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短　報
Note

Assessment of octopus beak digestion by a harbor seal (*Phoca vitulina*) under feeding experiment

By

Takahito MASUBUCHI* and Mari KOBAYASHI**†

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Summary：Seals are considered ecologically important because they are the top predator in coastal ecosystems. However, the dietary habits necessary for assessing their impact have been investigated using indirect methods because foraging behavior cannot be directly observed. Of these, stomach content analysis can directly identify the foraging organisms and the amount of food consumed during the day or so immediately prior to death. On the other hand, it has been pointed out that the digestion in the stomach may lead to underestimation of species identification and foraging volume estimation. In this study, we euthanized a harbor seal, which was scheduled to be killed for population control purposes, after feeding it one octopus per day for eight consecutive days and examined the effect of digestion by analyzing the remaining lower beaks in the stomach. As a result, it was estimated that both the upper and lower jaw plates were retained in the stomach for about 7 days, and then migrated to the intestine. The Lower-Hood Length (LHL) of the lower beak was suggested to be the most accurate method for reproducing the weight of the forehead plate, which is necessary for estimating the weight of a predated octopus.

Key words：harbor seal, Enteroctopus spp, beak, stomach, digestion

Introduction

Marine mammals have feeding habits that are dependent on organisms in water[1]. Since one group of marine mammals—the pinniped clade of seals—is not perfectly adapted to the sea, they require haul-out sites for rest and nursing and are strongly dependent on coastal organisms around the haul-out site[2].

Seals are considered ecologically important because they are the top predator in coastal ecosystems. Nevertheless, the effects of seals on their ecosystem is still unclear. In general, indirect methods such as stomach content analysis, fecal analysis, fatty acid analysis, and nitrogen and carbon stable isotopic analysis have been conducted for marine mammals[3-11]. The method of stomach content analysis in particular is a lethal method, unlike other methods. This method, which is used for various marine animals, enables researchers to identify foraged organisms and feeding amounts about one day prior to death[12, 13].

Past studies on the stomach contents of pinnipeds have shown that they have preyed on benthic fishes, epipelagic fishes, and cephalopods[14, 15]. In particular, harbor seals (*Phoca vitulina*) have been reported to prey on benthic fishes and octopuses[14, 16-18]. However, analyses of stomach contents have shown that prey organisms are affected by digestion in the stomach and that the identification of species and the amounts consumed may be underestimated[11, 12]. As digestion progresses and it becomes difficult to identify the species, hard parts such as otoliths and identifiable bones in fish and beaks in cephalopods have been used to identify the ingested organisms and calculate their weight[11, 14]. Among the hard parts, however, the beaks of cephalopods are retained in the stomach for more than eight days in cetaceans[19] and at least one day in pinnipeds[20, 21], which have reportedly led to overestimation. Accurate knowledge of the duration of intragastric beak retention is essential to prevent under- or overestimation of foraged weights in dietary analyses.

In this study, we conducted an experiment in which a harbor seal in Hokkaido, Japan, was fed with *Enteroctopus*

* Department of Bioindustry, Graduate school of Tokyo University of Agriculture
** NPO Marin Wildlife Center of Japan
† Corresponding author（E-mail : m3kobaya@nodai.ac.jp）

spp, a major component of its diet, and residual beaks in the seal's stomach were collected to evaluate the process of wear on the beaks.

Materials and methods

One harbor seal (age 0, female, 34 kg) that was captured during population control at Cape Erimo, Hokkaido, was used for the feeding experiment (Capture Permit No. 21-28-0003 by the Ministry of the Environment). We fed the seal with one dead octopus per day at 6 pm for eight days from 14 October 2016. In all cases, foraging time was within 30 minutes. Fecal material was checked every morning when the tank was cleaned. Euthanasia was administered one day after the end of the feeding regimen, and beaks were collected from inside the stomach. The degree of damage to the lower beak was classified from A to G based on the condition of the wing and crest sections (Table 1). Animal care and experiments were carried out in accordance with the Guide for Animal Experimentation of the Tokyo University of Agriculture (accessed, May 6, 2016). Technology conformed to the guidelines for the care and use of laboratory animals as described by the National Institutes of Health and Guidelines for the Procedure of Obtaining Mammal Specimens as Approved by the Mammal Society of Japan (accessed, June 24, 2016). Finally, the animal was euthanized by the anesthesia. This experiment was conducted under the animal experiment permission number 2022014 of Tokyo University of Agriculture. The eight common octopuses that were fed to the seal were caught in waters off Soya, in northern Hokkaido. The average weight of the octopuses was 4,127 g ± 258.3 (mean ± *SD*). Following Robinson and Hartwick (1983), we measured nine jaws (four jaws with upper beaks, five jaws with lower beaks ; Figure 1) that were collected from the stomach. The weight of each measured beak was back-calculated to estimate the weight of the octopus[22]. The difference between the weights of the octopuses that were fed and the back-calculated weights was used as the error weight. However, it was difficult to link each beak in the stomach to the feeding weight. Therefore, assuming that the error in weight for each octopus was small, we calculated weight by best-subset selection procedure from the feeding weight (for eight individuals) and back- calculated weight. The measurement of beaks with the smallest error measurement was considered.

Table 1 Assessment of degrees of beak digestion.

degree of damage	Assessment	Digestive condition
A	Retention 1 day	Without damage & small amount of soft tissue attached to non-pigment part of lower beak
B	2 days	Mild damage to the non-pigment of the crest part and wing part of lower beak
C	3 days	No non-pigment portion of lower beak
D	4 days	Damage to pigment lower crest part
E	5 days	Damage to pigment-lower-wing part
F	6 days	Greatly damaged pigment lower crest & pigment-lower-wing part
G	1 week	Less than 25% of lower beak

※The alphabet ranks of Figure 3

Results and Discussion

In this study, eight octopuses were fed, but seven pairs of beaks were collected from the stomach. We couldn't find one beak. We therefore assumed that beaks

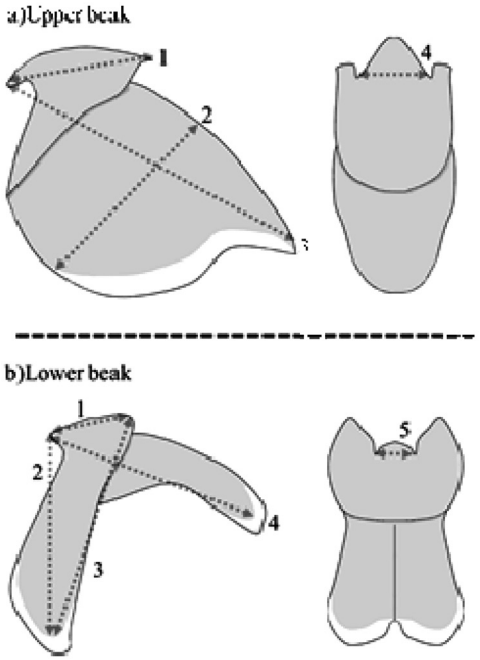

Figure 1 Measurements of upper and lower beak of the *Enteroctopus* spp. a) 1 : Upper-Hood Length (UHL), 2 : Pigment Upper-Lateral-Wall Length (PULWL), 3 : Upper-Crest Length (UCL), 4 : Upper-Rostral Width (URW) ; b) 1 : Lower-Hood Length (LHL), 2 : Total Standard-Lower-Wing Length (TSLWL), 3 : Pigment Lower-Wing Length (PLWL), 4 : Pigment Lower-Crest Length (PLCL), 5 : Lower-Rostral Width (LRW)

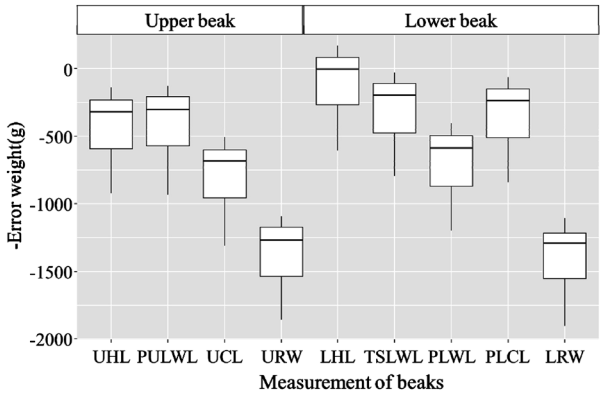

Figure 2 Error weight per measurement site. The error weight was calculated by subtracting the estimated weight from the feeding weight.

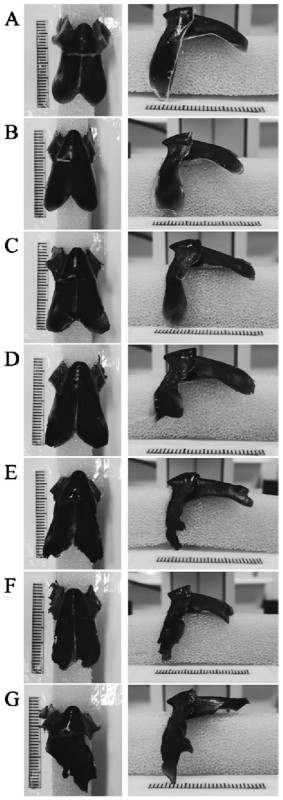

Figure 3 The degree of abrasion of the lower beak was ordered from A to G in accordance with the amount of damage and the beak's visual appearance. Details are described in Table 1.

were digested in the intestine after being held in the stomach for about seven days. It was not possible to determine that the process of wear for the upper beaks was greater than that for the lower beaks. The error weight results showed that the error of the lower beaks was less than that of the upper beaks (Figure 2). The hood length of the lower beak (LHL) had the lowest error weight. LHL was therefore considered to be the most suitable for back-calculating the weight of beaks in the stomach with advanced digestion. In the lower beak, the lower crest length (LCL) and lower wing length (LWL) were shown to be areas of particularly severe wear (Figure 2 ; Figure 3). Six of the seven wing sections and five of the seven crest sections of the lower beaks were found to be damaged (Figure 3). The effects of digestion were thus thought to start in the wing section and progress to the crest section.

The use of LCL and LWL has been reported to be suitable when estimating the weight of cephalopods[22, 23]. For the beaks obtained from the stomach contents, however, we considered it more desirable to use LHL for weight back-calculation, because the tissue of the beak was thicker and less affected by digestion.

When calculating the weight composition of recovered stomach contents, we believe that excluding species for which only the beak remains may underestimate the prey weight of the species. Therefore, it is necessary to infer the retention time in the stomach. The prey species of seals can be determined within half a day to one day of stomach content analysis from otoliths, beaks, bone fragments. Therefore, this paper is useful as an indicator for distinguishing prey within half a day to one day of stomach contents.

The present study suggests that the ability to assess when the beak was ingested could eliminate the bias in stomach content analysis that has been a problem in the past. However, the variety of cephalopods that seals feed on is very wide and the characteristics of the beaks, such as thickness and shape, vary considerably. It would therefore be necessary to assess the stomach retention period and digestion circumstances of the beaks of members of *Ommastrephidae*, the squid family of cephalopods, which are also commonly consumed by harbor seals.

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給餌下におけるゼニガタアザラシ胃内マダコ科頭足類の顎板残留評価

増渕隆仁*・小林万里*, **†

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要約：高次捕食者であるアザラシ類は沿岸生態系において生態学的に重要な位置を占めていると考えられるが，その影響評価に必要な食性については，採餌行動を直接観察できないことから間接的な手法の他，胃内容分析による調査が行われている。このうち，胃内容分析は死亡する直前の 1 日間程度の採餌生物と採餌量を直接特定することができるものの，胃内の消化状況によって種の同定や採餌量の推定が過小評価になる恐れが指摘されている。本研究では，個体数管理上の殺処分が予定されているゼニガタアザラシ 1 個体に対して 8 日間連続で 1 日 1 個体のマダコ科頭足類を給餌した後に安楽死させ，胃中に残った顎板を解析することにより消化の影響を検討した。その結果，顎板は上顎，下顎ともに胃内に 7 日間程度保持された後，腸内へ消化されると推定された。顎板は消化により摩耗が進行するが，捕食されたマダコ科頭足類の重量を推定するのに必要な額板重量の再現には，下顎の Lower-Hood Length（LHL）を使用することが最も精度が高いことが示唆された。

キーワード：ゼニガタアザラシ，マダコ科頭足類，顎板，胃内容物，消化

* 東京農業大学大学院生物産業学研究科（第一著者のみ：水産研究・教育機構）
** 北の海の動物センター
† Corresponding author（E-mail : m3kobaya@nodai.ac.jp）