

# Keratin decomposition by trogid beetles: evidence from a feeding experiment and stable isotope analysis

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**Abstract** The decomposition of vertebrate carcasses is an important ecosystem function. Soft tissues of dead vertebrates are rapidly decomposed by diverse animals. However, decomposition of hard tissues such as hairs and feathers is much slower because only a few animals can digest keratin, a protein that is concentrated in hairs and feathers. Although beetles of the family Trogidae are considered keratin feeders, their ecological function has rarely been explored. Here, we investigated the keratin-decomposition function of trogid beetles in heron-breeding colonies where keratin was frequently supplied as feathers. Three trogid species were collected from the colonies and observed feeding on heron feathers under laboratory conditions. We also measured the nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) stable isotope ratios of two trogid species that were maintained on a constant diet (feathers from one heron individual) during 70 days under laboratory conditions. We compared the isotopic signatures of the trogids with the feathers to investigate isotopic shifts from the feathers to the consumers for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . We used mixing models (MixSIR and SIAR) to estimate the main diets of individual field-collected trogid beetles. The analysis indicated that heron feathers were more important as food for trogid beetles than were soft tissues under field conditions. Together, the

feeding experiment and stable isotope analysis provided strong evidence of keratin decomposition by trogid beetles.

**Keywords**  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  · Food habit · Herons · Mixing model · MixSIR · SIAR · Trogidae

## Introduction

The decomposition of vertebrate carcasses is an important ecosystem function (Carter et al. 2007; Sugiura et al. 2013). Soft tissues of dead vertebrates are rapidly consumed by diverse arthropods and vertebrates (Janzen 1977). However, decomposition of hard tissues such as hairs and feathers is much slower because only a few animals can digest keratin, a protein that is concentrated in hairs and feathers (Waterhouse 1957). Researchers have considered that chewing lice (Ischnocera, Amblycera), clothes moths (Lepidoptera: Tineidae), carpet beetles (Coleoptera: Dermestidae) and keratin beetles (Coleoptera: Trogidae) can consume keratin materials (Waterhouse 1952, 1957; Trivedi et al. 1991; Hughes and Vogler 2006; Fukatsu et al. 2007). Various techniques have been used to clarify the food habits of those insects, including laboratory observations of feeding (Baker 1968), gut content analysis (Day 1952; Waterhouse 1952, 1953; Trivedi et al. 1991) and gene expression analysis of gut contents (Hughes and Vogler 2006).

Stable isotope ratios in consumers can be used to quantitatively estimate diet composition in field-collected individuals (Vanderklift and Ponsard 2003). Nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) stable isotope ratios have recently been used to analyse food web structure (e.g. Post 2002; Kolb et al. 2010; Hyodo et al. 2010). Generally, these ratios are constantly enriched at higher trophic levels, and hence, they can be used to estimate food–consumer relationships (Post 2002; McCutchan et al. 2003). Recently, Gómez-Díaz and González-Solís (2010)

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investigated  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in chewing lice and their hosts (seabirds) to determine if host feathers are the main diet of chewing lice under field conditions. Therefore, the use of stable isotope analysis would be appropriate for clarifying the trophic position and decomposition function of keratin consumers.

Here, we conducted field observations, a laboratory-feeding experiment and stable isotope analysis to clarify the trophic position and keratin decomposition function of trogid beetles in heron breeding colonies in central Japan. Trogidae, which includes about 300 species, is a cosmopolitan family that is found in various habitats (Scholtz 1986; van der Merwe 2008). Trogid adults are known to visit old vertebrate carcasses to lay eggs under the carcasses, and hatched larvae have been observed feeding on keratin materials in carcasses (Baker 1968; Scholtz 1986; van der Merwe 2008). Adults and larvae of trogid beetles are thought to be able to digest vertebrate skins, hairs and feathers (Baker 1968; Scholtz 1986; Hughes and Vogler 2006; van der Merwe 2008). Trogid adults and larvae, which are known as generalist keratin feeders, have been recorded in various bird nests and breeding colonies (Philips and Daniel 1977; Nasu et al. 2007; Tsukamoto et al. 2009; Yahiro et al. 2013). Therefore, trogid beetles may be potentially important consumers of keratin and may contribute to keratin decomposition under field conditions. However, studies have rarely explored whether trogid beetles use keratin materials as their main diet under field conditions. Therefore, the ecological function of trogids remains unclear. Herons (Ardeidae), which forage in lakes, rivers, ponds and flooded agricultural (paddy) fields, frequently breed on inland vegetation (e.g. deciduous secondary forests, coniferous plantations and bamboo forests in Japan) (Takano et al. 1985; Higuchi et al. 1996; Sugiura and Ikeda 2013). Colonial nesting by herons is known to produce chick carcasses (Ueno et al. 2006; Sugiura and Ikeda 2013), resulting in the accumulation of large numbers of feathers (keratin) on the ground under vegetation that is inhabited by colonies. However, no study has clarified how such a large quantity of keratin is decomposed.

We investigated assemblages of trogid beetles in six heron colonies and quantitatively measured the amount of feathers consumed by trogid adults under laboratory conditions. We also measured  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in trogid beetles and heron feathers to clarify their isotopic shifts and used mixing models to estimate the diets of trogid beetles under field conditions. Finally, we discussed how trogid beetles contribute to keratin decomposition.

## Materials and methods

### Study sites

To clarify the trophic status of trogid beetles, we conducted field observations and sampling at six sites in the southern

area of Ibaraki Prefecture, central Japan (C1–C6; Fig. S1; Table S1; cf. Sugiura and Ikeda 2013). Average monthly temperature ranged from 1.7 to 27.3 °C, and total annual precipitation was 1395.5 mm in 2012 (Tsukuba Meteorological Station; 36°03' N, 140°07' E). Six study sites were established in heron-breeding colonies, with a mean distance to the nearest study sites of 15.1 km and a range of 5.6–22.9 km (Fig. S1; Table S1). Two adjacent forest patches with colonies were considered a single study site (C6) because the distance between the two patches was only 150 m. Herons mainly used canopies of conifer trees as nesting sites in five of the study sites (C1–C3, C5–C6; Fig. S1; Table S1), whereas a bamboo canopy was used at site C4 (Fig. S1; Table S1).

Each breeding colony consisted of >10 pairs of herons, such as *Ardea cinerea* Linnaeus, *Egretta intermedia* (Wagler) and *Nycticorax nycticorax* (Linnaeus). Heron chick carcasses were supplied to the forest floor at the study sites from April to September, although very few were supplied at study site C4. We assessed the abundance of heron chick carcasses in late June 2012, when chick carcasses were most frequently dropped from nests (Sugiura and Ikeda 2013). We counted the number of heron chick carcasses on six and three line transects (transect size, 10×2 m) in site C6 and the other sites, respectively. The numbers of fresh carcasses ranged from 0.33 to 2.33 per 10 m<sup>2</sup> (Table S1). The number of chick carcasses supplied to the forest floor is known to increase with heron nest density (Ueno et al. 2006).

### Sampling

To investigate the species composition, feeding ecology and stable isotope ratios of trogid beetles, we randomly collected trogid adults from heron chick carcasses and/or the soil under carcasses at each study site in May–September 2012 (mean±SD 15.8±6.1 surveys per site). Feather traps, heron feathers that were placed on the ground (cf. Tsukamoto et al. 2009), were also used to collect trogids at two sites (C4 and C5).

Because the external morphology does not differ, we did not distinguish between sexes. Ages of the trogid adults could not be determined.

To investigate the density of keratin feeders, we counted the number of trogid beetles (adults and larvae) and other potential keratin feeders (tineid moths and dermestid beetles) in ten old heron chick carcasses at each study site on 22–23 July 2012. The fresh carcasses, of which soft tissues had remained undecomposed, were not included in this investigation. The density of keratin feeders could not be investigated at site C4 because no carcasses were found on those days. Beetle species were identified using colour pictorial guides (Kurosawa et al. 1985; Kawai et al. 2005; Okajima and Araya 2012). Tineid larvae were reared in the laboratory and eclosed adults were identified (Nasu et al. 2007).

To measure the stable isotope ratios of trogid beetles, we examined adults of three trogid species, *Trox niponensis* Lewis, *Trox opacotuberculatus* Motschulsky and *Trox mitis* Balthasar. Sample sizes of trogid beetles are provided in Table 1. To measure the stable isotope ratios of potential food items for trogid beetles, we sampled feathers and thigh muscles from fresh chick carcasses of three heron species, *A. cinerea*, *E. intermedia* and *N. nycticorax*, using scissors in May–September 2012. Our preliminary observations showed that trogid beetles were frequently found on heron chick carcasses in heron breeding colonies. Therefore, heron carcasses were considered to be the only food resource for trogid beetles at the study sites. Primary flight feathers from wings were used as one of the most keratin-rich materials, and thigh muscles were used as a representative soft tissue. Because soft tissues degraded rapidly, we only sampled muscle tissues at two sites, C5 and C6. We sampled both feathers and muscles from the same individuals, although we sampled only feathers from some individuals. Sample sizes of heron feathers and muscles are provided in Table 1. One flight feather and/or >100 mg of thigh muscle were sampled from 4 to 14 carcasses of each heron species at each study site (Table 1). We never injured live herons to sample tissues.

### Feeding experiment

To investigate the food sources of trogid beetles, we observed feeding behaviours of *T. niponensis*, *T. opacotuberculatus* and *T. mitis* under laboratory conditions (22 °C, 16 h in light, 8 h in darkness). We placed five adults of each trogid species collected from study site C6 in a plastic case (110×110×50 mm) with flight feathers of *A. cinerea*, *E. intermedia*, or *N. nycticorax*. We then determined whether these trogid species were capable of consuming feathers from *A. cinerea*, *E. intermedia*, or *N. nycticorax*.

To investigate the keratin-decomposition function of trogid beetles, we measured the amount of feathers that was consumed by trogid beetles under laboratory conditions (22 °C, 16 h in light, 8 h in darkness). We used 40 adults of each trogid species that was collected from the heron breeding colonies; *T. niponensis* and *T. opacotuberculatus* were collected from study site C6, and *T. mitis* was collected from study site C3. We used flight feathers from the heron species *E. intermedia*, which was commonly found at six study sites (Table S1). Feathers were sampled from study site C6 and oven-dried at 40°C for more than 72 h. After excluding rachises and calami from each feather, we used scissors to cut the feather remains

**Table 1**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of trogid beetles and heron chicks in heron breeding colonies

Animal group	Measured tissue	Study site <sup>a</sup>	Methods	N	Mean±SD ‰	
Species					$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Trogid beetle						
<i>Trox niponensis</i>	Whole body	C4	FT	15	11.2±0.9	-23.1±0.9
		C6	C	15	10.8±1.0	-24.3±0.6
<i>Trox opacotuberculatus</i>	Whole body	C1	C	15	9.8±1.5	-24.3±0.9
		C5	C	15	12.2±3.0	-24.2±1.2
			FT	7	13.2±2.9	-23.7±2.2
		C6	C	15	11.1±1.3	-23.3±1.3
<i>Trox mitis</i>	Whole body	C2	C	15	10.4±1.1	-23.1±1.6
		C6	C	15	10.4±0.9	-23.3±0.7
Heron						
<i>Ardea cinerea</i>	Feather	C5	C	10	13.4±4.3	-22.6±1.0
		C6	C	4	10.1±1.8	-22.4±0.7
	Muscle	C5	C	5	11.1±5.0	-22.9±0.4
		C6	C	5	9.5±1.2	-23.4±0.7
<i>Egretta intermedia</i>	Feather	C5	C	10	9.1±1.0	-22.1±1.5
		C2	C	7	8.8±0.7	-20.7±0.9
		C1	C	9	10.8±0.6	-21.1±0.8
	Muscle	C6	C	13	10.9±1.7	-20.6±0.7
		C4	C	11	11.5±2.5	-20.8±1.4
		C5	C	8	7.9±0.9	-23.9±0.6
<i>Nycticorax nycticorax</i>	Feather	C6	C	14	10.1±1.6	-22.6±0.7
		C6	C	10	7.8±1.3	-21.1±0.6
	Muscle	C6	C	9	8.3±1.5	-22.3±0.9

C collected from carcasses, FT collected by feather traps

<sup>a</sup> Study site code numbers are provided in Fig. S1 and Table S1

into small pieces (about 10×10 mm). We measured the weight of several feather pieces to the closest 0.1 mg using an electronic balance (Toledo Mettler AB204, Tokyo). Trogid adults were individually reared in plastic Petri dishes (55 mm diameter, 15 mm high) with pre-weighed feather pieces and wet tissue paper for 10 days. After 10 days, we collected feather remains from the Petri dishes. The feather remains were oven-dried at 40°C for more than 72 h and weighed. We estimated the amount of feathers consumed by each trogid adult by comparing the two dry weights. After the feeding experiment, all of the trogid adults were starved for more than 1 week to make sure their guts were clear; they were then euthanised, oven-dried at 40°C for more than 72 h and weighed to the closest 0.1 mg using an electronic balance. Five adults of *T. opacotuberculatus* that died during the experiment were excluded from the analysis.

To investigate isotopic shifts in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  between dietary items and consumers, we experimentally fed adults of two trogid species (*T. niponensis* and *T. opacotuberculatus*) with a constant diet during a period of 70 days under laboratory conditions. We used 40 adults of each trogid species that were collected from an old *E. intermedia* carcass at study site C6. Before the experiment, all of the trogid adults were starved for more than 1 week to make sure their guts were clear. Five adults of each trogid species were used for stable isotope analysis at the start of the experiment. As a constant diet, we used flight feathers from an adult carcass of *N. nycticorax* that was collected from Hamura Zoo (35°77' N, 139°33' E) in 2002. The feathers were oven-dried at 40°C for more than 72 h. The dried feathers (except for rachises and calami) were fed to trogid adults. Thirty-five adults of each trogid species were placed in plastic cases (110×110×50 mm) with feathers and wet tissue paper. We collected five individuals of each trogid species for stable isotope analysis on 5, 10, 15, 20, 30, 50 and 70 days after placement. Seventy days were considered sufficient to investigate isotopic shifts in trogid beetles based on previous studies with other arthropod species (Oelbermann and Scheu 2002; Gratton and Forbes 2006). We also estimated the assimilation weight of the feathers that were consumed by trogid adults over 70 days to determine if the experimental period was long enough to investigate isotopic shifts in trogid adults. Because we did not determine the assimilation efficiency of trogid beetles, we used values of 20–80 %, which correspond to known values for various animals (Begon et al. 1996).

#### Stable isotope analysis

We measured nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) stable isotope ratios in trogid adults (whole body) and heron chicks (feathers and thigh muscles). Teneral individuals of trogid beetles were not used in the stable isotope analysis because their isotopic ratios would be almost completely determined

by their larval diets. All of the adult beetles were starved for more than 72 h to ensure their guts were clear before the stable isotope analysis. After the gut contents were excluded, all of the samples were oven-dried at 60°C for more than 72 h. The whole body of each beetle was smashed using a bead cell disrupter (FastPrep FP120, Qbiogene, Inc., USA). Feathers and muscles were also oven-dried at 60°C for more than 72 h. We excluded rachises and calami from each feather and we used scissors to cut the feathers into small pieces (about <5 mm). Rachises and calami from feathers were not used in the analyses. Muscles were smashed using the bead cell disrupter. We used an isotope ratio mass spectrometer (Delta V Advantage; Thermo Fisher Scientific) coupled with an elemental analyser (Flash 2000; Thermo Fisher Scientific) to measure the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of trogids and herons. Isotope ratios were expressed as parts per thousand (‰) relative to the international standard:  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C} = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1,000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Pee Dee belemnite and atmospheric nitrogen were used as the carbon and nitrogen isotope standards, respectively.

Portions of the feathers were washed in Folch solution (containing chloroform and methanol, in a 2:1 ratio), rinsed thoroughly with distilled water to remove any surface contamination and oven-dried at 60°C for more than 72 h.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of washed feathers were not different from those of unwashed feathers. Therefore, we used feathers that were not washed in any chemical solution.

#### Data analysis

One-way analysis of variance (ANOVA) models were used to compare (1) body weight and feather consumption per individual adult among trogid species, (2)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among trogid species at study site C6, (3)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the trogid species *T. opacotuberculatus* between sampling methods at study site C5 and (4)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of each trogid species among study sites. The Tukey–Kramer method was also used to clarify differences in body weight and feather consumption among trogid species. Pearson product–moment correlation coefficients were calculated to clarify the relationship between body weight and consumption per individual adult in each trogid species. All of these analyses were performed using R version 2.15.1 (R Development Core Team 2012).

By applying mixing models to stable isotope ratios, it is possible to estimate the relative proportions of different food items in consumer diets (Moore and Semmens 2008; Parnell et al. 2010; Ikeda et al. 2010). Two Bayesian mixing models, MixSIR version 1.0.4 (Semmens and Moore 2008; Moore and Semmens 2008) and SIAR version 4.1.3 (Stable Isotope Analysis in R; Parnell et al. 2010) were used to estimate the contribution of each potential food item (feathers and muscles of *A. cinerea*, *E. intermedia* and *N. nycticorax*) to the diet of



each trogid species. MixSIR and SIAR were run in 200,000,000 and 500,000 iterations, respectively. We used the fractionation values (isotopic shift from diet to consumer) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in *T. niponensis* and *T. opacotuberculatus*, which were calculated using isotope ratios for the constant diet and trogid adults (individuals kept for 70 days; see above). For each trogid species, the median and 90 % Bayesian credible interval of the contribution of each food item were estimated using the mixing models.

## Results

### Density and species composition of trogid beetles

Three trogid species, *T. niponensis*, *T. opacotuberculatus* and *T. mitis*, were found on/under heron chick feathers in heron breeding colonies (Fig. 1a). One to three species of trogids were found in each colony (Table 2). The species composition of trogids differed among the study sites (Table 2). Although other potential keratin consumers, such as the tineid moth *Monopis pavlovskii* (Zagulajev) and the dermestid beetle *Dermestes maculatus* de Geer, were also found on heron carcasses, their densities were lower than trogid densities on the study dates (22–23 July 2012; Table 2).

### Feeding on feathers by trogids

Three trogid species, *T. niponensis*, *T. opacotuberculatus* and *T. mitis*, were observed feeding on feathers from three heron species (*A. cinerea*, *E. intermedia* and *N. nycticorax*) under laboratory conditions (Fig. 1b). The mean $\pm$ SD body weight significantly differed among the three species: *T. niponensis*, 6.5 $\pm$ 1.3 mg, *T. opacotuberculatus*, 13.1 $\pm$ 2.8 mg, *T. mitis*, 33.7 $\pm$ 6.7 mg (Fig. 2a; one-way ANOVA,  $F_{2,112}=423.5$ ,  $P<0.0001$ ). The mean $\pm$ SD consumption of *E. intermedia* feathers during 10 days also differed among the trogid species: *T. niponensis*, 3.0 $\pm$ 2.9 mg, *T. opacotuberculatus*, 11.5 $\pm$ 4.5 mg, *T. mitis*, 27.3 $\pm$ 17.8 mg (Fig. 2b; one-way ANOVA,  $F_{2,112}=50.84$ ,  $P<0.0001$ ). Large-sized species fed more on feathers than small-sized species (Fig. 2b). In addition, the

amount of feathers consumed per individual adult increased with body weight in each trogid species (Pearson product-moment correlation coefficients; *T. niponensis*,  $r=0.21$ ,  $df=38$ ,  $t=1.3$ ,  $P=0.20$ ; *T. opacotuberculatus*,  $r=0.43$ ,  $df=33$ ,  $t=2.7$ ,  $P=0.001$ ; *T. mitis*,  $r=0.41$ ,  $df=38$ ,  $t=2.8$ ,  $P=0.008$ ).

### $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of field-collected trogids

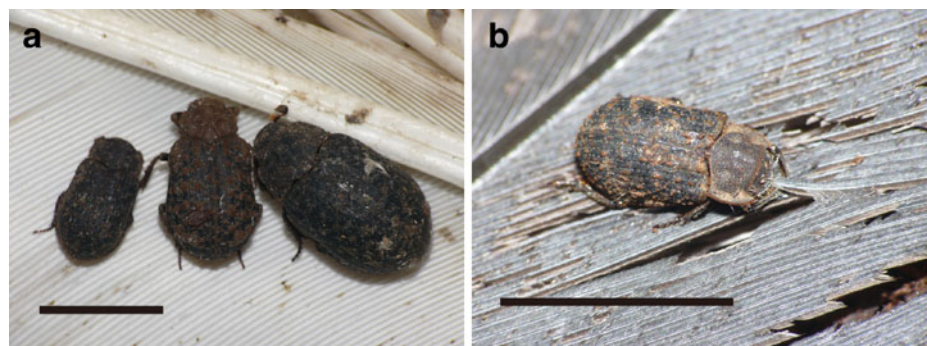
At study site C6, mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values varied among trogids, heron muscles and feathers (Fig. 3; Table 1). Mean values of  $\delta^{15}\text{N}$  did not differ among the three trogid species (one-way ANOVA,  $F_{2,42}=1.6$ ,  $P=0.22$ ), but  $\delta^{13}\text{C}$  differed significantly among the trogid species ( $F_{2,42}=5.8$ ,  $P<0.001$ ). In the herons, mean  $\delta^{13}\text{C}$  values were higher in feathers than in muscles, while  $\delta^{15}\text{N}$  values did not differ between feathers and muscles (Fig. 3; Table 1). In trogids, mean  $\delta^{13}\text{C}$  values were lower than in heron feathers, although mean  $\delta^{15}\text{N}$  values did not show any clear differences among trogids and heron feathers (Fig. 3; Table 1).

At study site C5, mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of *T. opacotuberculatus* did not differ between individuals that were examined using different sampling methods (Table 1; collection from heron carcasses vs. feather traps; one-way ANOVA,  $\delta^{15}\text{N}$ ,  $F_{1,20}=0.5$ ,  $P=0.47$ ;  $\delta^{13}\text{C}$ ,  $F_{1,20}=0.6$ ,  $P=0.50$ ). Therefore, the sampling method did not influence the isotopic signature of trogid beetles. However, values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in trogid beetles did differ among sampling sites (Fig. 4; Table 1). Mean values of  $\delta^{15}\text{N}$  and/or  $\delta^{13}\text{C}$  in *T. niponensis* and *T. opacotuberculatus* differed among study sites (one-way ANOVA, *T. niponensis*,  $\delta^{15}\text{N}$ ,  $F_{2,42}=5.3$ ,  $P=0.28$ ,  $\delta^{13}\text{C}$ ,  $F_{1,28}=18.7$ ,  $P<0.001$ ; *T. opacotuberculatus*,  $\delta^{15}\text{N}$ ,  $F_{1,28}=1.1$ ,  $P<0.01$ ,  $\delta^{13}\text{C}$ ,  $F_{1,28}=3.2$ ,  $P=0.05$ ), but values in *T. mitis* did not differ between two sites (Fig. 4; Table 1;  $\delta^{15}\text{N}$ ,  $F_{1,28}=0.002$ ,  $P=0.96$ ;  $\delta^{13}\text{C}$ ,  $F_{1,28}=0.1$ ,  $P=0.76$ ). Similarly, values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in heron species differed among sampling sites (Fig. S2; Table 1).

### Isotopic shifts in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

We measured  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in trogid adults (*T. niponensis* and *T. opacotuberculatus*) that were maintained

**Fig. 1** Photographs of a three trogid species, *Trox niponensis* (left), *Trox opacotuberculatus* (middle), and *Trox mitis* (right), on an *Egretta intermedia* feather. **b** *Trox niponensis* feeding on an *Ardea cinerea* feather. Scale bars: 5 mm



**Table 2** Density and species composition of trogid beetles in study sites

	Study sites <sup>a</sup>						Total
	C1	C2	C3	C4	C5	C6	
Mean±SD numbers per chick carcass							
Trogid adult	0.8±0.9	1.5±2.1	3.1±3.7	–	0.5±0.7	10.1±6.3	3.2±4.9
Trogid larva	0.7±1.6	0	0.3±0.9	–	0.1±0.3	0	0.2±0.8
Tineid larva	1.0±3.2	0	0	–	0	0	0.2±1.4
Dermestid adult	0.1±0.3	0	0	–	0	0	0.02±0.14
Dermestid larva	0	0.1±0.3	0	–	0.2±0.4	0.2±0.6	0.1±0.4
Species composition of trogids (%)							
<i>Trox niponensis</i>	8.5	0	12.9	100.0	0.6	48.1	40.8
<i>Trox opacotuberculatus</i>	83.0	5.9	3.2	0	98.1	49.3	52.3
<i>Trox mitis</i>	8.5	94.1	83.9	0	1.2	2.7	6.8
(N, total numbers of adults)	(47)	(34)	(31)	(51)	(162)	(1007)	(1332)

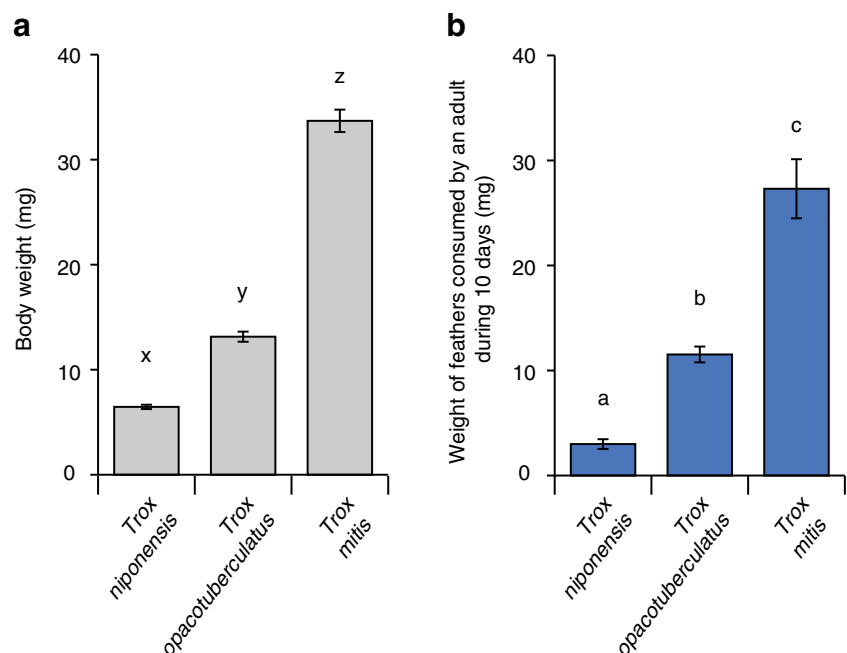
(–) The density of keratin feeders could not be investigated at the site because no carcasses were found on the study date

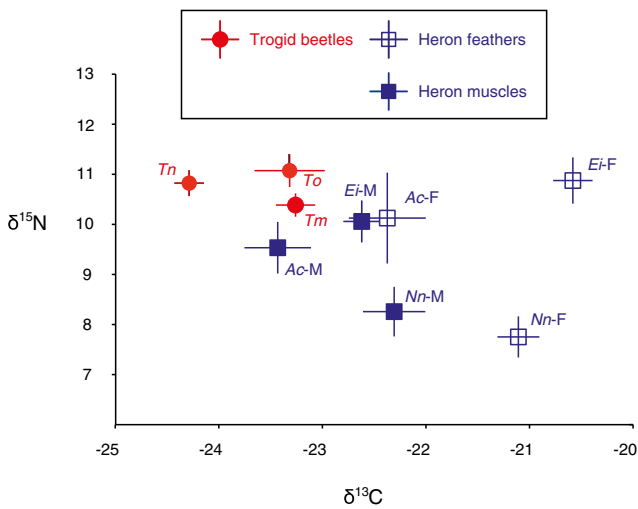
<sup>a</sup> Study site code numbers are provided in Fig. S1 and Table S1

on a constant diet (flight feathers from a *N. nycticorax* adult) under laboratory conditions over a period of 70 days. Values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  did not change during the feeding experiment in either trogid species (Table S2; Fig. S3). However, on the basis of our observations and estimations (Table S3), we thought that trogid adults assimilated enough of the constant diet to achieve turnover in stable isotopes. After the experiment, the mean±SD  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of individuals (*T. niponensis*,  $\delta^{15}\text{N}$ ,  $11.4\pm 0.5\%$ ,  $\delta^{13}\text{C}$ ,  $-23.8\pm 0.6\%$ ; *T. opacotuberculatus*,  $\delta^{15}\text{N}$ ,  $11.7\pm 0.5\%$ ,  $\delta^{13}\text{C}$ ,  $-22.8\pm$

$0.2\%$ ) were compared with values from their feather diet ( $\delta^{15}\text{N}$ ,  $11.2\pm 0.3\%$ ,  $\delta^{13}\text{C}$ ,  $-20.4\pm 0.5\%$ ). The mean±SD isotopic shifts in  $\delta^{15}\text{N}$  ( $\Delta\delta^{15}\text{N}$ ) were  $+0.2\pm 0.5$  and  $+0.5\pm 0.5\%$  in *T. niponensis* and *T. opacotuberculatus*, respectively (Table 3), suggesting that both trogid species were enriched in  $^{15}\text{N}$  relative to their diet. The mean±SD isotopic shifts in  $\delta^{13}\text{C}$  ( $\Delta\delta^{13}\text{C}$ ) were  $-3.4\pm 0.6$  and  $-2.4\pm 0.2\%$  in *T. niponensis* and *T. opacotuberculatus*, respectively (Table 3), suggesting that both trogid species were depleted in  $^{13}\text{C}$  relative to their diet.

**Fig. 2** **a** Mean dry weights (40°C) of three trogid species. **b** Mean dry weight of feathers consumed by a trogid adult during 10 days (22 °C, 16 h in light, 8 h in darkness). Different letters in both panels indicate significant differences (Tukey–Kramer method;  $P<0.05$ ). Bars indicate SE. Sample sizes for *Trox niponensis*, *T. opacotuberculatus*, and *T. mitis* were 40, 35, and 40, respectively

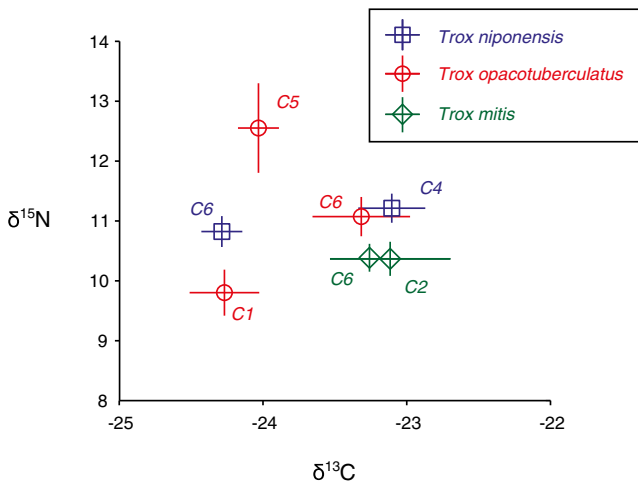




**Fig. 3** Mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of trogid adults (red closed circles), heron feathers (blue open squares), and heron muscles (blue closed squares) at study site C6. Bars indicate SE. Abbreviations are as follows: *Tn*, *Trox niponensis*; *To*, *Trox opacotuberculatus*; *Tm*, *Trox mitis*; *Ac*, *Ardea cinerea*; *Ei*, *Egretta intermedia*; *Nn*, *Nycticorax nycticorax*; *F*, feathers; *M*, muscles. Sample sizes for *Tn*, *To*, *Tm*, *Ac-F*, *Ac-M*, *Ei-F*, *Ei-M*, *Nn-F*, and *Nn-M* were 15, 15, 15, 4, 5, 13, 14, 10, and 9, respectively (Table 1)

Estimation of food items in trogid beetle diets under field conditions

We used two mixing models (MixSIR and SIAR) and fractionation values ( $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$ ) for each trogid species (Table 3) to estimate their diets under field conditions (Table 4). MixSIR tended to estimate a higher contribution of primary food items than did SIAR, and the Bayesian credible intervals were narrower in MixSIR than in SIAR (Fig. S4). However, both models showed similar results



**Fig. 4** Mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of *Trox niponensis* (blue squares), *T. opacotuberculatus* (red circles), and *T. mitis* (green rhombuses) at the study sites. Bars indicate SE. Code numbers next to circles, squares, and rhombuses indicate study site numbers, which are provided in Fig. S1 and Table S1. The sample size for each trogid species at each study site was 15 (Table 1)

**Table 3** Isotopic fractionation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in trogid beetles

Trogid species	Fractionation (mean $\pm$ SD‰)	
	$\Delta\delta^{15}\text{N}$	$\Delta\delta^{13}\text{C}$
<i>Trox niponensis</i>	0.2 $\pm$ 0.5	-3.4 $\pm$ 0.6
<i>Trox opacotuberculatus</i>	0.5 $\pm$ 0.5	-2.4 $\pm$ 0.2

(Table 4). At study site C6, the two trogid species most frequently used feathers of *E. intermedia* among the feathers and muscles of the three heron species (Table 4). The sampling method (i.e. collection from feathers of *E. intermedia*) reflected the results of the mixing models (i.e. feathers of *E. intermedia* were the main food). At site C5, feathers of *A. cinerea* and *E. intermedia* were frequently used by *T. opacotuberculatus*. Differences in sampling method (collection from *A. cinerea* feathers vs. feather traps) were not reflected in the results of mixing models (i.e., feathers of both *E. intermedia* and *A. cinerea* were identified as main food items).

Discussion

Gene expression analysis of gut contents (Hughes and Vogler 2006) has been used to clarify the food habits of trogid beetles. However, keratin digestion by trogids has rarely been investigated. In this study, we conducted a laboratory feeding experiment and stable isotope analysis to clarify the trophic niche of trogid beetles and their ecological function. Together, the feeding experiment and stable isotope analysis provided strong evidence of keratin digestion by trogid beetles (Tables 1 and 4; Figs. 2 and 3). However, this study may present some difficulties. For example, mixing models can be analysed only under the assumption that all possible sources of variability are controlled, all possible dietary sources are analysed and that the turnover rates of tissues for all species are measured (Moore and Semmens 2008; Parnell et al. 2010). However, we did not measure the tissue turnover rates of all species. Additionally, our sampling was unbalanced and sample sizes at some study sites were low. Therefore, our findings cannot be discussed without reservations. Considering these limitations, we discuss the feeding ecology of trogid beetles, isotopic shifts and their contribution to keratin decomposition.

Feeding ecology of trogid beetles

The feeding behaviours of trogid beetles have been observed under laboratory conditions (e.g. Baker 1968; Young 2006). Some studies have indicated that both adults and larvae of trogids mainly feed on keratin materials, such as bird feathers, reptile scales and mammal hairs (Baker 1968; Scholtz 1986), whereas other studies have indicated that adults and/or larvae

**Table 4** Proportion of food sources for three trogid species estimated by MixSIR and SIAR mixing models

Study site <sup>a</sup>		Estimated proportion of each potential item <sup>b</sup>					
		<i>Ardea cinerea</i>		<i>Egretta intermedia</i>		<i>Nycticorax nycticorax</i>	
Species		Muscle	Feather	Muscle	Feather	Muscle	Feather
C6	<i>Trox niponensis</i> (N=15) <sup>f</sup>						
	MixSIR	0.0 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	<i>0.7 (0.6–0.8)</i>	0.0 (0.0–0.2)	0.1 (0.0–0.2)
	SIAR	0.0 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	<i>0.6 (0.5–0.8)</i>	0.1 (0.0–0.1)	0.1 (0.0–0.2)
C6	<i>Trox opacotuberculatus</i> (N=15) <sup>c</sup>						
	MixSIR	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	<i>0.9 (0.8–0.9)</i>	0.0 (0.0–0.1)	0.0 (0.0–0.1)
	SIAR	0.1 (0.0–0.2)	0.1 (0.0–0.3)	0.1 (0.0–0.3)	<i>0.5 (0.3–0.7)</i>	0.1 (0.0–0.2)	0.1 (0.0–0.2)
C5	<i>Trox opacotuberculatus</i> (N=15) <sup>d</sup>						
	MixSIR	0.0 (0.0–0.1)	<i>0.5 (0.4–0.8)</i>	0.0 (0.0–0.1)	<i>0.4 (0.1–0.5)</i>	–	–
	SIAR	0.2 (0.0–0.4)	<i>0.4 (0.2–0.6)</i>	0.1 (0.0–0.2)	<i>0.3 (0.1–0.5)</i>	–	–
C5	<i>Trox opacotuberculatus</i> (N=7) <sup>e</sup>						
	MixSIR	0.0 (0.0–0.1)	<i>0.5 (0.4–1.0)</i>	0.0 (0.0–0.0)	<i>0.5 (0.0–0.6)</i>	–	–
	SIAR	0.3 (0.0–0.5)	<i>0.4 (0.1–0.6)</i>	0.1 (0.0–0.3)	<i>0.2 (0.0–0.4)</i>	–	–

(–) no carcasses in the study site

<sup>a</sup> Study site code numbers are provided in Fig. S1 and Table S1

<sup>b</sup> Values and those in parentheses are the median and 90 % Bayesian credible intervals, respectively. Proportions higher than 0.50 are shown in italics

<sup>c</sup> Individuals collected from *E. intermedia* feathers

<sup>d</sup> Individuals collected from *A. cinerea* feathers

<sup>e</sup> Individuals collected by feather traps

of some trogid species feed on insect carcasses and eggs as well as keratin materials (Roffey 1958; Young 2006). However, the types of foods that trogid beetles mainly consume under field conditions have remained unclear. Our combined feeding experiment and stable isotope analysis indicated that three trogid species, *T. niponensis*, *T. opacotuberculatus* and *T. mitis*, mainly assimilated heron feathers in heron breeding colonies. We also found a difference in the amount of feathers consumed per individual adult among the three trogid species (Fig. 2b). This difference could be caused by the differences in body sizes among trogid species (Fig. 2a) because the amount of feathers consumed per individual adult of each trogid species increased with body weight. The mixing models indicated that two trogid species assimilated feathers of *E. intermedia* and/or *A. cinerea* but not feathers of *N. nycticorax* (Table 4). We found no evidence of feeding specialisation, such as a morphological structure associated with feeding on a particular heron species. On the contrary, our laboratory experiments indicated that all three trogid species could consume feathers of all three heron species (including *N. nycticorax*), suggesting that the breadth of the diet does not differ among the three species. Body size is generally smaller in *N. nycticorax* than in *E. intermedia* and *A. cinerea* (Takano et al. 1985; Higuchi et al. 1996), suggesting that *N. nycticorax* chick carcasses would be harder for trogids to find compared to *E. intermedia* and *A. cinerea* under field

conditions. Therefore, the feather species that are used by trogids may depend on the relative availability of vertebrate species, but further investigation is needed.

#### Isotopic fractionation in trogid beetles

Nitrogen signatures generally show an increase in the isotopic ratio with increasing trophic level (mean fractionation value of  $\delta^{15}\text{N}$ , +3.4‰), whereas carbon shows only a slight increase (mean fractionation value of  $\delta^{13}\text{C}$ , +0.4‰) (Post 2002). The isotopic shifts in trogid beetles did not follow this general pattern (Table 3). Like other animal consumers, *T. niponensis* and *T. opacotuberculatus* were enriched in  $^{15}\text{N}$  relative to their diet. However, the difference between consumers and their diet was only 0.2‰ in *T. niponensis* and 0.5‰ in *T. opacotuberculatus* (Table 3). Unlike other animal consumers, trogid adults were depleted in  $^{13}\text{C}$  relative to their diet (Table 3). The difference between consumers and their diet was 3.4‰ in *T. niponensis* and 2.4‰ in *T. opacotuberculatus*. What factors caused these unusual isotopic shifts in trogid beetles? One possibility is that trogid beetles have higher lipid contents than their foods. Within an organism, lipids are depleted in  $^{13}\text{C}$  relative to proteins and carbohydrates (more negative  $\delta^{13}\text{C}$ ), and variation in lipid content among organisms or tissue types has the potential to introduce considerable bias into stable isotope analyses that



use  $\delta^{13}\text{C}$  (McCutchan et al. 2003; Post et al. 2007). Thus,  $\delta^{13}\text{C}$  is likely to be lower for samples with high lipid content than for samples with low lipid content (McCutchan et al. 2003; Post et al. 2007). Because samples were not subjected to lipid extraction prior to analysis in this study, the potential effects of these heron feather and trogid beetle specific compounds on carbon signatures of trogids remain unclear. Lipid content has been reported to differ among trogid species (Le Lagadec et al. 1998), suggesting that the difference in lipid content is the cause of the differences in  $\delta^{13}\text{C}$  content among the three trogid species.

A second possibility is that the assimilation rates of trogid beetles are much lower than we assumed (20–80 %). If this is true, the stable isotope ratios in individuals that were maintained on a constant diet for 70 days may not have reflected the stable isotope ratios in their diet. However, the duration of our feeding experiment (70 days) covered one-third of the adult activity period for a year (approximately 6–7 months; Kawai et al. 2005). Even if the assimilation rates of trogid beetles were low, the isotopic signature should have reflected the isotopic signature of trogids during the period to a greater or lesser extent.

#### Keratin decomposition by trogid beetles

Tineid moths, dermestid beetles and trogid beetles have been recorded as scavengers in various types of bird nests (Philips and Daniel 1977; Nasu et al. 2007; Tsukamoto et al. 2009; Yahiro et al. 2013). In Japanese heron colonies, trogids were more abundant than other keratin-feeding insects (Table 2) and trogid adults constantly consumed feathers (Fig. 1). Therefore, trogid beetles may contribute to keratin decomposition. Sites that have large accumulations of keratin are not only found in bird breeding colonies but also in other habitats where vertebrates are present in high density. However, keratin decomposition has not been investigated under field conditions. Further investigation is needed to clarify the contributions of scavenging insects to keratin decomposition in the field.

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**Author contributions** SS and HI conceived and designed the experiments. SS performed field collections and feeding experiments. HI performed stable isotope analyses. SS and HI analysed the data. SS wrote the manuscript.

**Competing financial interests** The authors declare there are no competing financial interests.

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