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Short communication

Diet of Mass-stranded Striped Dolphins (*Stenella coeruleoalba*) in Southern Japan (East China Sea)

Ayaka Matsuda¹, Tadasu K. Yamada², Yuko Tajima², Tatsuya Kunisue³, Masao Amano⁴,
and Takashi F. Matsuishi¹

¹*Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate,
Hokkaido 041-8611, Japan*

E-mail: matsuda@fish.hokudai.ac.jp

²*Department of Zoology, National Museum of Nature and Science, 4-1-1 Amakubo,
Tsukuba, Ibaraki 305-0005, Japan*

³*Center for Marine Environmental Studies, Ehime University, 2-5 Bunkyo-cho,
Matsuyama 790-8577, Japan*

⁴*Faculty of Fisheries, Nagasaki University, Bunkyo-machi, Nagasaki 852-8521, Japan*

Abstract

Striped dolphins (*Stenella coeruleoalba*) mass-stranded on 26 April 2013 at Minamisatsuma, Kagoshima Prefecture, in southern Japan (East China Sea). The diet of the mass-stranded striped dolphins was investigated to reveal their foraging pattern through analyses of the stomach contents and stable isotopes in muscle. Of 26 stomachs sampled, 25 contained hard parts of prey animals; no fleshy remains were found in any of the stomachs. The identified prey species represented four cephalopod families: Loliginidae, Onychoteuthidae, Histioteuthidae, and Ommastrephidae. Among these, ommastrephids had the highest abundance (42.4%) and frequency of occurrence (69.2%). A chi-square test revealed that the prey species consumed did not significantly differ between male and female dolphins, although deeper-water squids (Onychoteuthidae and Histioteuthidae) appeared only in the stomachs of females. The values of $\delta^{13}\text{C}$ ranged from -20.4 to -17.0‰ (mean \pm SD: $-18.2 \pm 0.9\text{‰}$), and values of $\delta^{15}\text{N}$ ranged from 10.2 to 12.5‰ ($10.8 \pm 0.5\text{‰}$), with a significant difference in $\delta^{15}\text{N}$ between sexes ($p < 0.05$).

Key Words

Stomach contents, Stable isotopes, Cetacean ecology, Delphinidae

Introduction

Studies of the carcasses of stranded cetaceans have provided fundamental information on many aspects of cetacean biology, such as population structure, sex ratio, diet and disease. Observations of dead stranded animals can reveal much about their live and, with a sufficient sample size, about the species as a whole (Wilkinson and Worthy 1999). In particular, the carcasses of mass-stranded dolphins may provide valuable biological information on offshore species (Mignucci-Giannoni et al., 2000). The diet of mass-stranded dolphins provides evidence of not only the prey species consumed by the species but may also indicate the animal's physical condition just before stranding.

Stomach contents analysis is a common and direct method of identifying prey items and to assess interactions between predators and prey (Fitch and Brownell, 1968). However, examining the stomach contents of cetacean carcasses only reveals which prey items are eaten just before the individual's death. Hence, stable isotope analysis of body tissues, including muscle, blood and blubber, is widely used to investigate animal diet because the composition of stable isotopes in a predator should correlate to that in their trophic level of prey (DeNiro and Epstein, 1978, 1981; Minagawa and Wada 1984; Peterson and Fry, 1987). Consequently, this method has become popular in marine mammal research (Newsome et al., 2010). Nitrogen-15 traces typically increase with the trophic levels in a food chain (Hobson and Welch, 1992; Cabana and Rasmussen, 1994; Thompson et al., 1995), and carbon-13 traces indicate the different potential primary sources (Rau et al., 1992; Havelange et al., 1997; Dauby et al., 1998), for example aquatic vs. terrestrial, inshore vs. offshore, or pelagic vs. benthic contributions to food intake (Hobson et al., 1995; Smith et al., 1996).

Accordingly, examination of the actual stomach contents of stranded cetaceans clearly indicates the prey species consumed, while the nitrogen and carbon stable isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively) in body tissues can indicate the ecological niche of the food organisms. Combining these two methods allows a better understanding of the diet of stranded cetaceans.

The striped dolphin *Stenella coeruleoalba* (Meyen, 1833) is widely distributed in warm-temperate to tropical offshore waters of the Atlantic, Pacific, and Indian oceans as well as in many adjacent seas (Jefferson et al., 2015). Around Japan, the northern limit of this species is south of the Sanriku coast on the Pacific Ocean (Kasuya, 1996), and it is uncommon in the Sea of Japan and East China Sea (Kasuya, 1999).

Kasuya (1999) identified three concentrations in the summer distribution of striped dolphins in the western North Pacific that the first, the southern offshore concentration in the Kuroshio Counter Current area south of 30 °N, the second, the northern offshore concentration in offshore water east of 145 °E off northern Japan in latitudes 35 °N – 42 °N and the third, the Japanese coastal concentration in nearshore waters off the Pacific coast of Japan, which covers from the central part of the Sanriku Region to Kyushu at around 30 °N. The diet of striped dolphins was studied only by captured individuals by the dolphin drive fishery on the east coast of Izu Peninsula of Japan by Miyazaki et al. (1973) and Tobayama (1974). These two previous studies reported that the commonest prey species were the lantern fishes, Myctophidae (Miyazaki et al. 1973, Tobayama 1974) and the most dominant squids were Euploteuthidae followed by Loliginidae (Tobayama 1974).

Although Striped dolphin is widely distributed in the Pacific Ocean around Japan from Sanriku coast to the East China Sea, their feeding habits have only been studied from captured dolphins off the Izu Peninsula.

School structure of striped dolphins in the western Pacific was studied by Miyazaki and Nishiwaki (1978). Miyazaki and Nishiwaki (1978) classified striped dolphin schools into three types: juveniles, adults, and mixed schools, and the adult and mixed schools could each be further categorized as a mating or non-mating group. In an adult school, fully mature females seem to gather together and are then joined by socially mature males, thus ultimately forming a mating school (Miyazaki and Nishiwaki, 1978).

Striped dolphins' mass-stranded on the 26th of April 2013 at Minamisatsuma, Kagoshima Prefecture, in southern Japan (East China Sea) (Figure 1) (Ishikawa 2014). Thirty-one striped dolphins came ashore and 29 of these died in spite of efforts to free them. In Japan, striped dolphins usually strand singly; however, six incidents of mass stranding of this species were recorded between 1988 and 2013, including the event at Minamisatsuma. At Minamisatsuma, 27 of the carcasses, plus one fetus ($n = 28$), were successfully collected for scientific investigation.

This study analyzed the stomach contents and the stable isotope ratios in muscle of the striped dolphins that mass stranded at Minamisatsuma. From diet analysis, we aimed to reveal the foraging habits of the group.

Materials and Methods

Sample collection

Whole stomachs and muscle samples were collected from the mass-stranded striped dolphins. Stomachs were collected from 26 dolphins (Table 1) having a body length of 205.0–251.4 cm. Body length of each individuals were measured in laboratory. Of these, 11 specimens were males and 15 were females, including two that were pregnant (Specimen ID: NSMT M42161 and NSMT M42173). All males were confirmed to be matured by testis weight ($15.5 \text{ g} \leq$) (Miyazaki 1977) and all females had scars from ovulation on their ovaries. Stomach contents were removed in the laboratory and stored in 80% ethanol until sorting. For stable isotope analysis, muscle samples were excised from 27 carcasses, plus one fetus ($n = 28$; Table 1), and stored at -20°C .

Stomach contents analysis

The lower beaks of cephalopods found in the stomachs were sorted, counted, and identified to the lowest possible taxonomic level by referring to published guides (i.e. Clarke, 1986; Kubodera, 2005) and to the beak voucher collection in the National Museum of Nature and Science (Tsukuba, Japan). To investigate the quantitative composition of the stomach contents, percentage numerical composition ($\%N$) and the frequency of occurrence ($\%F$) of the prey species were determined using the following equations:

$$\%N_i = \frac{n_i}{\sum n_i} \times 100$$
$$\%F_i = \frac{m_i}{M} \times 100$$

where i denotes the prey species, and n_i is the number of prey i that were found in all stomachs sampled; m_i is the number of striped dolphin that fed on prey species i , and M is the total number of striped dolphins sampled. A chi-square test was used to test for differences in $\%F$ of each prey species between the male and female dolphins.

Stable isotope analysis

To remove the influence of variations in the lipid content, each muscle sample was rinsed repeatedly with a chloroform–methanol mixture (2:1) prior to analysis. After drying at 50°C for 48 h, samples were ground into a homogeneous powder using a sample

grinder (As One, BM-1S) at 3,000 rpm for several minutes. Approximately 0.5 ± 0.3 mg of each sample was packed into a tin cup for the analysis $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in an elemental analyzer (Thermo-Scientific, FLASH 2000) connected to an isotope ratio mass spectrometer (Thermo-Scientific, DELTA V) which located in Atmosphere and Ocean Research Institute, The University of Tokyo. The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated as follows:

$$\delta^{13}\text{C or } \delta^{15}\text{N (‰)} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, respectively. Vienna Pee Dee Belemnite and atmospheric N_2 , respectively, were used for the carbon and nitrogen stable isotope standards. Welch's t -test was used to compare the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between males and females.

Results

Stomach contents

Twenty-five of the 26 stomachs collected contained hard parts of prey. No fleshy remains were found in any of the 26 stomachs. Stomach contents were sorted into cephalopod beaks (lower and upper), remnants of cephalopod eyes (uncounted), remnants of cephalopod gladii (uncounted), 75 fish eyes, as well as parasitic nematodes. A total of 151 cephalopod lower beaks and 145 upper beaks were counted. The average number (\pm SD) of lower and upper beaks per a stomach sample was 5.8 ± 5.3 and 5.6 ± 5.2 , respectively. The prey species identified represented four cephalopod families: Loliginidae, Onychoteuthidae, Histioteuthidae, and Ommastrephidae (Table 2). Among these, ommastrephids accounted for 42.4% of the abundance and 69.2% of the frequency of occurrence. A chi-square test showed no significant differences between the prey species consumed by male and female dolphins, though species of Onychoteuthidae and Histioteuthidae appeared only in females (Specimen ID: NSMT M42137 and NSMT M42159).

Stable isotope ratios

The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the fetus (specimen NSMT M42173f) were -19.3‰ and 11.0‰ , respectively (Figure 2). Excluding the fetus, the values of $\delta^{13}\text{C}$ across the other samples ranged from -20.4 to -17.0‰ , and the values of $\delta^{15}\text{N}$ ranged from 10.2 to 12.5‰ . The mean and standard deviation was $-18.2 \pm 0.9\text{‰}$ for $\delta^{13}\text{C}$, and $10.8 \pm 0.5\text{‰}$ for $\delta^{15}\text{N}$ (Figure 3). The $\delta^{15}\text{N}$ values differed significantly between the sexes (Welch's t -test, $p < 0.05$; Figure 2).

Discussion

In Japan, the stomach contents of stranded striped dolphins have seldom been studied for comparison with captured dolphins (Miyazaki et al., 1973; Tobayama, 1974). Our study presents important data to better understand the feeding behavior of striped dolphins. The mass-stranded striped dolphins examined had fed mainly on squids belonging to the families Ommastrephidae and Loliginidae, and most of the cephalopod beaks in the stomachs had not yet darkened indicating that the cephalopods were not full-grown. Ray-finned fishes, particularly Myctophidae (lanternfish), are reported as the main prey of captured and incidentally caught striped dolphins on the Pacific coast of central Japan (Miyazaki et al. 1973; Tobayama 1974), but myctophids were not found in our stomach samples of the mass-stranded dolphins collected in southern Japan. In this study, the appearance of fisheyes in the stomach contents indicated that some kind of fish

had been eaten, but the species was not identified only by fisheye.

Stomach contents analysis revealed cephalopod beaks, no fresh remains, and no fish otoliths. In a feeding experiment with common dolphins (*Delphinus delphis*) and bottlenose dolphins (*Tursiops truncatus*), it was established that from the time of feeding, eight hours are required to digest mackerel, after this time period the external shapes of the fish were not discernable (Tobayama 1974). Therefore, it is probable that the mass-stranded striped dolphins examined had not fed on prey fishes soon before stranding.

The remains of species of Onychoteuthidae and Histioteuthidae, largely oceanic squids, were found only in the stomachs of females (Specimen ID: NSMT M42137 and NSMT M42159). Results of the stable isotope analysis showed a significant difference in $\delta^{15}\text{N}$ values between males and females (Figure 2). Specimen ID NSMT M42159 (female) had the highest $\delta^{15}\text{N}$ value and this individual fed on oceanic squid. This suggested that Specimen ID NSMT M42159 (female) used prey species which belong to higher trophic level. From the result of stomach contents analysis and stable isotope analysis, it was suggested that some individuals among females may have behaved like a fission–fusion society. Fission–fusion sociality is a highly flexible and complex form of social structure in which members within a community frequently belong to or separate from a group (e.g., Würsig 1978; Smolker et al. 1992). Fission–fusion sociality in cetaceans has been reported in several delphinid species as bottlenose dolphins (*Tursiops* spp.; e.g., Smolker et al. 1992), common dolphins (e.g., Bruno et al. 2004), dusky dolphins (*Lagenorhynchus obscurus*; e.g., Würsig and Würsig 1980), Guiana dolphin (*Sotalia guianensis*; Lunardi and Ferreira, 2014) and spinner dolphins (*Stenella longirostris*; e.g., Karczmarski et al. 2005) which is closely related to striped dolphin.

A schooling system has been documented for striped dolphins in the western Pacific (Miyazaki and Nishiwaki, 1978). Miyazaki and Nishiwaki (1978) classified striped dolphin schools into three types: juveniles, adults, and mixed schools, and the adult and mixed schools could each be further categorized as a mating or non-mating group. In an adult school, fully mature females seem to gather together and are then joined by socially mature males, thus ultimately forming a mating school (Miyazaki and Nishiwaki, 1978). Among the sampled mass-stranded females in our study, two individuals had a fetus and showed signs of lactation (Specimen ID: NSMT M42161 and NSMT M42173). Total body length of the sampled dolphins ranged from 205.0 to 251.4 cm. Based on size–age estimations made by Miyazaki and Nishiwaki (1978), this mass-stranded school probably comprised all adults, and all males were confirmed to be matured by testis weight ($15.5\text{ g} \leq$) (Miyazaki 1977) and all females had scars from ovulation on their ovaries. From these results it was suggested that they were an adult mating school. The result of stomach contents and stable isotope analysis correspond to different time ranges. From measured diet–tissue turnover times of bottlenose dolphin’s skin, it was estimated that the half-lives for $\delta^{15}\text{N}$ ranged from 14 to 23 days (Browning et al. 2014). About muscle of the gerbil (*Meriones unguiculatus*), carbon turnover was estimated as 27.6 days (Tieszen 1983). In our study, excluding the one female which has the highest $\delta^{15}\text{N}$ value, the $\delta^{15}\text{N}$ values were significantly different between the sexes (Welch’s *t*-test, $p < 0.05$). This differences of the $\delta^{15}\text{N}$ values between the sexes might indicate that the males had recently joined the school.

Stable isotope ratio of striped dolphin around Japan were reported in Endo et al. (2010). In Endo et al. (2010), stable isotope ratios of carbon and nitrogen were measured in red meat products from striped dolphins caught off Taiji (n=6) and Nago (n=5) sold in Japan. The mean and standard deviation of Taiji and Nago striped dolphin were $-18.0 \pm$

0.4‰ for $\delta^{13}\text{C}$, and $12.0 \pm 0.4\text{‰}$ for $\delta^{15}\text{N}$ and $-17.4 \pm 0.5\text{‰}$ for $\delta^{13}\text{C}$, and $12.7 \pm 1.2\text{‰}$ for $\delta^{15}\text{N}$ respectively (Endo et al. 2010) The $\delta^{15}\text{N}$ value was significant different both of between Taiji striped dolphins and our samples (Welch's *t*-test, $p < 0.05$), and Nago striped dolphin and our samples (Welch's *t*-test, $p < 0.05$). From the difference, the trophic level of our sampled striped dolphin was lower than previous study. The reason of the difference was unclear because the biological data (e.g. body length, sex and season) in Endo et al. (2010) was not describe for they measured sold samples.

In compare with 13 other small toothed whales around Japan, striped dolphin had the lowest $\delta^{15}\text{N}$ value (Matsuda unpublished data). The niche occupied of striped dolphin needs to be considered in relation to the stable isotope ratios of other cetaceans.

Dissection of specimen NSMT M42173 revealed a fetus. Stable isotope values for a cetacean fetus are rarely reported since these specimens are difficult to collect. In marine ecosystems, nitrogen and carbon isotope ratios in animals become higher (3 to 4‰ and 0 to 1‰, respectively) with a one-step higher trophic level (DeNiro and Estein, 1978, 1981; Minagawa and Wada, 1984). The values of both stable isotopes were higher in the fetus than in the adults ($\delta^{15}\text{N} : \Delta 3.102$; $\delta^{13}\text{C} : \Delta 0.14$) (Figure 3). The body length of neonates of striped dolphins at birth is 92.5–107.5 cm (Kasuya, 1972). Thus, the fetus in our sample (body length 90.8 cm) was inferred to be almost at full-term. The value of $\delta^{15}\text{N}$ during fetal development is presumed to resemble that in the maternal tissues because of shared nutrition, though recent fetal–maternal isotopic fractionation in humans revealed higher fetal values as compared with maternal values (de Luca et al., 2012; Burt and Amin, 2014). However, the mechanisms underlying the elevated $\delta^{15}\text{N}$ value in the fetus as compared with that in the mother remain to be determined. The stable isotope differences between the fetus and the pregnant mother might be attributable to a different nitrogen metabolism status, as found among individuals and between populations (Duggleby and Jackson, 2002; Kinaston et al., 2009; Burt and Amin, 2014; Tsutaya and Yoneda, 2015).

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Diet of mass-stranded striped dolphin

Table 1. Data collected on striped dolphins that mass-stranded at Minamisatsuma, Kagoshima Prefecture, in southern Japan, in April 2013, including the analyses performed to determine diet. Specimen NSMT M42173f is a fetus removed from NSMT M42173. + means study and – means non-study. NSMT = National Museum of Nature and Science, Tsukuba, Japan

| Museum ID | Sex | Body length (cm) | Stomach contents analysis | Stable isotope analysis |
|--------------|-----|------------------|---------------------------|-------------------------|
| NSMT M42133 | F | 223.5 | + | + |
| NSMT M42134 | F | 224.3 | + | + |
| NSMT M42135 | M | 220.3 | + | + |
| NSMT M42136 | M | 243.5 | + | + |
| NSMT M42137 | F | 227.3 | + | + |
| NSMT M42138 | F | 223.8 | + | + |
| NSMT M42139 | F | 227.6 | + | + |
| NSMT M42140 | M | 240.9 | + | + |
| NSMT M42141 | M | 242.0 | + | + |
| NSMT M42142 | M | 231.7 | + | + |
| NSMT M42158 | M | 235.3 | + | + |
| NSMT M42159 | F | 215.3 | + | + |
| NSMT M42160 | M | 240.5 | + | + |
| NSMT M42161 | F | 228.5 | - | + |
| NSMT M42162 | F | 231 | + | + |
| NSMT M42163 | F | 221.3 | + | + |
| NSMT M42164 | M | 240.9 | + | + |
| NSMT M42165 | M | 230.3 | + | + |
| NSMT M42166 | F | 229.1 | + | + |
| NSMT M42167 | M | 225.8 | + | + |
| NSMT M42168 | F | 231.2 | + | + |
| NSMT M42169 | F | 224.9 | + | + |
| NSMT M42170 | M | 251.4 | + | + |
| NSMT M42171 | F | 225.7 | + | + |
| NSMT M42172 | F | 230.4 | + | + |
| NSMT M42173 | F | 228.8 | + | + |
| NSMT M42173f | M | 90.8 | - | + |
| NSMT M42174 | F | 223 | + | + |

Diet of mass-stranded striped dolphin

Table 2. Total number of prey specimens, percentage of abundance, frequency of occurrence, and relative rank of each prey taxa among the 26 stomachs collected from striped dolphins that mass-stranded in Minamisatsuma, Kagoshima Prefecture, in southern Japan, in April 2013.

| Taxa | No. of specimens | | | Numerical abundance (total) | | Frequency of occurrence (total) | |
|------------------------------------|------------------|--------|-------|-----------------------------|------|---------------------------------|------|
| | male | female | Total | % | rank | % | rank |
| CEPHALOPODA | | | | | | | |
| Loliginidae | | | | | | | |
| <i>Uroteuthis edulis</i> | 4 | 1 | 5 | 3.3 | 4 | 15.4 | 4 |
| Loliginid spp. | 29 | 19 | 48 | 31.8 | 2 | 65.4 | 2 |
| Onychoteuthidae | | | | | | | |
| <i>Onykia (Onykia) loennbergii</i> | 0 | 1 | 1 | 0.7 | 6 | 3.8 | 6 |
| <i>Onychoteuthis</i> sp. | 0 | 1 | 1 | 0.7 | 6 | 3.8 | 6 |
| Histioteuthidae | | | | | | | |
| <i>Histioteuthis</i> sp. | 0 | 1 | 1 | 0.7 | 6 | 3.8 | 6 |
| Ommastrephidae | | | | | | | |
| <i>Todarodes pacificus</i> | 4 | 1 | 5 | 3.3 | 4 | 15.4 | 4 |
| Ommastrephid spp. | 34 | 30 | 64 | 42.4 | 1 | 69.2 | 1 |
| Unknown squids | 16 | 10 | 26 | 17.2 | 3 | 61.5 | 3 |
| Total | 87 | 64 | 151 | | | | |