth. Similar seasonal shifts in sunfish vertical behavior have been previously observed from long-term satellite tracks (Dewar et al. 2010; Potter and Howell 2011), suggesting that vertical distribution of ocean sunfish is driven primarily by thermal preferences and/or prey vertical distribution. Previous studies suggest that surfacing by sunfish between deep excursions is related to behavioral thermoregulation (Dewar et al. 2010; Potter and Howell 2011). Behavioral thermoregulation is commonly seen in many species of fishes, for example, both endothermic (e.g. Pacific bluefin tuna *Thunnus orientalis*, Kitagawa et al. 2001) and ectothermic fishes (e.g. blue shark *Prionace glauca* Carey and Scharold 1990) alternate between deep excursions into cold water and 'rewarming' periods in the surface mixed layer.

Possible foraging behaviours also differed with sunfish body size and season. The seabed-associated 'head-down' behavior observed in small sunfish may indicate benthic foraging. Ocean sunfish are thought to be suction feeders because of their skull structure (Gregory 1933), but could also squirt water from their mouth (Norman and Fraser 1949). Closely-related thread-sail filefish blow water to excavate prey from sandy substrates (Kawase and Nakazono 1996), and it is possible that small sunfish use similar benthic foraging techniques. However, it is difficult to say what and how they feed by the behavioural data in the present study.

It was successful to obtain evidences that large sunfish (TL >120 cm) feed on gelatinous zooplanktons in deep water by the digital still camera with light source. The large sunfish fed on various gelatinous zooplanktons like scyphozoa (lion's mane *Cyanea capullata*), a kind of ctenophore, a chain of siphonophore (at least two species of Prayidae). There were two different type foraging events; one was accompanied

with deceleration and the other was accompanied with acceleration. The former swimming behaviour is consistent with foraging on slow moving prey, however it is still a question why they accelerated to catch such a slow moving preys. The digital still camera should be unable to detect all of the feeding events because of its low sampling intervals (30 sec in 2012 and 4 sec in 2013) and couldn't get images in shallow bright water (<40 m) because of high gain for deep dark water, therefore, feeding events were extracted using swimming speed recorded by the accelerometers. Total 69% of true feeding events were detected from change of swimming speed, however, false detection became over 60%. This high false detection rate might be due to low sampling interval of the digital still camera, which couldn't record all true feeding events. The detected feeding events with deceleration occurred mostly in deep water during the day, while the detected feeding events with acceleration occurred more commonly in shallower water in large sunfish. The depth distribution of the detected feeding events was consistent with the peak occurrence of siphonophore in the still images. Gelatinous planktons like jellyfish occur at highest densities below the chlorophyll maximum layer or in the middle part of the main pycnocline (Ichikawa et al. 2006; Vereshchaka and Vinogradov 1999; Vinogradov 2005). From vertical temperature profile in July, major thermocline existed above 200 m and pycnocline should coincident with thermocline, therefore siphonophore might be abundant at this layer. The occurrence of feeding events with acceleration in shallow water might be related to their prey searching. In the still images taken in shallow water, silhouette of the objects were distinct, enables to identify the prey objects from a distance. Sunfish might search their preys by a vision (Pope et al. 2010), therefore they can find prey from a distance and rush the prey. In addition, some siphonophores have bioluminescence for bait attraction (Haddock and Case 1999, Haddock et al. 2005), might enable ocean sunfish to find in deep water. On the other hand, the detected feeding events occurred more commonly in shallow water (<50 m) in the medium

sunfish in July which swam within surface layer.   Medium sunfish might feed different preys in shallow warm water.

In conclusion, the behavioural data suggested that in July, small sunfish possibly forage on benthic preys, medium sunfish might forage in shallow water, and large sunfish mainly swam into deep water where their preys were abundant and foraged for gelatinous planktons mainly siphonophore.   Feeding on siphonophore by large sunfish was often observed in cold water (<12℃).   On the other hand, mean of ambient temperature of sunfish were 12-15℃ in all size classes.   Depth range of small sunfish was smaller than large sunfish, because small sunfish should have lower thermal tolerance.   This might be because smaller sunfish didn't use gelatinous zooplanktons like siphonophore, which were consumed by large sunfish.

Table 4-1 Summary of deployment.   Shaded lines indicate the data were not used.

MS or MA in ID indicates released in July or November, respectively.

ID	TL (cm)	Release date	Duration (hours)	Data logger	Camera	Note
MS091	51	July 5, 2009	8	W1000-PD2GT		
MS092	54	July 5, 2009	7	W1000-PD3GT	DSL400	Swam into set-net
MS093	58	July 6, 2009	6	W1000-PD3GT	DSL400	
MS094	49	July 8, 2009	7	W1000-PD2GT		
MS095	51	July 8, 2009	7	W1000-PD3GT	DSL400	
MS101	87	July 14, 2010	8	W1000-3MPD3GT		
MS102	164	July 14, 2010	7	W1000-3MPD3GT		
MS103	84	July 16, 2010	8	W1000-3MPD3GT		
MS104	130	July 16, 2010	8	W1000-3MPD3GT		
MA101	105	November 8, 2010	8	W1000-3MPD3GT		
MA102	105	November 16, 2010	8	W1000-3MPD3GT		
MA103	105	November 17, 2010	8	W1000-3MPD3GT		
MS121	95	July 17, 2012	142	W1000-3MPD3GT		
MS122	136	July 17, 2012	142	W1000-3MPD3GT	DSL400 with Light	
MS123	148	July 17, 2012	142	W1000-3MPD3GT		
MS124	200	July 17, 2012	142	W1000-3MPD3GT	DSL400 with Light	
MS125	125	July 18, 2012	118	W1000-3MPD3GT	DSL400 with Light	
MA121	67	November 13, 2012	72	W1000-3MPD3GT		
MA122	94	November 13, 2012	72	W1000-3MPD3GT	DSL400 with Light	DSL didn't work, Dead
MA123	74	November 15, 2012	-	W1000-3MPD3GT		Fail to retrieve
MS131	105	July 12, 2013	118	W1000-3MPD3GT	DSL400 with Light	DSL didn't work
MS132	125	July 13, 2013	118	W380-PD3GT	DVL400	
MS133	191	July 13, 2013	94	W1000-3MPD3GT	DSL400 with Light	

Table 4-2　Summary of feeding events of the sunfish in long deployment in July

ID	TL (cm)	No. of deep excursion	No. of images with prey items	Feeding events with acceleration	Feeding events with deceleration
MS121	95	-	-	33	242
MS131	105	60	-	39	978
MS125	125	6	6	23	214
MS132	125	15	-	5	896
MS122	136	31	57	25	917
MS123	148	43	-	6	944
MS133	191	30	112	187	664
MS124	200	10	10	36	343
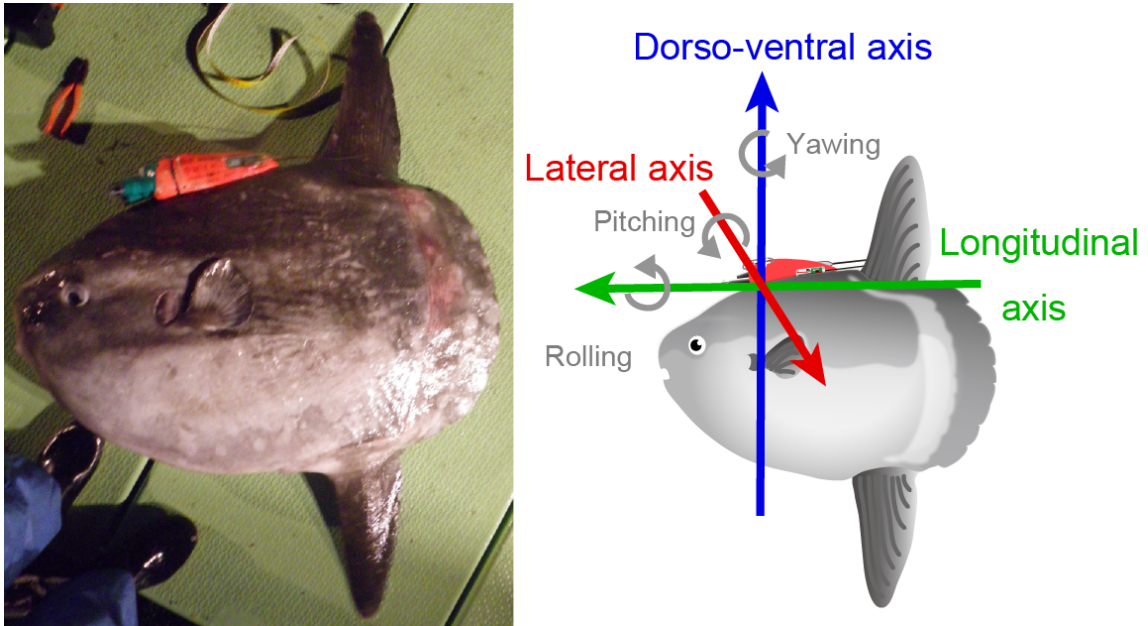
Fig. 4-1   The instruments package deployed on a sunfish (MA132: TL 94 cm) (left), and a diagram of tri-axis of acceleration and magnetism recorded by the accelerometers (right).
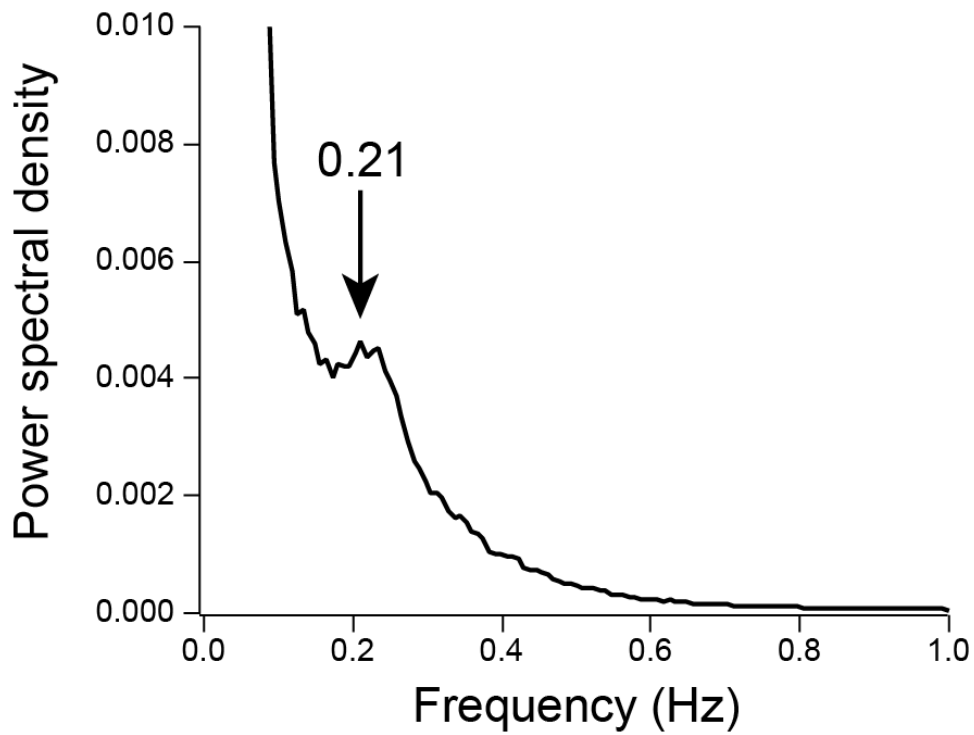
Fig. 4-2 Example of power spectral density (PSD) calculated from lateral accelerations obtained from the largest individual (MS124: TL 200 cm). An arrow indicates a peak frequency of 0.21 Hz.
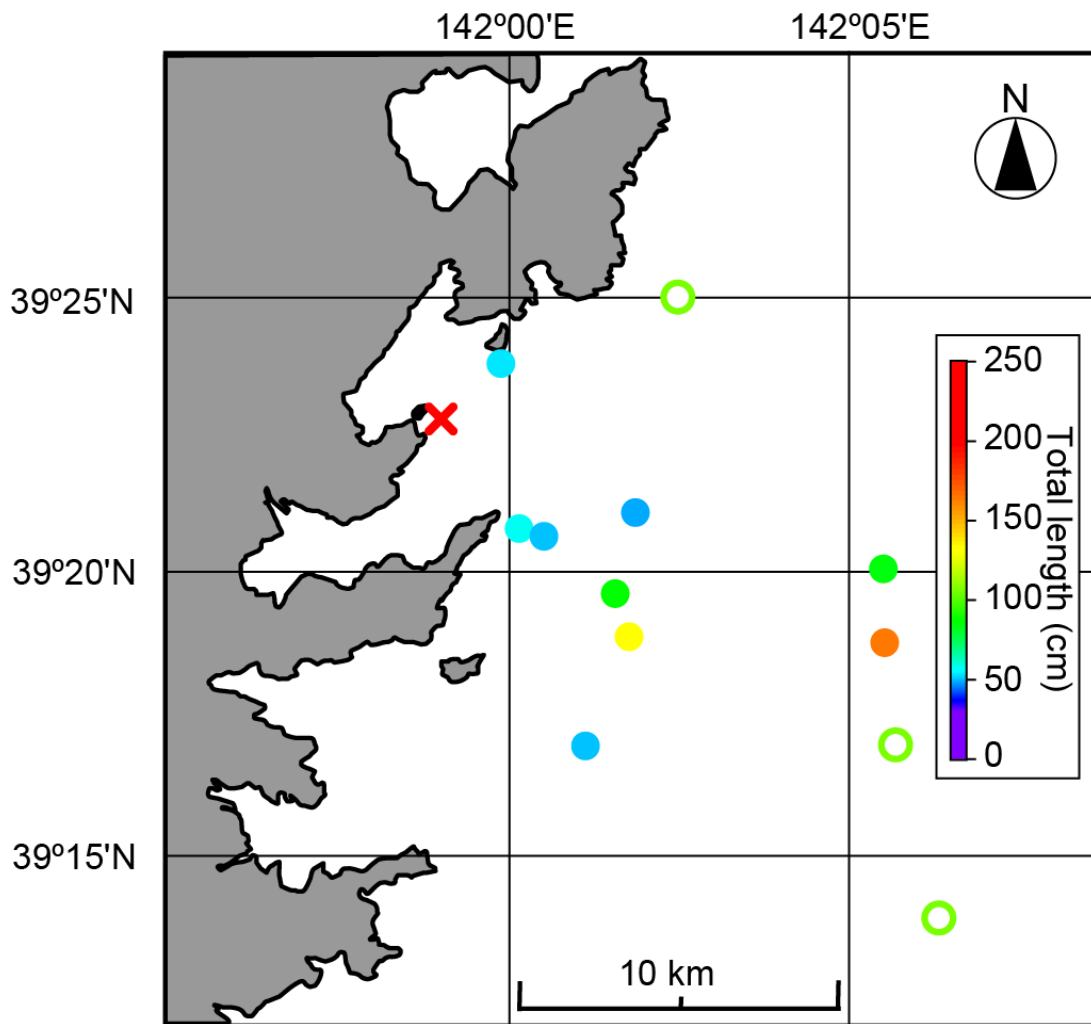
Fig. 4-3 The package retrieved locations of short deployment in 2009 and 2010. Solid circles indicate in July and open circles indicate in November. The colours of the locations indicate body size of the sunfish. A red cross indicates the location of sunfish release.
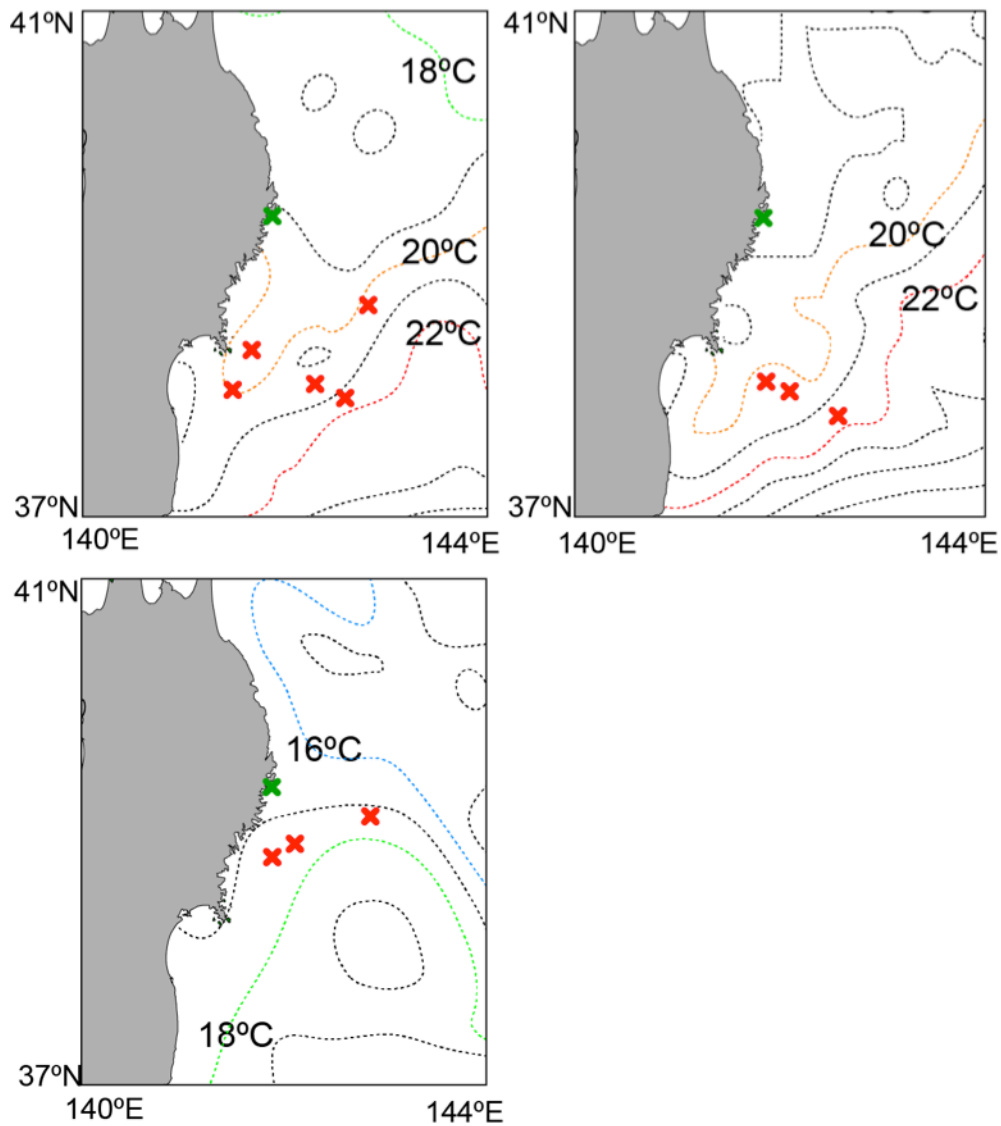
Fig. 4-4 The package pop-up locations of long deployments via Argos system in July 2012 (upper left), in July 2013 (upper right) and in November 2012 (lower). Red crosses indicate the pop-up locations and green crosses indicate the locations of sunfish release. Broken lines indicate sea surface temperature at the day of package pop-up from the web site (Japan Meteorological Agency: http://www.data.kishou.go.jp/kaiyou/db/kaikyo/daily/sst_jp.html).
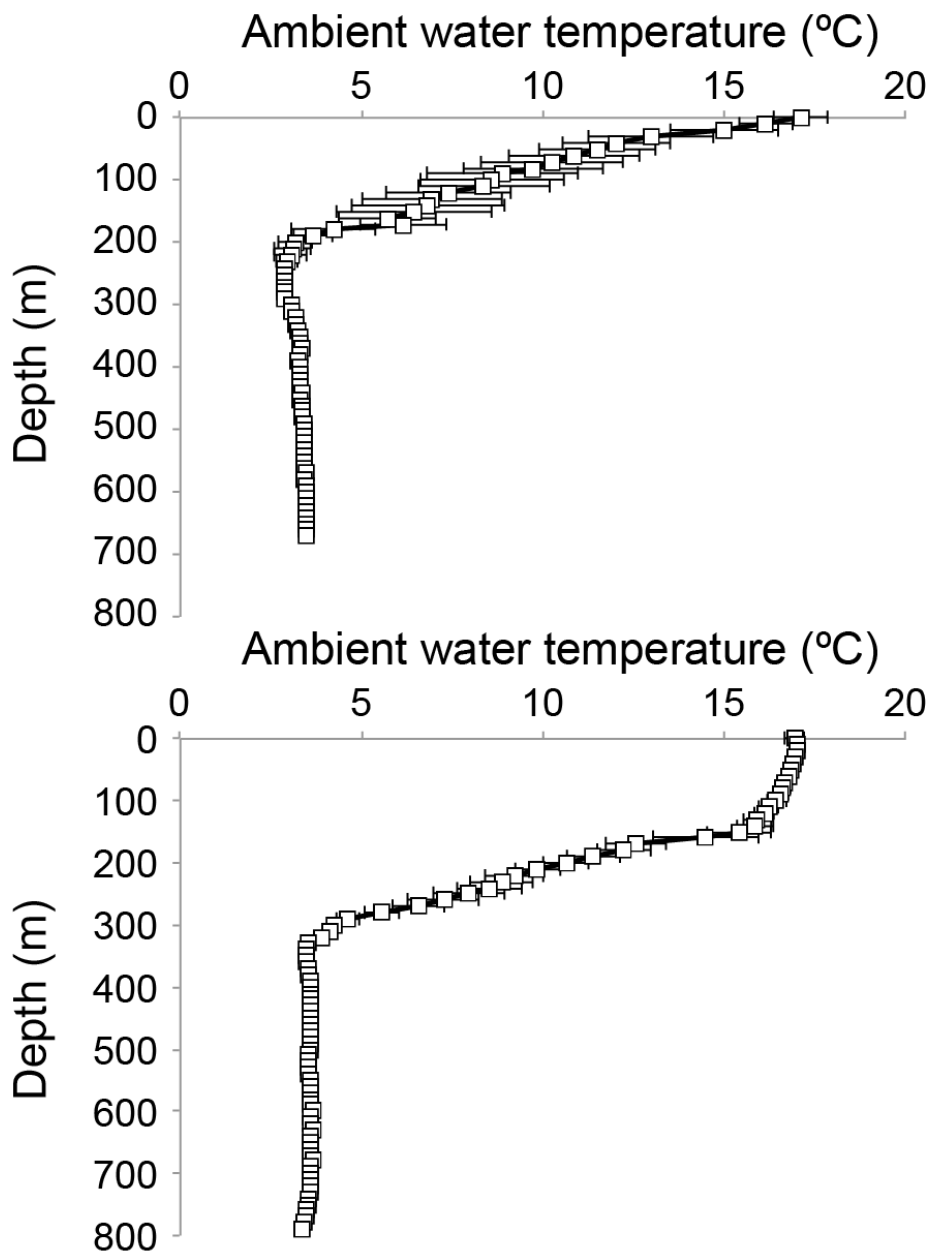
Fig. 4-5 Vertical temperature profiles in July (upper) and in November (lower) recorded by the data-loggers deployed on the sunfish (MS131 in July and MA122 in November).
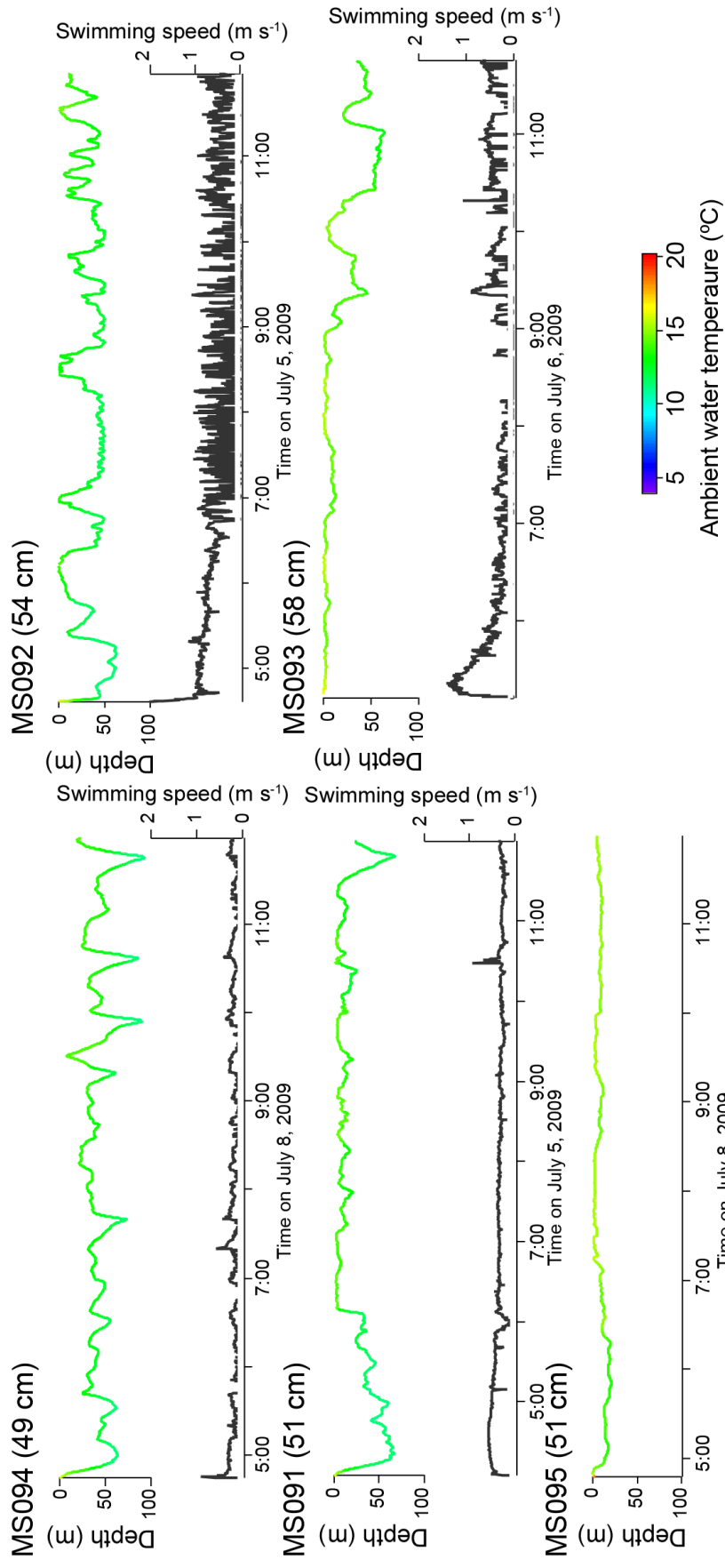
Fig. 4-6 Time series data of the depth and swimming speed obtained by short deployments on five small sunfish in July 2009. Colours of depth indicate ambient water temperature. Swimming speed of M095 failed to record because of a problem in a speed sensor. The sunfish (MS092) was found to swim into another set-net at 7:00 by the still images.

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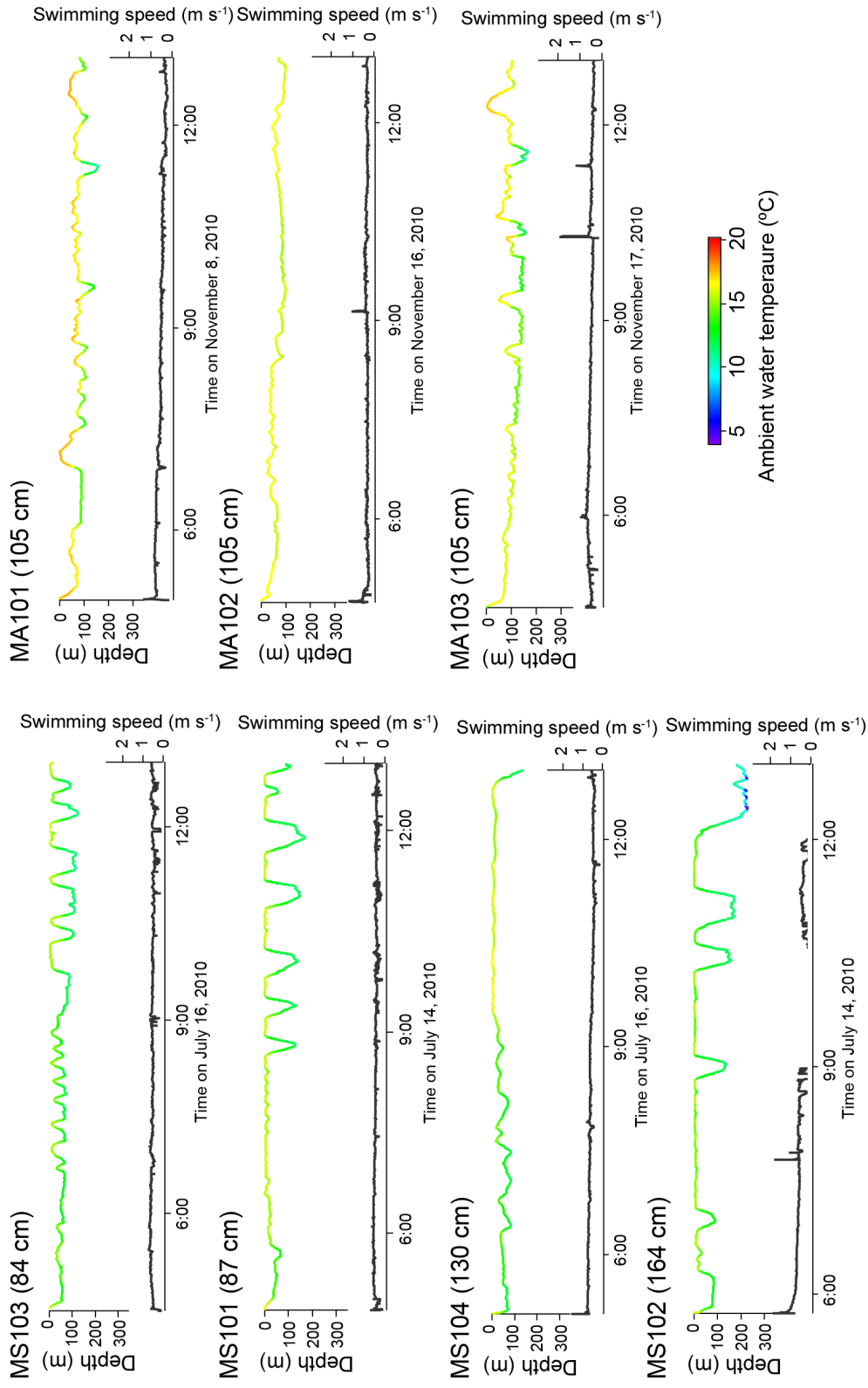
Fig. 4-7 Time series data of the depth and swimming speed obtained by short deployments on seven medium and large sunfish in July and November 2010. Colours of depth indicate ambient water temperature.

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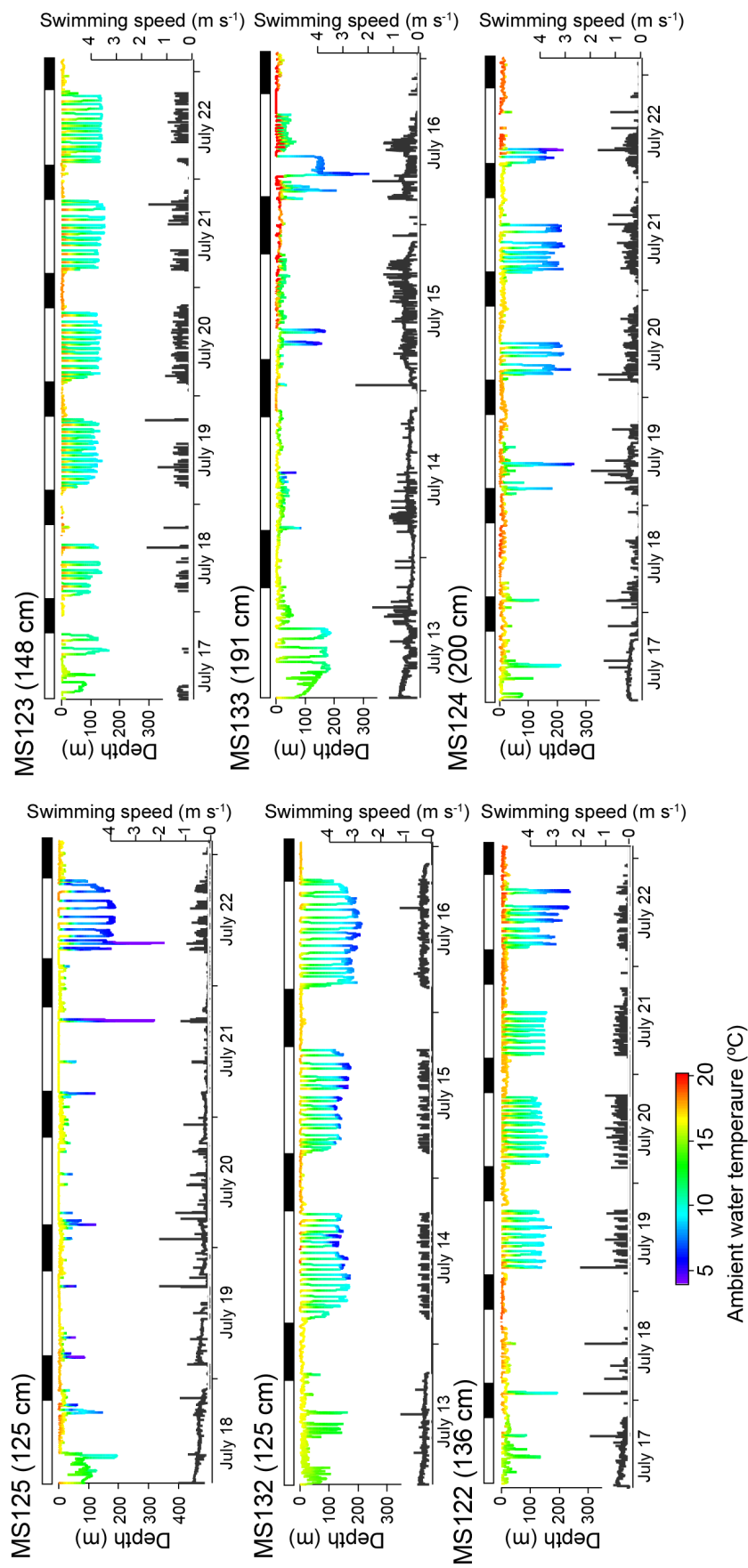
Fig. 4-8 Time series data of the depth and swimming speed obtained by long deployments on six large sunfish in July 2012 and 2013. Colours of depth indicate ambient water temperature. Open and filled squares above the graph indicate daytime (open) and nighttime (filled).

50
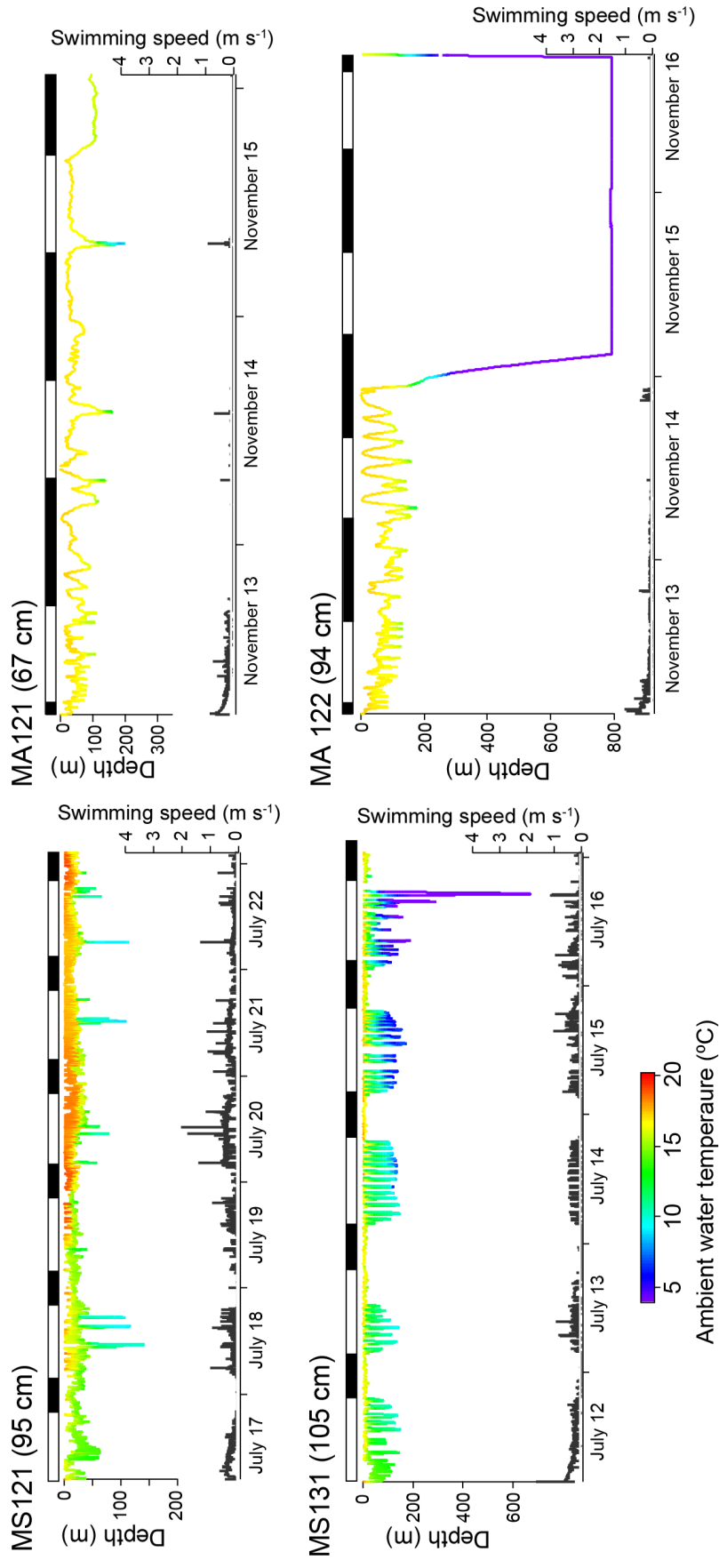
Fig. 4-9 Time series data of the depth and swimming speed obtained by long deployments on two medium sunfish in July 2012 and 2013 and two medium sunfish in November 2012. Colours of depth indicate ambient water temperature. Open and filled squares above the graph indicate daytime (open) and nighttime (filled). The sunfish (MA122) was dead 2 days after release and sank to 800 m.

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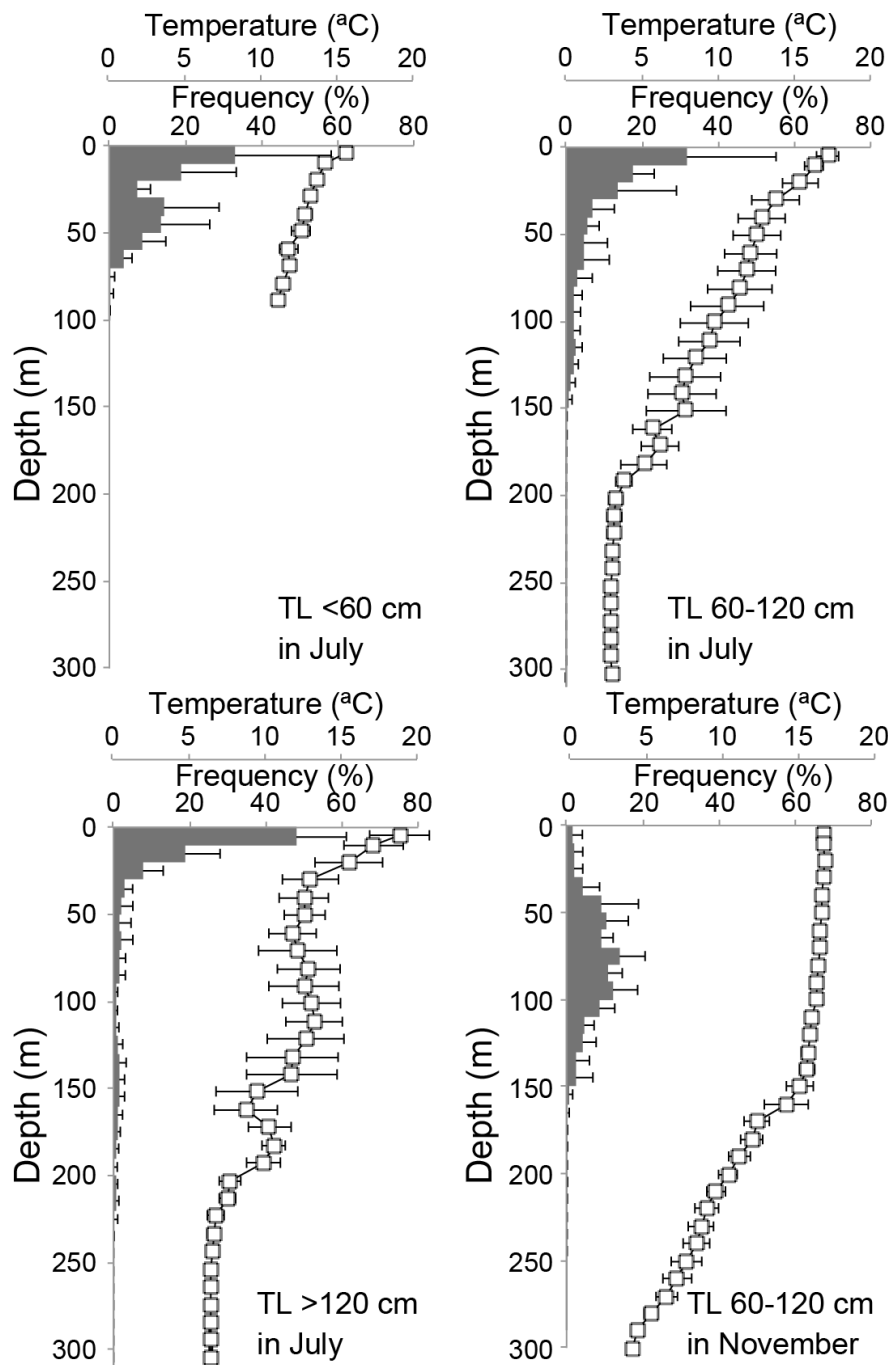
Fig. 4-10 Depth distribution with vertical temperature profiles of each body size and season. Error bars of distributions indicate standard deviation among individuals. Vertical temperature profiles were recorded by MS094 (TL 49 cm), MS131 (TL 105 cm), MS133 (TL 191 cm) and MA122 (TL 94 cm), respectively. In November, behavioural data were collected from only medium sunfish (TL 60-120 cm).
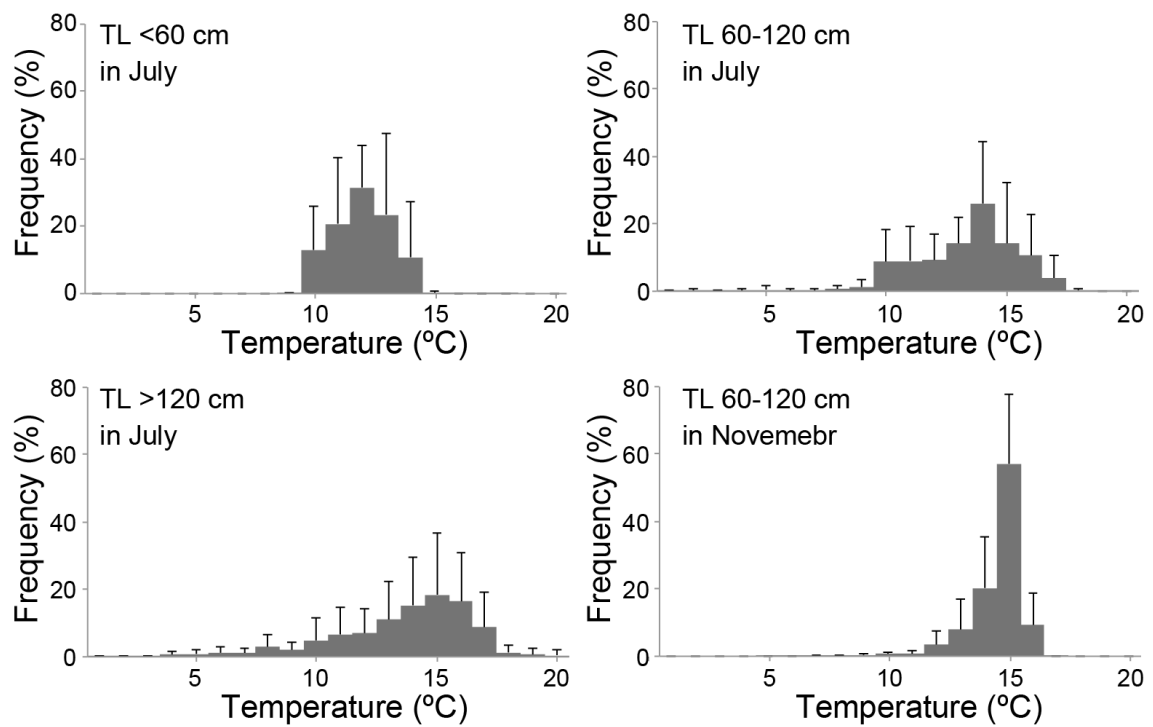
Fig. 4-11 Temperature distribution of each body size and season. Error bars indicate standard deviation among individuals.
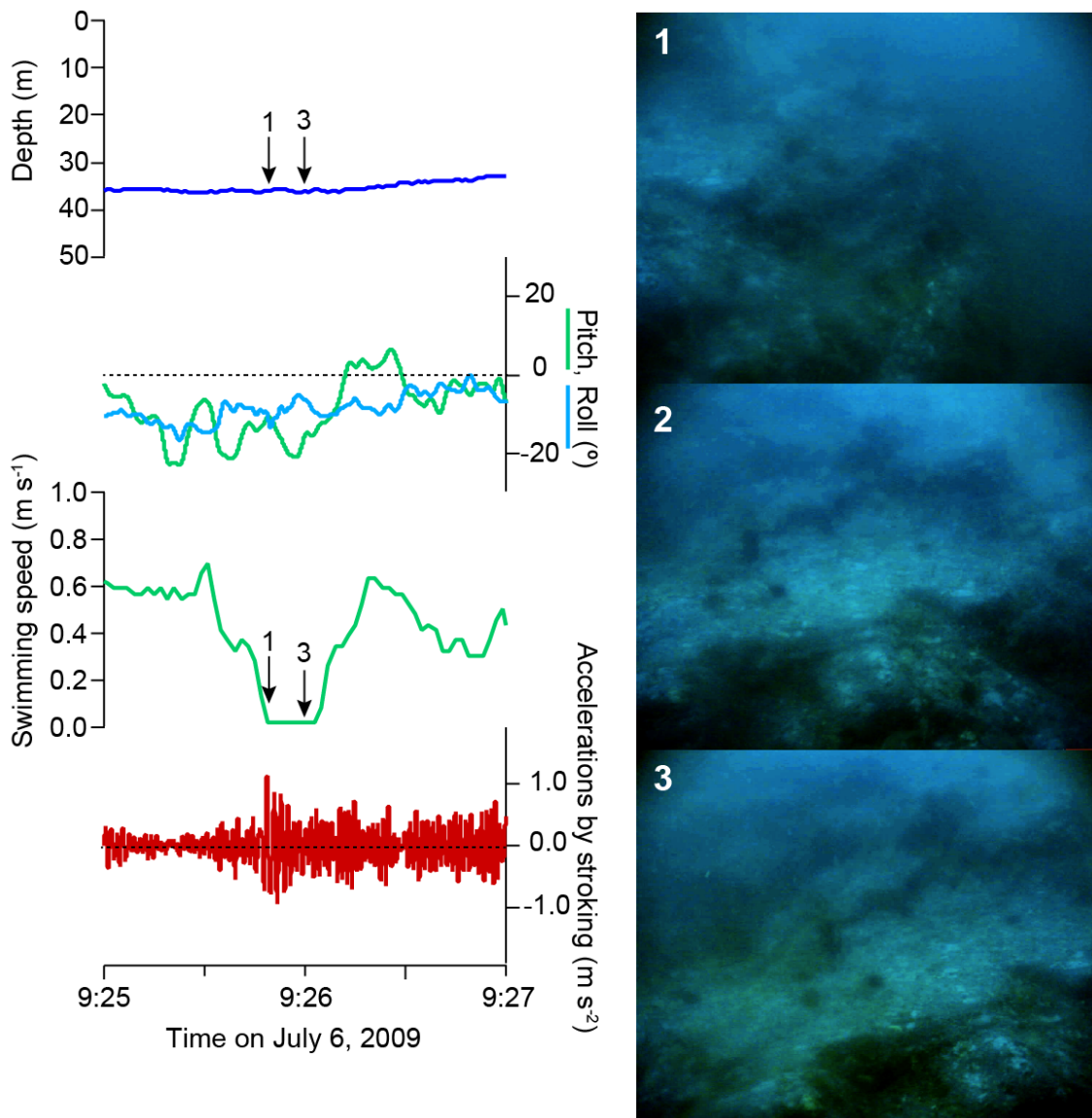
Fig. 4-12 A behaviour possibly associated with benthic foraging and still camera images obtained from MS093. Negative pitch means downward and negative roll means right side. Accelerations by stroking were the specific components of lateral acceleration. Arrows with number indicate the time of still camera images.
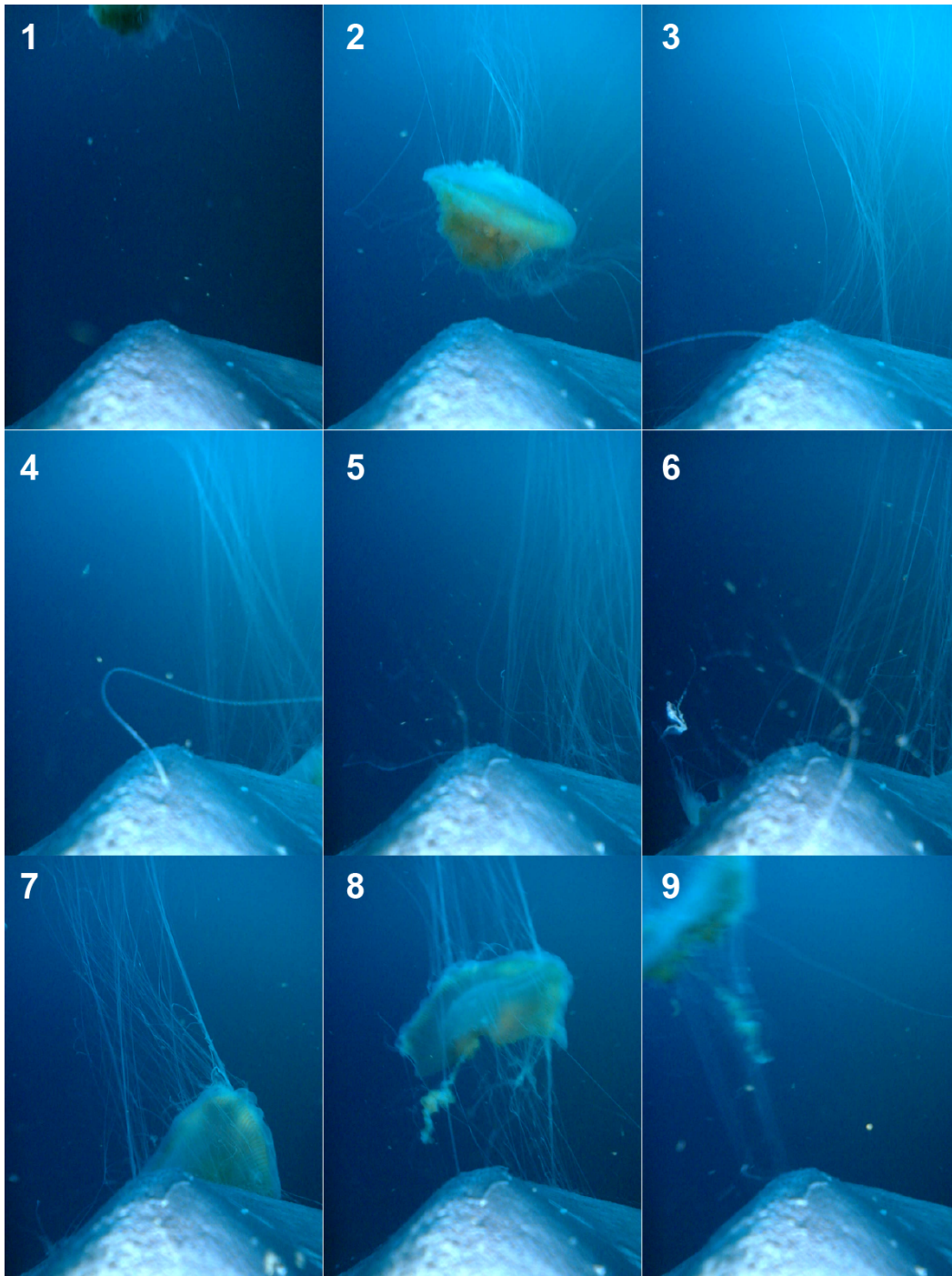
Fig. 4-13 Series of images at every 4 sec of feeding on a lion's mane jellyfish (*Cyanea capillata*) from the sunfish of 191 cm in 2013 (MS133). The sunfish approached to the jellyfish (1-2), ate (3-6), and ejected the jellyfish (7-9). The sunfish didn't eat whole body of the jellyfish, but ate gonad, oral arms and a part of bell.
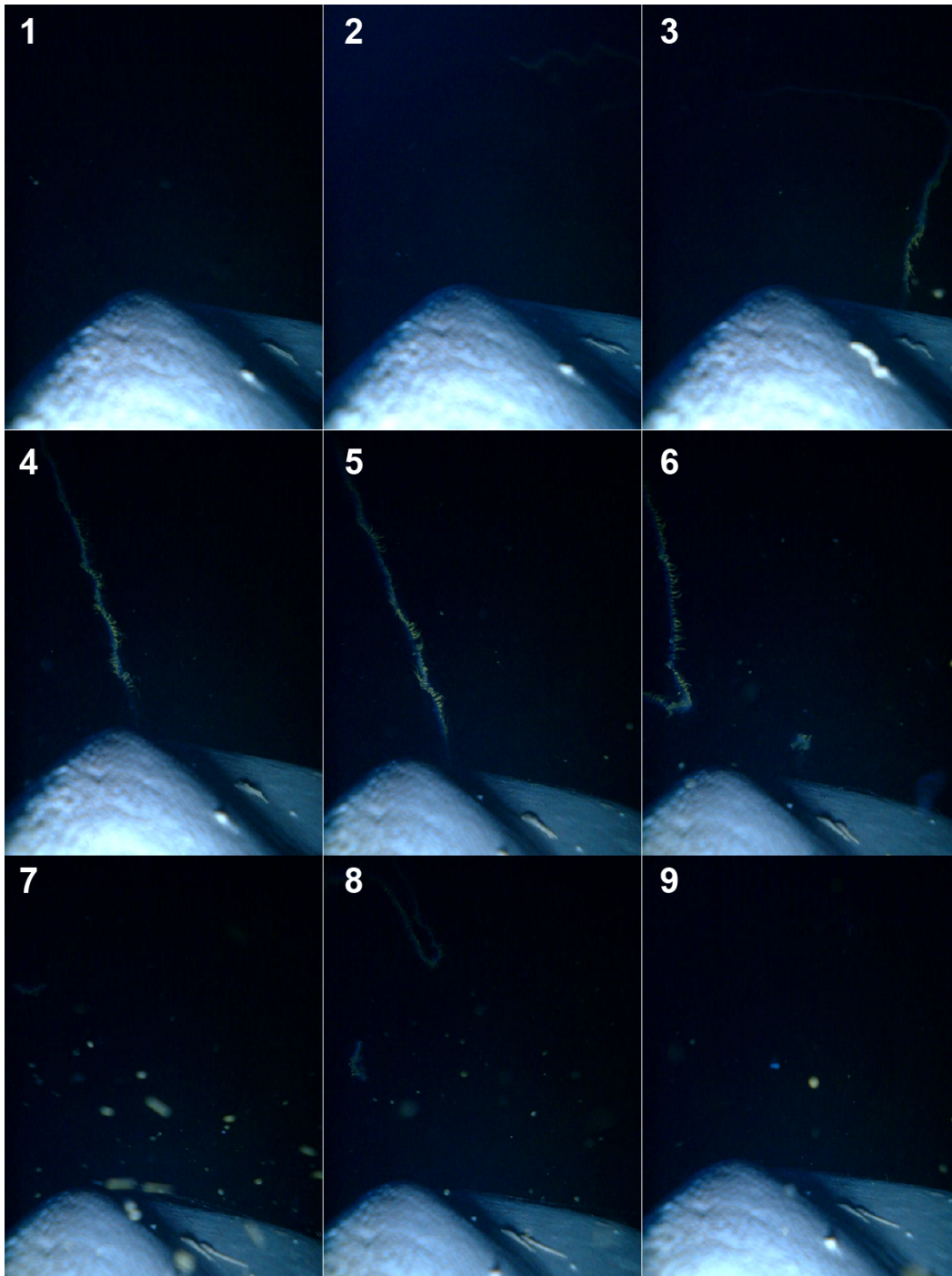
Fig. 4-14　Series of images at every 4 sec of feeding on a chain of siphonophore (Prayidae *sp*.) from the sunfish of 191 cm in 2013 (MS133).　The sunfish approached to the siphonophore (2), ate (3-5), and ejected the siphonophore (6-8).　The sunfish didn't eat whole chain of siphonophore.
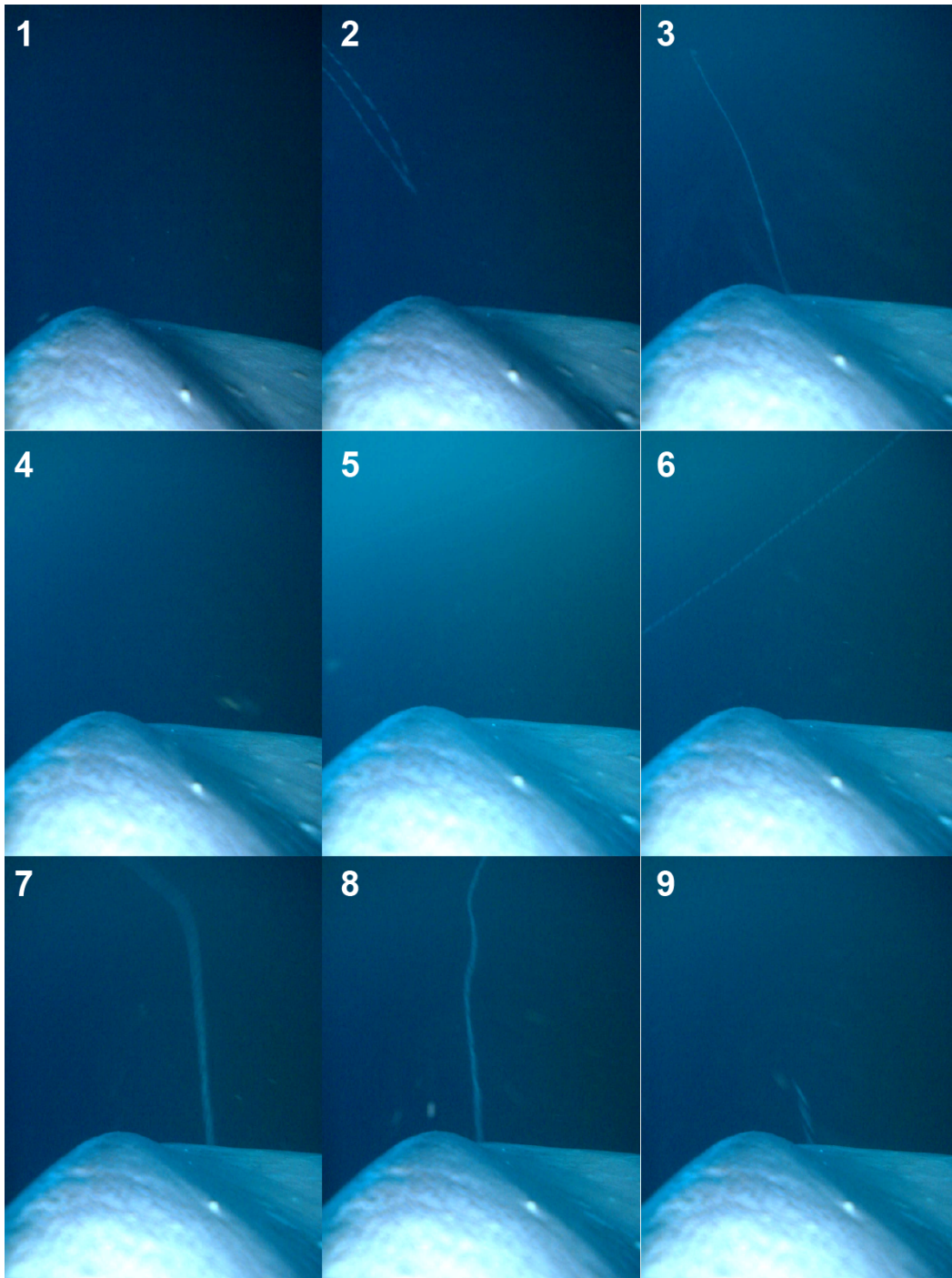
Fig. 4-15 Series of images at every 4 sec of feeding on a chain of siphonophore (species can't be identified) from the sunfish of 191 cm in 2013 (MS133). The sunfish approached to the siphonophore (2), ate (3), and approached again (4-6), ate (7-8) and finished eating (9). The sunfish ate whole chain of siphonophore.
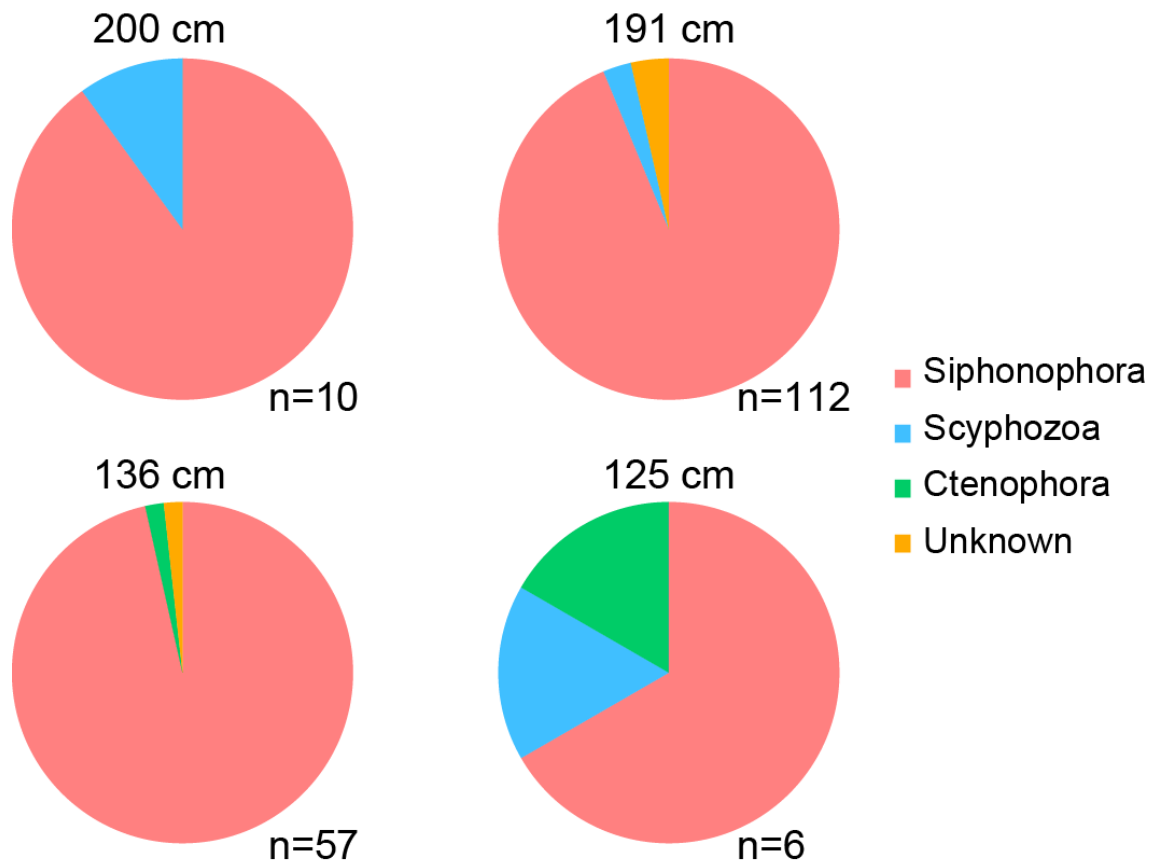
Fig. 4-16 Proportion of prey items seen in DSL images from four sunfish in July 2012 and July 2013. There was no prey item in DSL images from the sunfish of 105 cm in 2013. Siphonophora was the most observed prey item in all individuals.
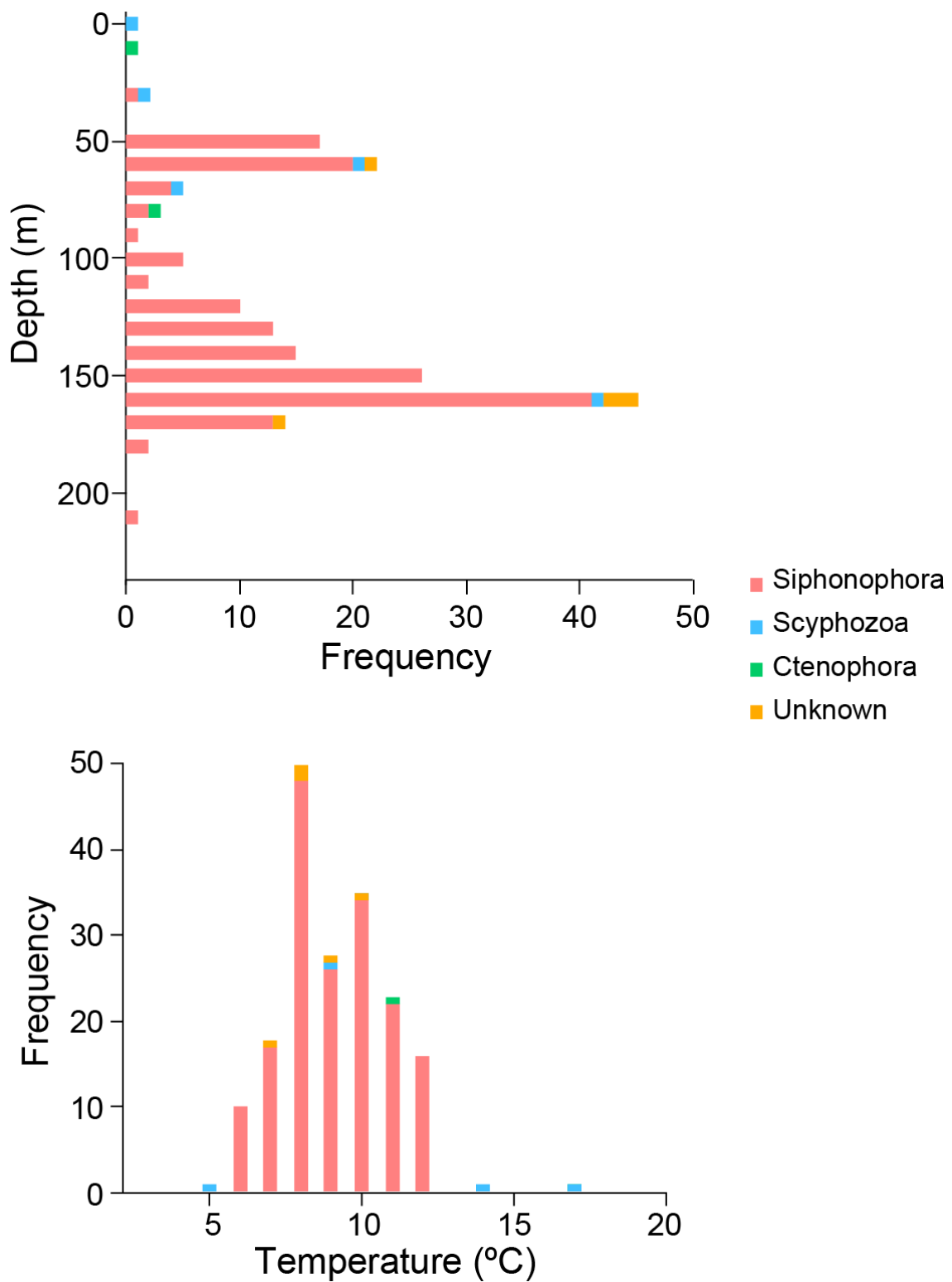
Fig. 4-17 Occurrence depth and temperature of each prey item in July. Most consumed prey Siphonophora peaked at 160 m depth and 8℃.
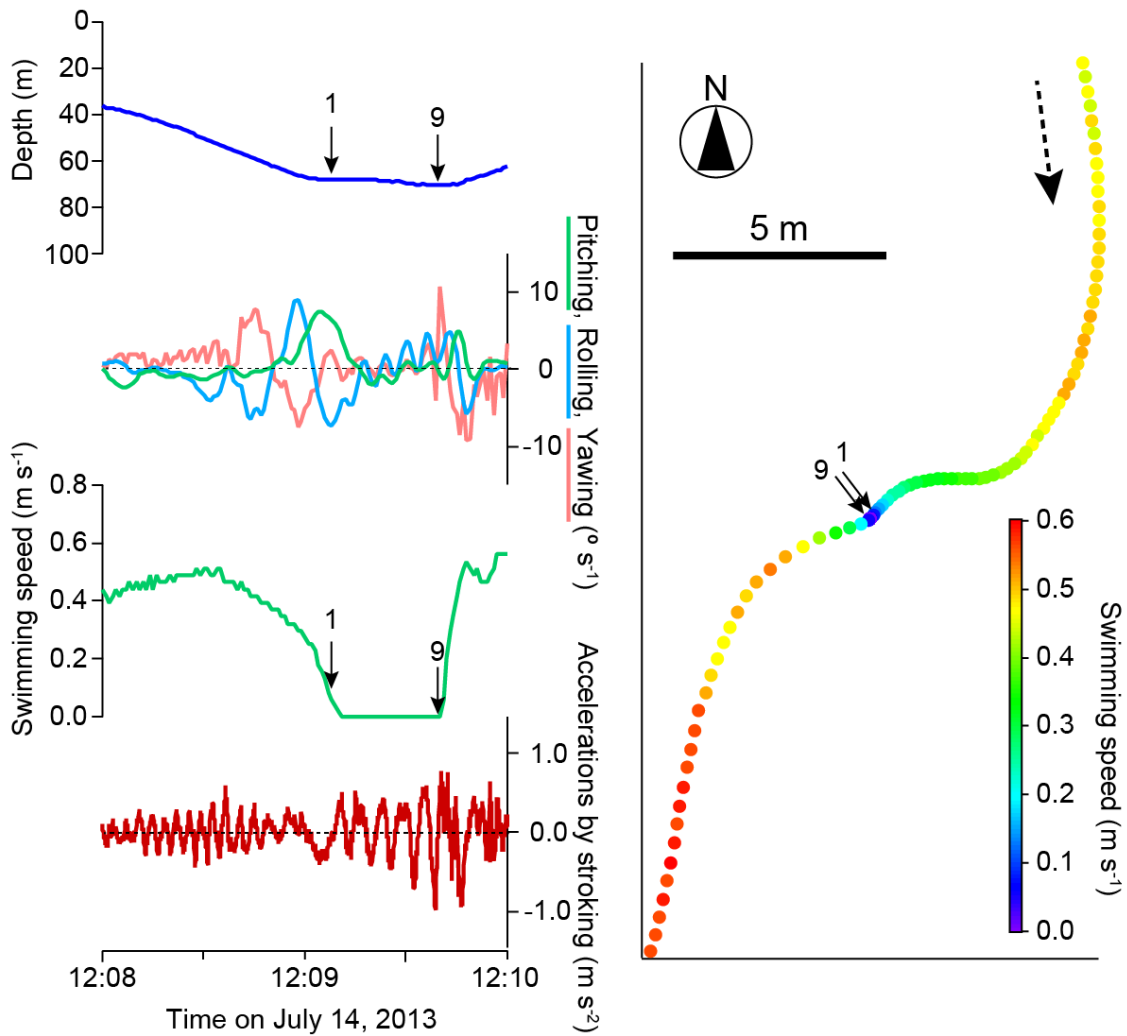
Fig. 4-18   Feeding behaviour on lion's mane (Fig. 4-13) recorded by accelerometer and calculated horizontal path during the feeding event.   Pitching, rolling and yawing indicate change of angle in pitch, roll and yaw, respectively.   The colours of the horizontal path indicate swimming speed.   A dashed arrow in the path indicates the direction of movement.   The arrows with number indicate the locations of DSL images in Fig 4-13.
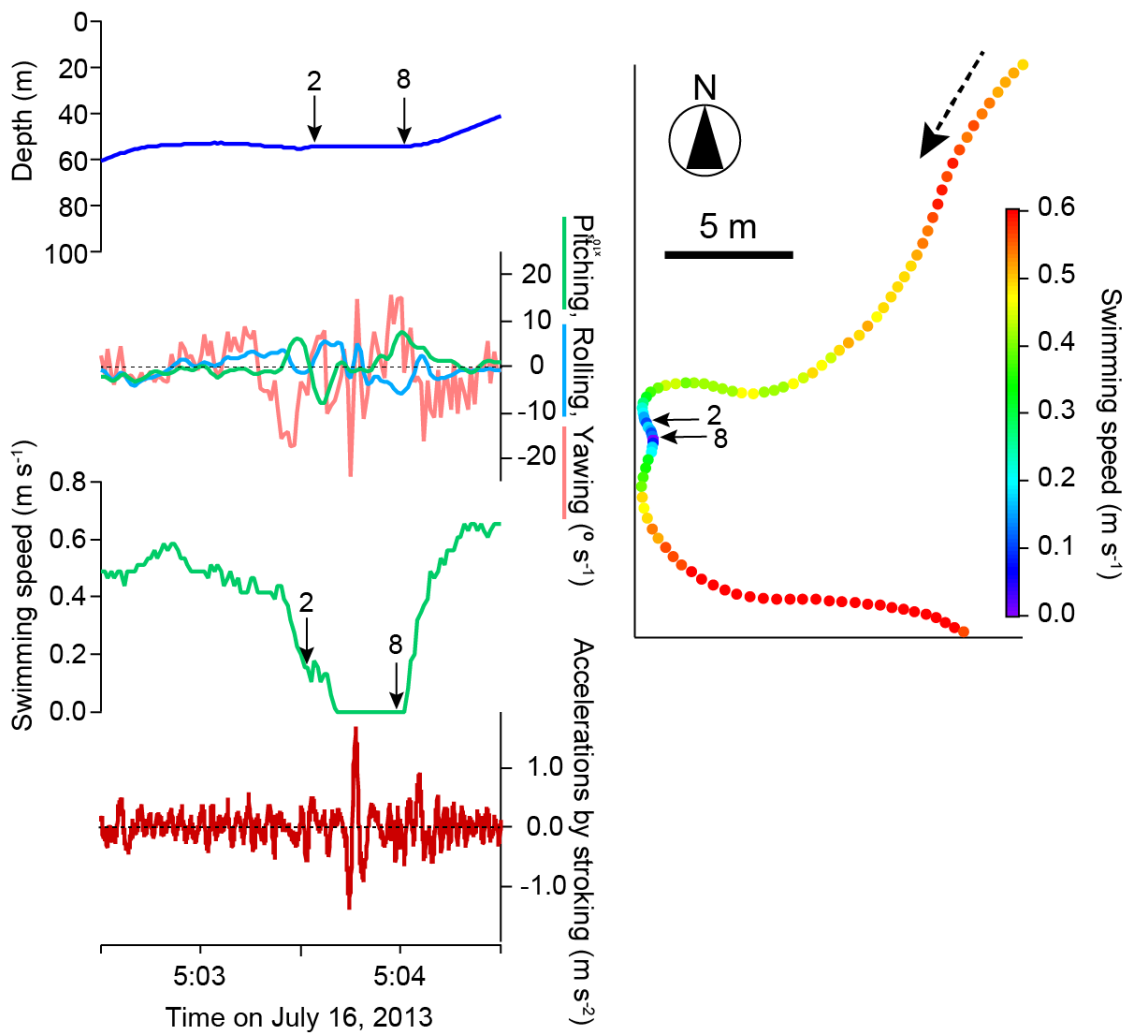
Fig. 4-19 Feeding behaviour on a chain of siphonophore (Fig. 4-14) recorded by accelerometer and calculated horizontal path during the feeding event.   Pitching, rolling and yawing indicate change of angle in pitch, roll and yaw, respectively.   The colours of the horizontal path indicate swimming speed. A dashed arrow in the path indicates the direction of movement.   The arrows with number indicate the locations of DSL images in Fig 4-14.
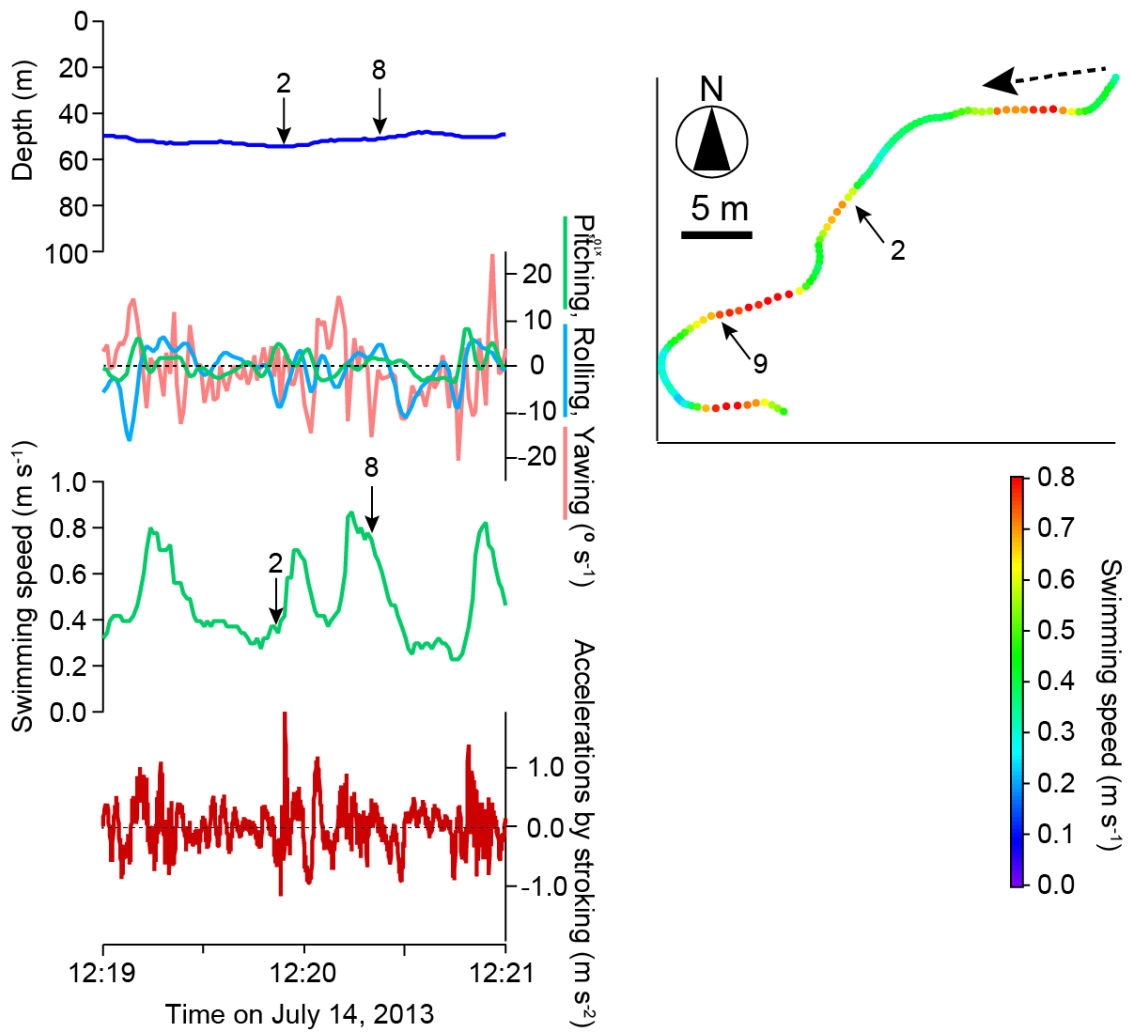
Fig. 4-20 Feeding behaviour on a chain of siphonophore (Fig. 4-15) recorded by accelerometer and calculated horizontal path during the feeding event. Pitching, rolling and yawing indicate change of angle in pitch, roll and yaw, respectively. The colours of the horizontal path indicate swimming speed. A dashed arrow in the path indicates the direction of movement. The arrows with number indicate the locations of DSL images in Fig 4-15.
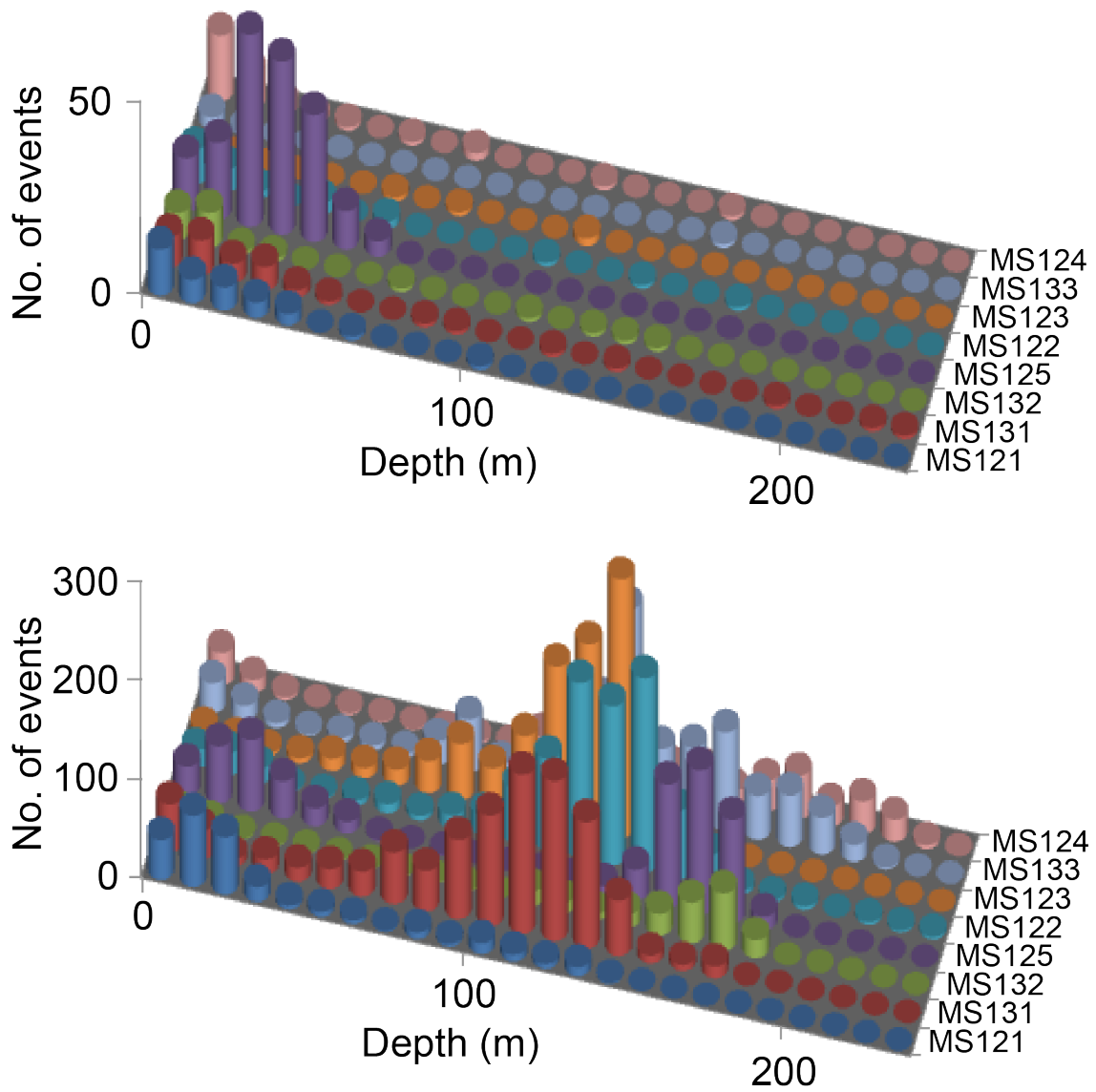
Fig. 4-21 Vertical distribution of the number of detected feeding events with acceleration (upper) and deceleration (lower) of the sunfish obtained by long deployments in July.
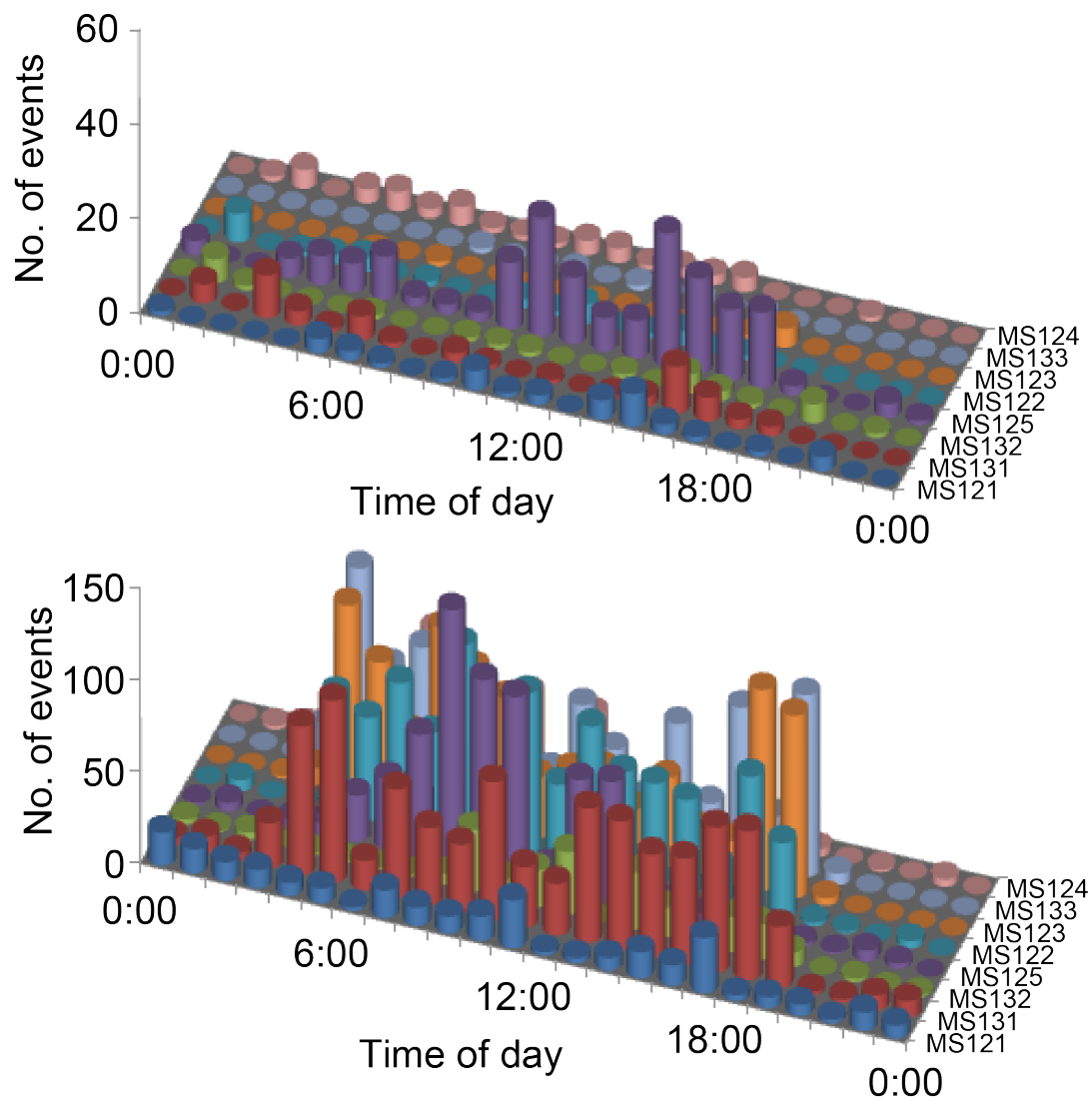
Fig. 4-22 Mean hourly number of detected feeding events with acceleration (upper) and deceleration (lower) of the sunfish obtained by long deployments in July.

Chapter 5   Thermoregulation

Introduction

From the behavioural study in Chapter 4, behavioural thermoregulation of ocean sunfish was suggested.   The ocean sunfish is often observed lying motionless at the sea surface, which seems to be 'basking' (Pope et al. 2010).   However, recent studies have revealed that ocean sunfish repeatedly approach from the surface to over 500 m depth (Dewar et al. 2010; Hays et al. 2009; Potter and Howell 2011; Sims et al. 2009a, 2009b).   These deep excursions were found for direct to foraging by Chapter 4. There are several hypotheses of the reason of surfacing; to be sleeping, to be eliminated their external parasites by seabirds, and to rewarm after excursion to deep cold water (Abe et al. 2012, Cartamil and Lowe 2004).   Sunfish stayed at the surface after every deep excursion (Dewar et al. 2010), so it is difficult to be considered the main function of this behaviour is parasite elimination.

The body temperature of ocean sunfish should be affected by ambient water temperature because of their ectothermy.   Behavioural thermoregulation of ectothermic species was often reported that they regulated their body temperature by moving between different temperature environments (e.g. Dubois et al. 2009, Kiefer et al. 2007). Behavioral thermoregulation is commonly seen in many species of fishes, for example, both endothermic (e.g. bluefin tuna, *Thunnus orientalis*, Kitagawa et al. 2001) and ectothermic fishes (e.g. blue shark, *Prionace glauca*, Carey and Scharold 1990) alternate between deep dives into cold water and 'rewarming' periods in the surface mixed layer.

In this chapter, the thermal regulation hypothesis of surfacing was examined by direct measurement of their body temperature under natural condition.

Materials and methods

The field experiment was conducted at the same time of foraging behaviour study in Chapter 4. In 2013, body temperature of the same ocean sunfish was measured additionally using a depth temperature recorder (LAT1810ST, 11 mm in diameter, 35 mm in length and 5 g in air, and it has a 150-mm stalk temperature sensor, Lotek Wireless Inc., Canada), which recorded depth and two sites of temperature at 1 Hz. The depth temperature recorder was attached ventral side of the instrument packages. When the instrument package was attached to the sunfish, another tiny vertical hole was pierced and the stalk sensor of the depth temperature recorder was inserted into the hole, thus muscle temperature at 15 cm deep from its body surface was measured (Fig. 5-1). When the instrument package detached from the sunfish, the stalk sensor was pulled out from the sunfish by buoyancy of the package.

R ver.3.0.2 (R project) was used to analyze temperature data. For the body temperature analysis to estimate the changes in k necessary to account for the observed rates of body warming and cooling, a function of heat exchange with the environment and internal heat production was used (Holland and Sibert 1994). Heat loss (or gain) is proportional to the difference between the body temperature of sunfish and the water in which it is swimming:

$$\frac{dT_b(t)}{dt} = k\big(T_a(t) - T_b(t)\big) + \dot{T}_0 + \epsilon, \qquad (1)$$

where k is whole-body heat-transfer coefficient (°C min^{-1} °C^{-1}), $T_a(t)$ is ambient water temperature (°C) as a function of time t, $T_b(t)$ is body temperature of sunfish (°C) as a function of time t, $\dot{T}_0$ is the rate of temperature change due to internal heat production (°C min^{-1}), and ε is white Gaussian noise, and t is time (min). Response time lag of body temperature behind ambient water temperature were observed in measured body temperature, therefore τ (min) was added as response time lag of body temperature as follows:

$$\frac{dT_b(t)}{dt} = k\big(T_a(t-\tau) - T_b(t)\big) + \dot{T}_0 + \epsilon, \tag{2}$$

two different conditions about k were compared as follows:

$$(a) \; k = a \; constant \; value,$$

$$(b) \; k = \begin{cases} k_1; \; T_a(t-\tau) < T_b(t) \\ k_2; \; T_a(t-\tau) \geq T_b(t) \end{cases},$$

where k_1 and k_2 are two values for the heat-transfer coefficient. The parameters for each model were estimated using maximum-likelihood method. First, Eq. (2) was discretized as follow:

$$\frac{T_b(t+\Delta t) - T_b(t)}{\Delta t} = k\big(T_a(t-\tau) - T_b(t)\big) + \dot{T}_0 + \epsilon, \tag{3}$$

$$T_b(t+\Delta t) = T_b(t) + \Delta t\{k\big(T_a(t-\tau) - T_b(t)\big) + \dot{T}_0 + \epsilon\}, \tag{4}$$

where Δt is minimum scale of discretized time (I used time scale in minute in following analyses). Then, the parameters were parameterized as $\Delta t k \rightarrow k$, $\Delta t \dot{T}_0 \rightarrow \dot{T}_0$ and $\Delta t \varepsilon \rightarrow \varepsilon$. Then, Eq. (4) was transformed as follows:

$$T_b(t+\Delta t) = (1-k)T_b(t) + kT_a(t-\tau) + \dot{T}_0 + \epsilon, \tag{5}$$

$$= \mu(t) + \epsilon.$$

$T_b(t+1)$ can be assumed to follow as normal probability distribution whose average is $\mu(t)$ and dispersion is σ^2, therefore probability of observed body temperature of each time $P(T_b(t))$ were calculated as follows:

$$P\big(T_b(t+1); T_a(t-\tau), T_b(t), k, \dot{T}_0, \sigma\big) = \frac{1}{\sqrt{2\pi\sigma^2}}\left(e^{-\frac{1}{2\sigma^2}(T_b(t+1)-\mu(t))^2}\right), \tag{6}$$

and likelihood (L) and logarithmic likelihood ($\ln L$) of time series of body temperature $T_b(1), \cdots, T_b(N)$ was calculated as follows:

$$L = \frac{1}{(2\pi\sigma^2)^{\frac{N}{2}}} \prod_1^N e^{-\frac{1}{2\sigma^2}(T_b(t+1)-\mu(t))^2}, \tag{7}$$

$$\ln L = -\frac{1}{2\sigma^2}\sum_{1}^{N}\ln\big(T_b(t+1)-\mu(t)\big)^2 - \frac{N}{2}\ln\sigma^2 - \frac{N}{2}\ln(2\pi), \qquad (8)$$

and Akaike information criteria (AIC) was calculated as follows:

$$\text{AIC} = -2\ln L + 2l, \qquad\qquad (9)$$

where l is the number of parameters. *Optim* function in R was used to maximize ln$L$ by changing all parameters and calculated AIC of models (a) and (b). Then, the model which showed the lowest AIC was regarded as parsimonious model. To examine whether their heat-transfer coefficient change in dead condition or not, body temperature change of a dead sunfish (TL 32 cm) was recorded by putting the dead sunfish alternately in Styrofoam cartons of 18℃ and 8℃ seawater, measured body and water temperature using the depth temperature recorder, and analyzed the data similar to sunfish in the field. In case of the dead sunfish, the rate of temperature change due to internal heat production $\dot{T}_0$ was assumed as 0.

To examine effect of body size, the heat-transfer coefficient calculated above was compared with body size. The body mass of sunfish was estimated from total length (*TL* in cm) of the sunfish using the following equation (Watanabe and Sato 2008).

$$BM = 52.96\times(\frac{TL}{100})^{2.96},$$

where *BM* is estimated body mass in kg. In addition, using the behavioural data obtained by long deployment on the eight sunfish in July, relationship between cooling in deep water and warming at the surface was obtained and compared among body sizes. Deep excursions were defined as getting into colder than 15℃ for longer than 5 min. Then, cooling index (cumulative thermal difference between the threshold temperature 15℃ and ambient water temperature during staying in deep cold water) and warming index (cumulative thermal difference between ambient water temperature during staying at the surface and the threshold temperature 15℃ during staying at the surface after

deep excursion) were compared. The overnight surfacing was excluded from the analyses. To test whether there was a relationship between warming index and cooling index and interaction of body size, GLMM and AIC were used to select the most parsimonious model. A function *glmm* contained in *repeated* package of R was used to calculate AIC. Warming index was set as a response variable in the models. Warming index only or interaction between warming index and estimated body mass were set as explanatory variables, and individual was set as a random effect.

Results

Total of 13 days of behavioral data with body temperature were obtained from the three sunfish. As mentioned in the previous chapter, all sunfish showed typical day-night behavior, repetitively approached from the surface to over 100 m during day and stayed at shallow depth (0-20 m) during night (Fig. 5-2). Ambient water temperature was 16-22°C at the surface and minimum 3°C at depth over 200 m. Before deep excursions, body temperature of the sunfish was 16-17°C similar to ambient water temperature at the surface, and decreased maximum 6°C with ambient water temperature decreased during deep excursions (Figs. 5-2 and 5-3). After every deep excursion, the sunfish stayed at the surface and their body temperature rebounded to similar to the sea surface temperature (Figs. 5-2 and 5-3). Body temperature ranges of the sunfish (12-21°C) were narrower than the range of ambient water temperature (3-22°C), and the minimum body temperature was higher than the minimum ambient water temperature (Fig. 5-4). Mean body temperatures of three sunfish (MS131, MS132 and MS133) were 16.1, 16.4 and 16.9°C, and mean ambient water temperatures were 15.1, 14.6 and 15.8°C, respectively. Body temperature of the dead sunfish was also changed by ambient water temperature (Fig. 5-5).

Calculated AIC were lower in variable k model (*b*) than constant k model (*a*) in all individuals in the field, and became minimum at response time lag of body

temperature τ was 4-8 min, and estimated response time lag τ was larger in larger individuals (Table 5-1, Fig. 5-6). The estimated parameters for AIC minimum model of each k condition are presented in Table 5-1. The values of k_1 and k_2 produced by variable k model (*b*) generate warming and cooling curves which match the observed body temperature changes (Figs. 5-3 and 5-5). All individuals in the field showed lower heat-transfer coefficient during cooling (k_1) than heat-transfer coefficient during warming (k_2) and the rate of temperature change due to internal heat production ($\dot{T_0}$) was 0 (Table 5-1). The ratios of k_1 and k_2 were similar among body sizes (Table 5-1). Both values of k_1 and k_2 decreased with body size of sunfish (Fig. 5-7). In case of dead sunfish, both models (*a*) and (*b*) showed similar lowest AIC, and against the individuals in the field, k_1 was larger than k_2 in model (*b*) of the dead sunfish.

From the AIC selection of relationship between warming and cooling index, lower AIC score was obtained from the model including only cooling index (3759.3) than the model including interaction between cooling index and body size (3759.7), indicating that warming index increased with increase of cooling index but there was no interaction effect of body size. The estimated coefficient of cooling index was positive (0.1477 ± 0.0269), indicating warming index increased with increase of cooling index (Fig. 5-8).

Discussion

Some behavioural reports of ocean sunfish suggested their vertical movements might be related to behavioural thermoregulation (Cartamil and Lowe 2004, Hays et al. 2009, Sims et al. 2009a, 2009b), but no evidence was indicated. In the present study, measured body temperature of the sunfish decreased during the sunfish stayed at deep cold water, but rebounded during they stayed at the surface after dives. This indicates a function of surfacing is behavioural thermoregulation for recovering body temperature by warm surface water.

As the results of the heat-transfer coefficient k estimation, the sunfish in the field showed smaller heat-transfer coefficient k during cooling than during warming, though the dead individual showed similar heat-transfer coefficient k both during cooling and warming. These suggest that sunfish might be able to physiologically regulate their whole-body heat-transfer. This enables them to be able to keep mean body temperature 1-2℃ warmer than mean ambient water temperature without heat production. Similar body temperature change was observed in other ectothermic fish, blue sharks *Prionace glauca*, that cooling rate was smaller than warming rate, enabling to keep 4℃ warmer mean body temperature than mean ambient water temperature (Carey and Scharold 1990). In case of endothermic fish, Pacific bluefin tuna *Thunnus orientalis* has similar heat-transfer coefficient k during cooling and warming, however, they can keep their body temperature by heat production (Kitagawa et al. 2001). The heat-transfer coefficient k during cooling of the dead sunfish was an extension of the regression line of the heat-transfer coefficient during cooling of the sunfish in the field (fig. 5-7). This might suggest that heat loss was physical constraints and living sunfish could increase heat gain during warming. Response time lag of body temperature behind ambient water temperature was estimated to be larger in larger individuals including the dead small sunfish. This might be because larger sunfish had smaller heat-transfer coefficient k.

Warming indexes (amount of heat gained during staying at the surface after deep excursion) increased with increase of cooling index (amount of heat lost during staying in deep water), indicating that sunfish need to gain larger amount of heat when they lost larger amount of heat. However, there was no interaction effect of body size in the relationship between warming and cooling index. The heat-transfer coefficient estimation indicated that larger sunfish had smaller heat-transfer coefficient k during cooling, however, heat-transfer coefficient k during warming was also smaller in larger sunfish. Ratio of heat-transfer coefficient k between warming and cooling was similar,

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indicating that larger sunfish need more time to recover their body temperature than smaller sunfish.   Fish use their gills to transfer oxygen from the surrounding water into their blood, and fish gills have 2 - 48 time s larger surface area than body surface area (Gray 1954).   This larger surface area of gill may work as a heat exchanger.   There is no evidence of relationship between body surface area and gill surface area according to body size difference in ocean sunfish, however, this might be affected the relationship between heat-transfer coefficient and body size.

The results of the present study suggest that surfacing behaviour of ocean sunfish is behavioural thermoregulation to rewarm using warm surface water, and the thermoregulation was reinforced by physiological change such as small heat-transfer coefficient k during cooling and large heat-transfer coefficient k during warming.   All sunfish showed similar mean muscle body temperature (16-17ºC), indicating this might be suitable body temperature of this species.   These physiological and behavioural thermoregulation make them be able to forage deep cold water.

Table 5-1 Results of estimations of whole-body heat-transfer coefficient. k_2/k_1 indicate ratios of k_1 (heat-transfer coefficient during cooling) and k_2 (heat-transfer coefficient during warming).

Total length (cm)	k condition	k	k_1	k_2	$\dot{T}_0$	t	AIC	k_2/k_1
105	Constant	8.06×10^{-3}			8.63×10^{-3}	6	-42529	-
105	Variable		6.23×10^{-3}	2.28×10^{-2}	0	5	-43747	3.66
125	Constant	6.41×10^{-3}			1.20×10^{-2}	8	-35318	-
125	Variable		4.33×10^{-3}	2.83×10^{-2}	0	7	-37762	6.53
191	Constant	3.47×10^{-3}			8.40×10^{-5}	7	-41653	-
191	Variable		2.17×10^{-3}	8.75×10^{-3}	0	7	-42710	4.03
32 (dead)	Constant	2.32×10^{-2}			0	4	-1667	-
32 (dead)	Variable		2.51×10^{-2}	1.97×10^{-2}	0	4	-1687	0.78
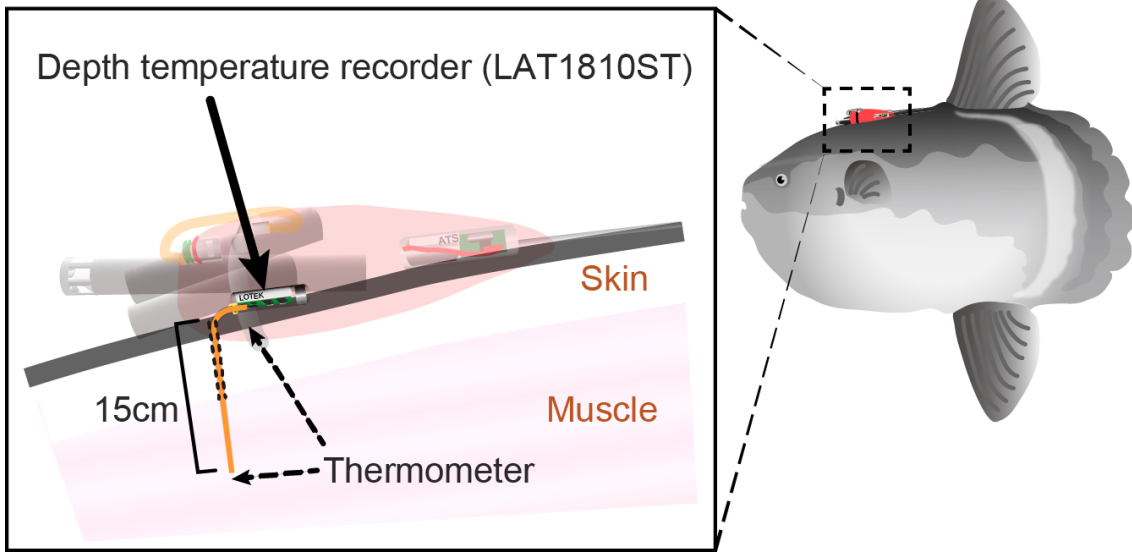
Fig. 5-1 A diagram of how to measure body temperature of sunfish using a depth temperature recorder (LAT1810ST).
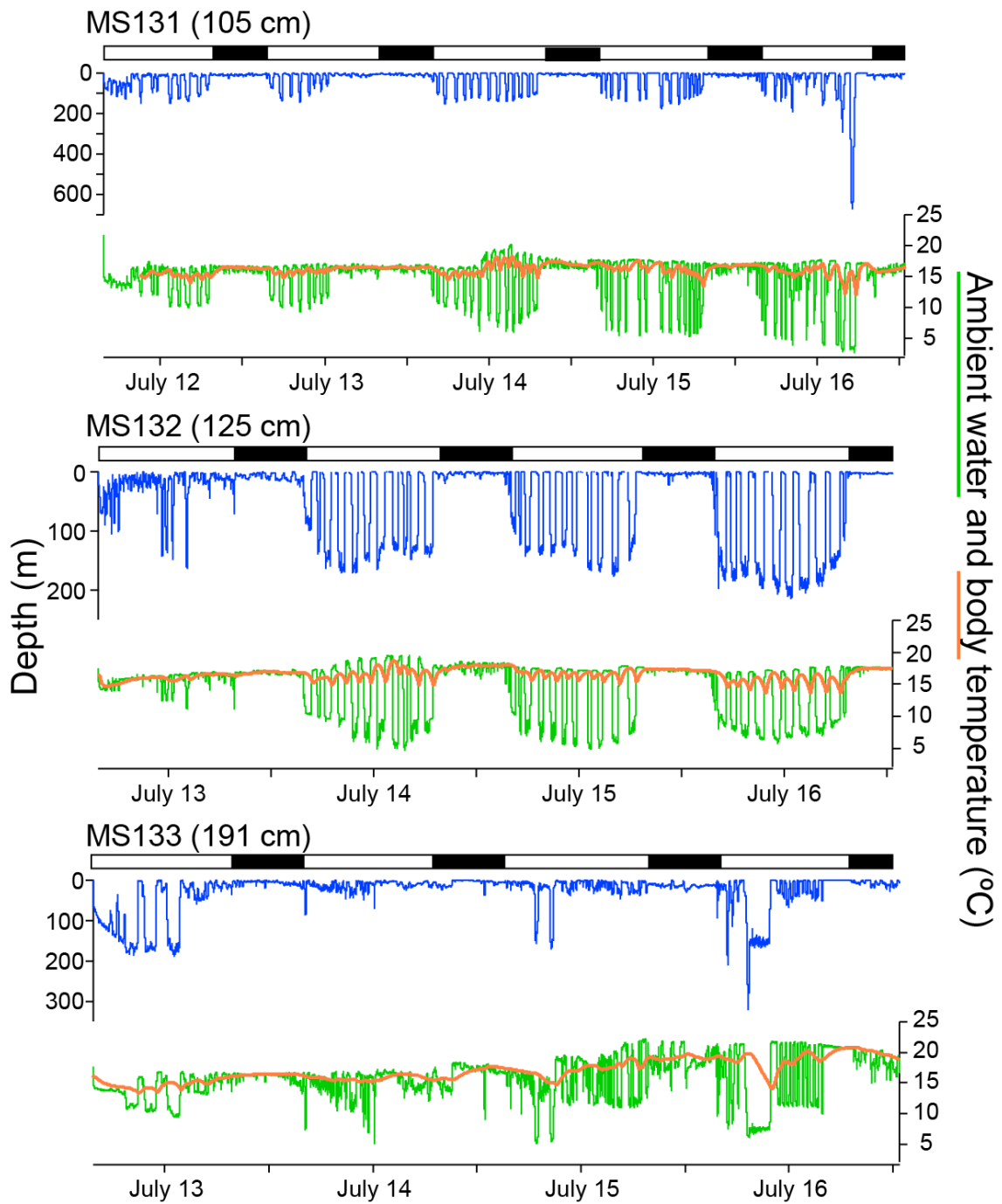
Fig. 5-2 Time series data of the depth, ambient water temperature (green) and body temperature (orange) of the sunfish in July 2013.
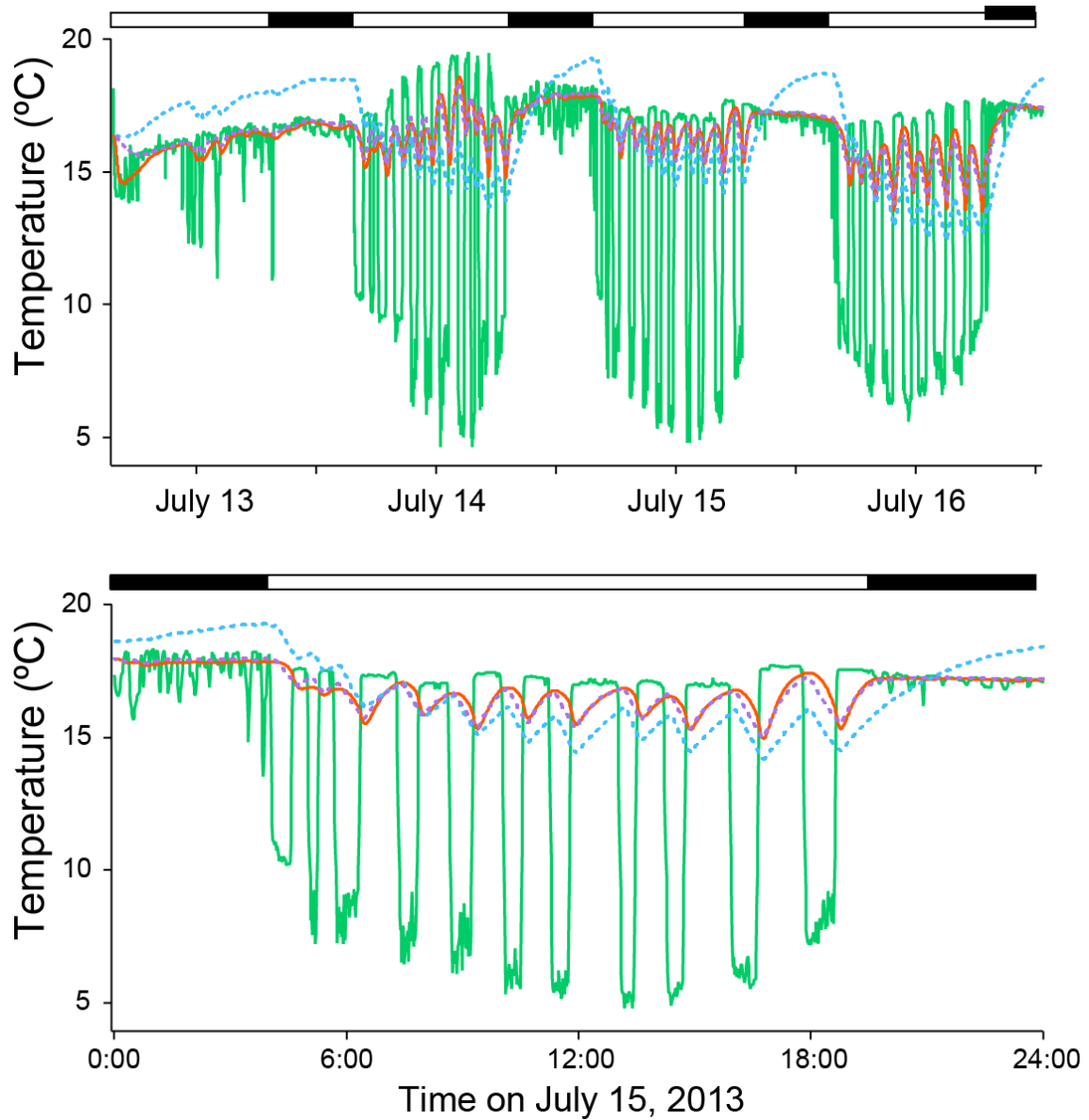
Fig. 5-3 Change of measured body temperature (orange) with ambient water temperature (green) of a sunfish (MS132) of whole deployment (upper) and enlarged (lower). Blue broken lines indicate calculated body temperature from *k* constant model (model (a)), and purple broken lines indicate calculated body temperature form *k* variable model (model (b)).

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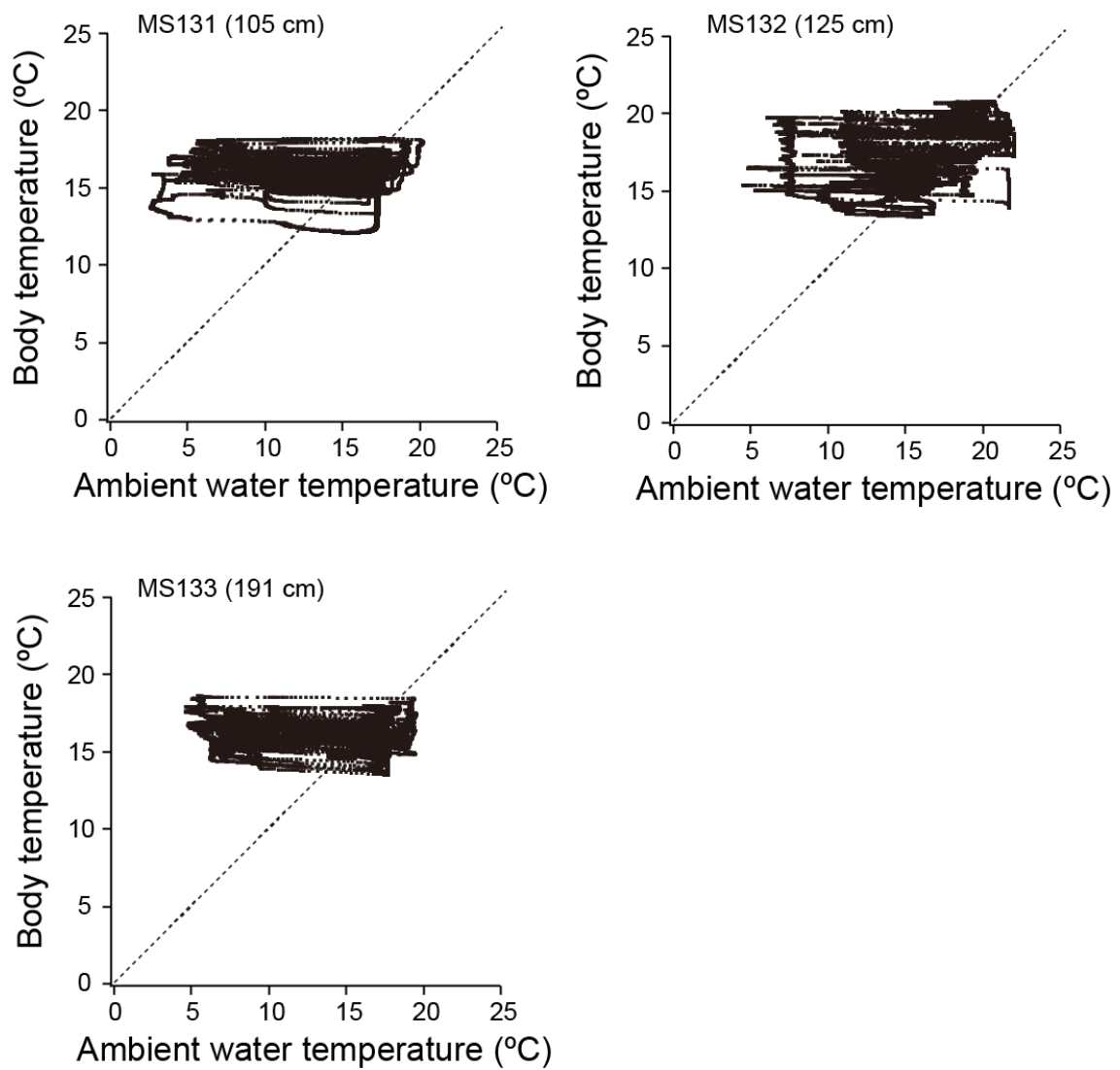
Fig. 5-4 Relationship between ambient water temperature and measured body temperature. Colours of plots indicate different individuals. A broken line indicates body temperature equal to water temperature.
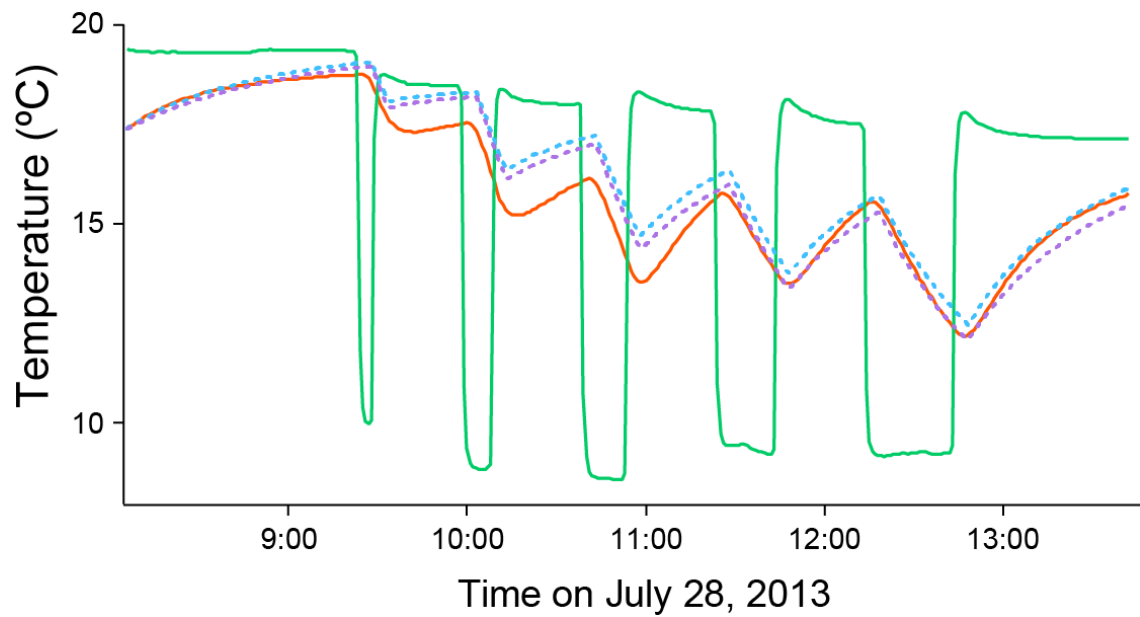
Fig. 5-5 Change of measured body temperature (orange) with ambient water temperature (green) of a dead sunfish. Blue broken lines indicate calculated body temperature from constant k model (a), and purple broken lines indicate calculated body temperature form variable k model (b).
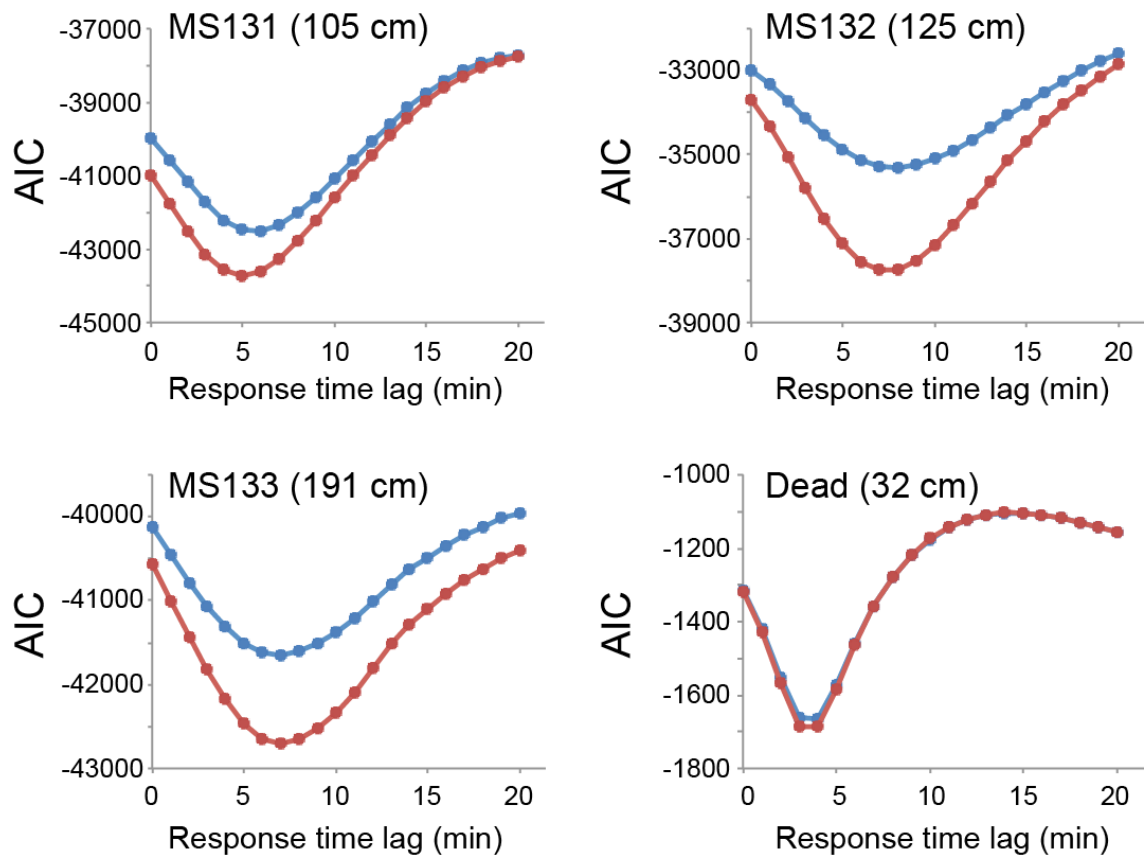
Fig. 5-6 Relationship between response time lag (τ) and AIC of model (a) (blue) and model (b) (red). The model (b) showed lower AIC than the model (a) in all individuals, however, in case of the dead sunfish, both models (a) and (b) showed similar AIC. AIC became lowest at response time lag were 4-7 min.
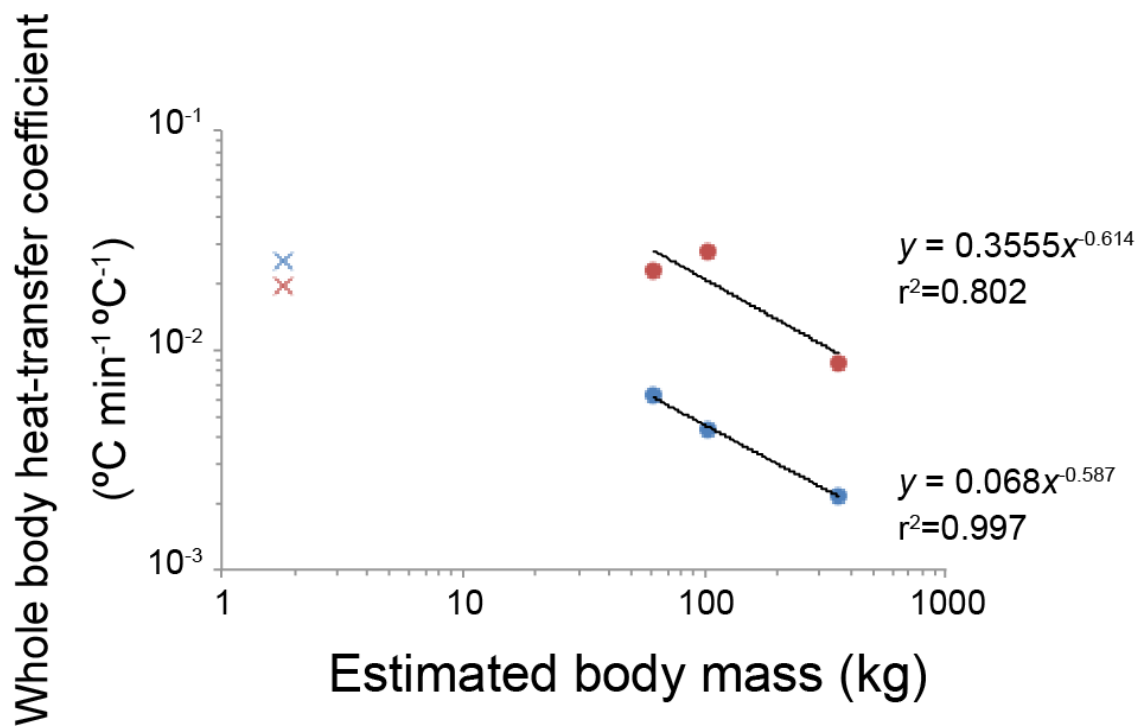
Fig. 5-7 Relationship between estimated body mass and whole-body heat-transfer coefficient. Blue and red indicate heat-transfer coefficient during cooling and warming, respectively. Circles show the sunfish in the field and crosses show the dead individual.
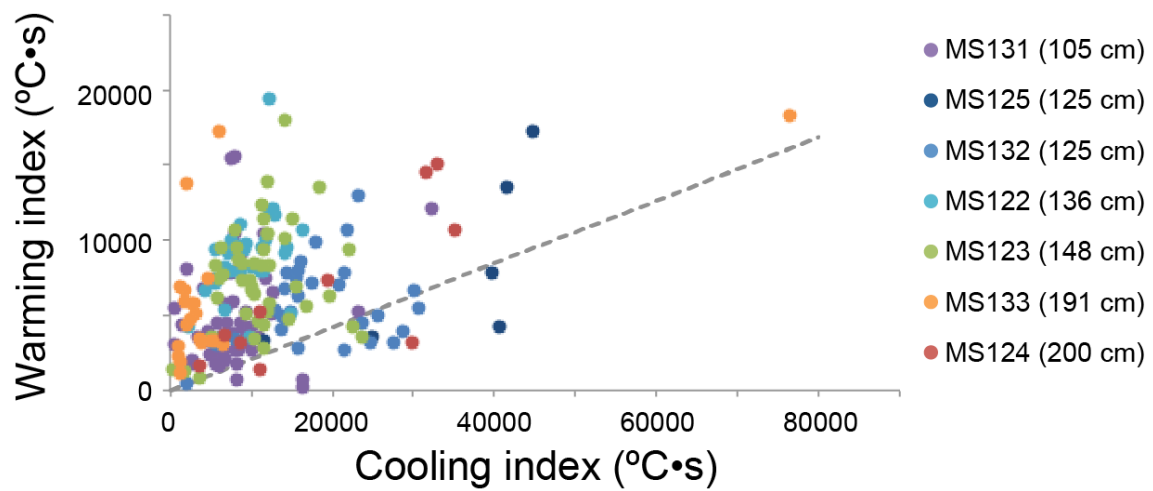
Fig. 5-8 Relationship between cooling index and warming index. A broken line indicates warming index was 4.72 (mean of the heat-transfer coefficient ratios between cooling and warming of three sunfish in the field) times of cooling index. 180 out of 205 points (88%) were included above the line.

Chapter 6   General discussion

The purpose of the present study was to clarify ecology of the mysterious species like ocean sunfish by various approaches, and the present study is the first to describe the actual feeding behaviour of ocean sunfish under natural condition and a role of surfacing as symbolic behaviour of this species.

Dietary analyses in the present study suggested that smaller sunfish might feed on benthic crustacean while larger sunfish might feed on gelatinous zooplankton.   This was consistent with feeding habit of sunfish speculated by behavioural data in the present study.   The other studies using stable isotope techniques also suggested benthic feeding in juvenile sunfish (Syväranta et al. 2012, Harrod et al. 2013).   However, few evidence of benthic feeding of the smaller sunfish in the field was obtained in the present study, therefore it is necessary to obtain additional behavioural data of feeding from small sunfish.   The larger sunfish obviously fed on gelatinous zooplanktons such as lion's mane jellyfish and various siphonophores.   Lion's mane jellyfish was also suggested as putative diet of larger sunfish by the stable isotope analyses in the present study.   However, the sunfish only ate a part of the jellyfish such as gonads and oral arms and left bell.   Gonads and oral arms of the jellyfish have higher energy than bell of the jellyfish (Doyle et al, 2007).   Ocean sunfish might eat only energy rich parts of jellyfishes.   There has been no study which reported the evidence of feeding of ocean sunfish on siphonophores, however, behavioural and visual data in the present study indicated that larger ocean sunfish mostly fed on a chain of siphonophore at deep depth around 120-160 m.   Siphonophores were known to do diel vertical migration, during day they stayed deep (300-500 m) and came to shallow (100-200 m) during night (Silguero and Robinson 2000).   Studies using satellite tracking suggested that ocean sunfish occupying significant greater depth during the day (Hays et al. 2009, Sims et al. 2009a).   This behaviour matches the strategy of normal diel vertical migration and has

been attributed to a strategy of near continual feeding on vertically migrating prey (Sims et al. 2009a, Hays et al. 2009).   However, the ocean sunfish, at least larger individuals, in the present study were obviously diurnal, that they foraged only during the day and were almost inactive during the night, indicating their diel pattern might not be related to diel vertical movements of their prey.   From the results of foraging behaviour in the present study, ocean sunfish fed prey with deceleration in deep water and with acceleration in shallow water, suggesting that sunfish might find their prey by vision. Visual acuity of ocean sunfish, calculated from peak ganglion cell densities, was between 3.51 and 4.33 cycles per degree, comparable to adult sharks (2.8–3.7 cycles per degree; Kino et al. 2009).   They were diurnal possibly because they might need light for finding their prey, accordingly they couldn't find their prey during the night.

In the present study, vertical movements of ocean sunfish changed with seasonal difference of ocean thermal structure.   Other studies suggested that ocean sunfish might shift from predation on seasonally abundant surface medusa during the summer to vertically migrating gelatinous species found at deeper depths during the winter months (Hays et al. 2009, Pope et al. 2010).   The ocean sunfish in the present study mainly swam near surface water (>20 m) in July, and swam within mixed layer (<100 m) in November.   The medium sunfish in July mainly foraged in surface water, however, the large sunfish in July mainly foraged in deep water against those studies. The stable isotope analyses of amino acids in the present study suggested that the medium and large sunfish foraged in different water masses; the medium sunfish foraged in warm water and the large sunfish foraged in cold water, however, both the medium and large sunfish swam to similar region in the field experiments.   From the vertical temperature profiles in July, there was a large gap in temperature between surface and deep water.   The foraging spots estimated by stable isotope analyses of amino acids might indicate not horizontal difference but vertical difference of foraging site.

Body temperature of ocean sunfish measured in the present study had narrow range of 14-20ºC than range of ambient water temperature (2-22ºC). There was no evidence that ocean sunfish have endothermy, therefore they should maintain their body temperature using ambient temperature like other many ectothermic fishes. As results of catch survey in the present study, ocean sunfish were mostly caught at temperature 15-17ºC similar to the mean of their body temperature (16-17ºC) obtained from the present study. As results of long deployments in the present study, all sunfish migrated to further offshore. Other studies using satellite tracking suggested that ocean sunfish mainly swam offshore habitat and rarely came into coastal area (Dewar et al. 2010, Potter et al. 2011). From the catch survey, ocean sunfish were caught from colder temperature (<12ºC) than their thermal preference (15-17ºC), might because the sunfish caught by coastal set-nets might stray into coastal area from offshore. Studies using satellite or acoustic tracking suggested that vertical movements of ocean sunfish might be related to behavioural thermoregulation (e.g. Cartamil and Lowe 2004, Dewar et al. 2010, Hays et al. 2009, Sims et al. 2009a). Cartamil and Lowe (2004) demonstrated that a significant relationship between maximum dive depth and the ensuring post-dive surface period. In the present study, the body temperature of the sunfish, which decreased during the sunfish swam within deep cold water, but rebounded during they stayed at the warm surface after deep excursions, was recorded first. This indicates a function of surfacing is behavioural thermoregulation for recovering body temperature by surface warm water. Additionally, changing of body temperature indicated that whole-body heat-transfer differed between cooling and warming, suggesting that ocean sunfish might be able to efficiently gain heat from the warm surface water by changing their whole-body heat-transfer. This might enable ocean sunfish to extend their foraging time in deep cold water. Accordingly mean body temperature of ocean sunfish showed 1-2ºC warmer than mean of ambient water temperature. Similar body temperature change was observed in other ectothermic fish:

blue sharks *Prionace glauca*, that cooling rate was smaller than warming rate, enabling to keep 4℃ warmer mean body temperature than mean ambient water temperature (Carey and Scharold 1990). However, physiological mechanism to change whole-body heat-transfer remains a mystery. One possibility is they can change blood flow between cooling and warming. For example, sea turtles change their blood flow velocity between warming and cooling conditions (Hochscheid et al. 2002). Fish gills have 2 - 48 times larger surface area than body surface area (Gray 1954), and this larger surface area of gill might work as a heat exchanger. Ocean sunfish might increase blood flow through their gills and increase heat exchange at their gills during warming at the surface. Thermal preference of ocean sunfish was suggested at 15-17℃ by both the catch survey and the direct measurement of their body temperature, however, main preys of larger sunfish, such as siphonophora, live deep cold water (5-12℃). The behavioural and physiological thermoregulations enable them to increase foraging time in deep cold water. From the heat-transfer coefficient estimation, smaller individuals had larger heat-transfer coefficient, indicating that smaller individuals can't stay longer in deep cold water. The result of nitrogen stable isotope ratio of amino acid and foraging behaviour suggested that medium sunfish foraged within surface mixed layer. Ontogenetic shift in feeding habit of ocean sunfish might be related to ability to resist to cold temperature.

There remain a lot of mysteries about ecology of this species, for example, seasonal sifts in prey types, physiological thermal regulation, spawning grounds, however, the present study has revealed a lot of important information about ecology of ocean sunfish from various different perspectives. Furthermore, it can be possible to clarify how they adapted to pelagic habitat by comparing with both their related forms and other pelagic fishes. These approaches should be useful to know about ecology of various pelagic fishes including commercially important fishes as well as deep-sea

species, and should reach evolutionary studies: how fish species belong to various taxa live in and adapt to pelagic habitat.

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