Population ecology of the red wing grasshopper, Celes skalozubovi akitanus (Orthoptera, Acrididae) in Japan

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Research Article

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Abstract

The red wing grasshopper, *Celes skalozubovi akitanus* has been declined rapidly in Japan, and only a few populations have been recorded. Information about ecological characteristics such as food plants, phenology and mating behavior are strictly limited, and population demographics of the grasshopper in its habitat is still unknown. Consequently, conservation actions for *C. s. akitanus* population are urgently needed in Japan. We studied the population structure and basic ecology of *C. s. akitanus*, to enable the conservation management of the grasshopper and its habitat. A field survey using the transect counts and mark release recapture method was conducted for two years (2016 and 2017). We revealed that *C. s. akitanus* in the study area fed on a variety of grassland plants but not on the dominant *Miscanthus sinensis* and *Pteridium aquilinum.* The recapture rate of *C. s. akitanus* is considerably high, about 68% or more, and the two isolated populations were found in the studied grassland. The estimated total population size is less than 120 to 140 individuals, indicating that the studied population is extremely small. Our results showed that this species has a strong sedentary behavior because most of the adults move less than 5 m in one season, and strongly prefer a bare ground within the grassland. The continuing implementation of annual prescribed burning and partial mowing in the habitat is important for the conservation of *C. s. akitanus*. In addition, creating continuity between two populations is effective for the survival of populations in this study area.

INTRODUCTION

Celes skalozubovi akitanus (Shiraki 1910) is a grasshopper characterized by thick and large body size and rose red colored hindwings, which is distributed in Japan, the Korean Peninsula, Northeast China and the Russian Far East (Furukawa 1939; Bay-Bienko and Mishchenko 1951; Storozhenko et al. 2015; Kano et al. 2016). In Japan, although *C. s. akitanus* has been recorded from Honshu area north of Fukui Prefectures, since the record from Niigata Prefecture in 1986, the species had not been found for about 30 years until recently rediscovered (Kano et al. 2016). Therefore, *C. s. akitanus* in Japan has been categorized as “Critically endangered (CR)” in the 4th Version of the Japanese Red Lists (Ministry of the Environment Government of Japan 2020), and designated as “nationally endangered species of wild fauna” of the law “Act on Conservation of Endangered Species of Wild Fauna and Flora” in 2016. More recently several authors have reported the discovery or rediscovery of *C. s. akitanus* populations in Japan, as the following Prefectures: Yamagata (Nagahata 2015), Fukushima (Okatsu et al. 2018), Niigata (Sakai 2017), Nagano (Uchida et al. 2016), Yamanashi (Watanabe 2018).

A knowledge about the ecology and biology of *C. s. akitanus* in the literature is exceedingly limited. Its habitat environment also has not been understood well. The recently discovered populations were founded in coastal (natural) grasslands (Sakai 2017) or semi-natural grasslands that are maintained by prescribed burning and partial mowing for long term period (Nagahata 2015; Okatsu et al. 2018). Some authors previously described the habitat of *C. s. akitanus* in Japan is “grassland” (Kiyosawa 1956; Yamasaki and Baba 1964; Yamasaki 1967) and “understory vegetation in sparse pine forest” (Furukawa 1965). Recent literatures pointed out that the habitat is “open forest road and undergrowth in sparse pine

forest" (Mitoki 2006; Ichikawa 2015; Kano et al. 2016), and consequently, these have become the description as if the species inhabits forest environment.

C. s. akitanus in Japan is the most threatened species and thus conservation measures were urgently required. Although revealing and accumulating the specific ecological information, such as life history, feeding plants and reproductive behavior is imperative for the conservation of the species population, those basic information remains unknown (Kano et al. 2016). There is only one study which shows the food plants of *C. s. akitanus* adults using fecal analysis (Yamamoto and Uchida 2018). The results of their study suggested that *C. s. akitanus* feeds on several plant species (36 species assigned to 19 families) in their studied grasslands and may avoid feeding on a species of the family Poaceae (*Miscanthus sinensis*). Furthermore, the population size and mobility in their habitat also should be clarified. Understanding the structure and dynamics of small population in fragmented habitats is considered crucial to sustaining such population over a long period time.

The aims of the present study are that (1) to elucidate whether the species inhabits not only the grassland but also forest environments, as noted by some literatures in the past, (2) to obtain the information about the basic ecology of *C. s. akitanus*, and (3) to determine the detailed population demographics and the mobility of the species adults for planning a conservation management. To achieve these aims, we conducted a field observation and transect counts survey of *C. s. akitanus* at its habitat, semi-natural grassland in Yamagata Prefecture, Japan, and performed mark release recapture surveys for two years.

MATERIALS AND METHODS

Study area

This study was conducted in a semi-natural grassland (area of ca. 50 ha) located at the foot of Iide mountains in Oguni town, Yamagata Prefecture, Japan (37°57'N; 139°42'E) (Fig. 1). In this grassland, *C. s. akitanus* was first discovered on 25 June 2015 (Nagahata 2015). This area belongs to a cool temperate climate, and the mean annual temperature and precipitation is 10.8℃ and 2972.8 mm, respectively (for 30 years: 1981–2010, Japan Meteorological Agency 2010, http://http://www.data.jma.go.jp). The studied grassland is located a south-facing gentle slope with undulating, at approximately 400–600 m a.s.l., surrounded by a deciduous broad-leaved forest comprised mainly of *Fagus crenata* Blume, including of japanese ceder, *Cryptomeria japonica* (L.f.) D.Don plantations in part. This semi-natural grassland has been managed by the people living in this area and had utilized as a *Miscanthus* grassland for livestock feeds and making thatched roof until around 1960s (Nagahata 2019). The grassland has maintained by traditional managements, prescribed burning in early May and mowing partially once a year. Currently, in the grassland bracken harvesting was carried out from late May to June. The vegetation occupied mainly by *Pteridium aquilinum* (L.) Kuhn, *Miscanthus sinensis* Anderss, *Pueraria lobate* (Wild.) Ohwi. In a wet and dry part, *Phragmites australis* (Cav.) Trin. Ex

Steud. and *Arundinella hirta* (Thunb.) occupied, respectively. *Potentilla freyniana* Bornm., *Viola mandshurica* W.Becker, and *Ixeris dentate* (Thunb.) Nakai grow in a sunlit place.

To conduct the transect counts survey and the mark release recapture, we established Site A and B within the studied grassland (Fig. 1). Site A is extremely small area of about 600 m^2 in a peak on the slope covered by *Miscanthus sinensis* and *Pueraria lobate*. The grass height is about 1.5 m in August, but only the top of site has a *Zoysia* type grassland with bare ground and low grass height (about 20 cm). Site B is located on the ridge of the grassland, area of about 7500 m^2, with covered by *Pteridium aquilinum*, *Miscanthus sinensis*, and *Pueraria lobata*. The grass height is about 1.5 m in late August with almost no bare grounds. In both sites A and B, we set the pathway which mowed 1.5 m on both sides of it for field observations in summer. Mowing was carried out early July and August. Moreover, the mowing plot of 3 × 3 m was set in both study sites for creation of bare grounds and low grass height areas. Three mowing plots in Site A and six plots in Site B were established.

Transect counts survey

To understand the habitat range of *C. s. akitanus* and whether the species inhabits not only grassland but also forest, we conducted a transect counts survey in the semi-natural grassland and the surrounding forest (Fig. 1). The transect route is about 3 km long and was designed to include (1) the mown grassland area (corresponding to Site A and B), (2) the unmown grassland area around Site A and B, and (3) the forests within and adjacent to the grassland (Fig. 1). During July to October 2016 and May to June 2017 we carried out 20 times transect walks about 5 hours with counting of *C. s. akitanus* in the 1.5 m area of both route sides.

Food plants, mating and oviposition

Feeding behavior of *C. s. akitanus* was investigated by field observation and cage experiment subsidiarily. Field observation was conducted mainly at the study sites A and B through the survey of transect counts and mark release recapture (as mentioned later). When the feeding behavior of *C. s. akitanus* was observed, the researcher recorded the individual status (stage and sex), feeding plants information (plant species and its part) and its time.

Five nymphs (last instar) of *C. s. akitanus* (2 males and 3 females) collected from the study sites, were examined simultaneously for feeding experiment in a cage (acrylic case, 43×26×32 cm) under natural photoperiod in Yamagata-city (Yamagata Prefecture). In this experiment, fresh leaves of several plants (10 species) found at the studied grassland were placed in the rearing case. One specimen (male adult) was reared at different cage in single because this individual became an adult. We observed and recorded these specimens' behavior during 14–16 and 17–19 July 2017. Based on these observations, food preferences of *C. s. akitanus* were classified into the following four categories: I) prefer to feed, II) feed on subsidiary, III) only take a bite not feed, IV) not feed.

The behavior of mating and oviposition were investigated by field observation which is the same method our feeding behavior study. When the mating and oviposition behaviors were found by the researcher, the position of mated pairs, both the male and the female behaviors and the time of mating were recorded.

Flying behavior

To understand the flying habits and the movement ability of *C. s. akitanus* adults, a photograph of this species in flight and jumping was taken using a camera (Body: Nikon D810, Lens: Nikon 35 mm). There are two flight types “jumping” and “flying” in movement behavior of grasshoppers (Suzuki 2014). Indeed, when two grasshoppers, *Gastrimargus marmoratus* and *Eusphingonotus japonicas* disperse a long distance, they use “flying” form (Suzuki 2014). The “jumping” is defined as the condition of being merely spreading hindwings and enlarging hind legs, which is usually used for movement of shorter distance. The “flying” is defined as the flight of spreading hindwings and flexing hind legs. In our study, the flight type of this species was determined by the form of its hind legs and hindwings while flying using photography.

Mark release recapture (MRR)

Mark release recapture was conducted at the study area, Site A and B, where a population of *C. s. akitanus* was recorded by transect counts survey (Fig. 1). MRR was performed from 7 August to 25 October 2016 and from 10 August to 26 October 2017. Sampling events in 2016 and 2017 was done 21 and 20 times respectively at irregular intervals. Each sampling was conducted discretionally during the activity period of *C. s. akitanus* adults (08:30–17:30), because we could figure it out by preliminary investigation in 2015. When a weather on the study area was bad (e.g. rainy and/or windy), our sampling was not conducted because of finding the species is very difficult. Sampling adults was conducted mainly by hands and regarding individual flying long distance, insect net was used auxiliary. We marked identification number on the thorax in lateral of captured individual by the oily white pen (ultrafine), and recorded captured points, then released immediately at the captured point. To clarify detailed life span of the species adult, we calculated total days that marked individuals were recaptured at last, and mean days for each year and sex.

Based on the result of MRR, we calculated the average distance between capture point and recapture point for each individual of *C. s. akitanus* and the maximum distance from the first capture point to the recapture point for each individual.

Statistics

Our MRR data was analyzed with Jolly-Seber model (for open population) using the module POPAN 5.0 (Arnason and Schwarz 1999) in the program MARK version 9.0 (White and Burnham 1999). We estimated population size in years independently. Three primary parameters: survival rate (φ), capture probability (p), and the probability of entering the population ($pent$) were used for estimation population size. These parameters may be constant (.), dependent on sex (g), respond to time (t) in a factorial or

interactive (*g*t*). For each response variable we first used the interaction between sex and time (*g*t*) as explanatory variable and afterwards tested sex (*g*) and time (*t*) independently. We derived the daily population size (*Ni*) and the total population size (*N*). Firstly, we calculated the full model and then performed a Goodness-of-Fit Test (program RELEASE GOF) to confirm the data quality. Based on the lowest value for the corrected Akaike information criterion (AICc) (Burnham and Anderson 2002), the best model was selected.

RESULTS

Habitat environment

Our transect counts showed that *C. s. akitanus* was recorded only the semi-natural grassland and did not appear in the forest area, indicating that the species is undoubtedly grassland species. We could not find any individuals of the grasshopper in the unmown area around Site A and B within the grassland. In addition, local populations of *C. s. akitanus* were confirmed in each study area, which corresponds to two study sites (Fig. 1 Site A and B). Both populations area limited a narrow space such a ridge in the studied grassland. No other populations were found in the grassland.

Feeding behavior and food plants

Three examples of the feeding behavior of *C. s. akitanus* were observed in the study area. First, at Site A on (11:14) 21 August 2016, an adult female fed a leaf of *Trifolium repens*. Second, at Site B on (14:38) 13 August 2017, an adult female fed a leaf of *Artemisia japonica* withered on the ground surface. In this example, feeding marks were observed at both living (fresh) and dead (dry) leaves. Third, at Site B on (11:35) 26 August 2017, an adult female fed a radical leaf of *Ixeridium dentatum*.

Our cage experiment showed that food plants of *C. s. akitanus* is not single species, whereas it was limited family and species, especially, the family Asteraceae and Fabaceae (Table 1). The pteridophyte, *P. aquilinum* and the monocotyledon, *M. sinensis* and *A. hirta* were not fed by *C. s. akitanus*. To test whether this species feeds the monocotyledon plant, we introduced leaves of *Setaria viridis* (the family Poaceae) to reared individuals. Although this plant doesn't grow in the studied grassland, *C. s. akitanus* fed it well (Table 1).

Table 1
Food preferences of *Celes skalozubovi akitanus* in the cage experiment

		14–16 July 2017	17–19 July 2017	
Family	Species	Last instar nymphs (five individuas)	Last instar nymphs (four individuals)	Only adult male
Fabaceae	*Pueraria lobate*	I	I	I
	Lespedeza bicolor	I	I	I
	Desmodium podocarpum	II	II	IV
Asteraceae	*Artemisia japonica*	I	I	I
	Artemisia princeps	II	-	-
	Solidago virgaurea var. *asiatica*	-	III	III
	Aster ageratooides	IV	III	IV
Rosaceae	*Potentilla freyniana*	III	IV	IV
	Persicaria conspicua	-	II	II
Primulaceae	*Lysimachia clethroides*	-	III	III
Violaceae	*Viola mandshurica*	IV	IV	IV
Poaceae	*Miscanthus sinensis*	IV	IV	IV
	Arundinella hirta	IV	IV	IV
	*Setaria viridis**	I	I	II
Dennstaedtiaceae	*Pteridium aquilinum*	IV	IV	IV

*non-native plant species in the studied grassland

I, prefer to feed; II, feed on subsidiary; III, only take a bite but not feed; IV, not feed; -, did not provide

Mating

We observed only two mating behaviors of *C. s. akitanus* in the field. First case, on (11:45) 23 August 2016 in the study route of Site B, a pair of the species have mated on a dead grass of bare ground, and the male twisted his abdomen to left side of the female abdomen. The time of this mating process was unknown. The other, on (16:50) 7 August 2017 in the study route of Site B, this mating form was the same as first case, and the time of mating process was unknown. In our MRR survey, some females were

found in pairs with males. We didn't observed males mounting on females other than the mating described above.

We observed only one mating behavior in our rearing experiment. In this case, firstly, a male recognized visually a female and the male moved his antenna up and down, and then rubbed his wings and hind legs (this may be stridulation of the species). The female, however, didn't react to this male's behavior and consequently the mating was not occurred. Subsequently, when the female walked pass the male, a mating occurred after the male mounted the female. The male twisted his abdomen to right side of the female abdomen.

Oviposition

In the study field, two female oviposition behaviors were observed. In the first case, on (14:50) 1 September 2015 at the place adjacent to Site A, when it was rainy, the researcher found a female walking on asphalt with doing an oviposition behavior which open and close the beaked tip of the abdomen toward the ground surface. The female was moved to Site A by the researcher, in order to avoid roadkill. At 15:00, the female had dug the ground for 3 minutes, and then started walking. At 17:48, the female had laid eggs in the ground, and continued until 18:25. After that for 3 minutes, the female buried again the oviposition site using her hind legs skillfully.

The latter case, on (12:20) 16 September 2017, a female ovipositing in Site B was found. We didn't find even the same female in Site B when we investigated at 10:55 of the same day. Thus, this female oviposition should be begun after that. At 12:42, the female finished egg laying, and infilled and beat using her hind legs the oviposition site for 3 minutes.

Additionally, on (15:45) 16 September 2015, a female digging in the ground was found at Site A, but its oviposition was not observed. On 6 September 2017, moreover, we focused on the investigation of female oviposition under bad (rainy) weather condition, assuming that in such weather female may lay eggs in softy and moist soil. However, we didn't observe not only oviposition behavior but also the existence of female adult.

Flying behavior

C. s. akitanus male escaped into grassland after vertically jumping with spreading hindwings height of 1.0–1.2 m from the ground. Females also took such behavior similar to male, but often jumped with no spreading hindwings, and the height of jumping is less than 0.5 m. The observation of jumping with spreading hind legs were 37 examples for males and 29 examples for females (Fig. 2a). In contrast, the observation of flying with flexing hind legs was only one male (Fig. 2b). When *C. s. akitanus* jumps without spreading hindwings, it takes frequently with facing up posture (Fig. 2c).

Population demographics

In Site A, we marked *C. s. akitanus* adults of 14 individuals (male: 7 and female: 7) in 2016 and 22 individuals (male: 12 and female: 10) in 2017 (Table 2). The recapture rate at Site A was 100% in 2016 and 77.3% (male: 75% and female: 80%) in 2017. In Site B, we marked *C. s. akitanus* adults of 50 individuals (male: 30 and female: 20) in 2016 and 82 individuals (male: 49 and female: 33) in 2017 (Table 2). The recapture rate at Site B was 76% (male: 76.7% and female: 75%) in 2016 and 68.3% (male: 67.3% and female: 69.7%) in 2017. There was no significant difference in the captured individuals between male and female (Wilcoxon rank sum test, Site A, 2016: P = 0.7664, 2017: P = 0.6908, Site B, 2016: P = 0.2719, 2017: P = 0.1077).

Table 2
Number of marked individuals, recaptured individuals, recapture events and recapture ratio of *Celes skalozubovi akitanus* on Site A and B

Site	Year	Sex	Number of marked	Number of recaptured	Recapture events	Recapture ratio (%)
A	2016	Male	7	7	48	100
		Female	7	7	49	100
		Total	14	14	97	100
	2017	Male	12	9	17	75
		Female	10	8	14	80
		Total	22	17	31	77.3
B	2016	Male	30	23	80	76.7
		Female	20	15	62	75
		Total	50	38	142	76
	2017	Male	49	33	115	67.3
		Female	33	23	91	69.7
		Total	82	56	206	68.3

In the study area, the total population size estimated by the best model at Site A was 13.6 individuals in 2016 and 30.0 individuals in 2017 (Table 3). At Site B, the total population size estimated by the best model was 128.8 individuals in 2016 and 92.4 individuals in 2017 (Table 3). The estimated population size of males was relatively higher than that of females at Site A in 2017 and Site B in both years 2016 and 2017. At Site A in 2016, the estimated population size of both males and females were approximately the same. The daily population size estimated by the best model has decreased from the peaks in early or mid-August to October of 2016 and 2017 (Fig. 3).

Table 3
The best models and the estimates of total population size for each study site

Site	Year	Best models	AICc	No. Par	Male (± SE)	Female (± SE)	Total
A	2016	φ(.) p(.) Pent(g*t) N(g)	349	7	6.9 (0.69)	6.7 (0.61)	13.6
	2017	φ(.) p(.) Pent (t) N(g)	245	7	16.4 (2.91)	13.6 (2.61)	30
B	2016	φ(t) p(t) Pent (t) N(g)	650	34	77.6 (0.00)	51.2 (0.00)	128.8
	2017	φ(t) p(t) Pent (t) N(g)	897	39	56.5 (2.84)	35.9 (2.16)	92.4

life span

To understand the life span of *C. s. akitanus* adult, we calculated the mean days and max day between first captured and last recaptured in both years. As a result, there were no significant differences among sexes and studied years (Table 4). At Site A, the max day between first captured and last recaptured were 48 days for male and 37 days for female in 2016, and 44 days for male and 45 days for female in 2017. At Site B, the max day between first captured and last recaptured were 32 days for both male and female in 2016, and 43 days for male and 64 days for female in 2017. The maximum life span which is counted from the day of adult emerging in rearing was 64 days for male and 92 days for female.

Table 4
Mean days and max day between first capture and last recaptured of *Celes skalozubovi akitanus* in Site A and B

Site	Year	Sex	Mean days between first capture and last recaptured (mean ± SE)	Max day between first capture and last recaptured
A	2016	Male	30.86 ± 14.02	48
		Female	23.71 ± 12.30	37
			P = 0.4015	
	2017	Male	19.56 ± 11.95	44
		Female	20.38 ± 15.70	45
			P = 0.795	
B	2016	Male	12.91 ± 9.40	32
		Female	16.07 ± 9.98	32
			P = 0.3464	
	2017	Male	18.64 ± 13.33	43
		Female	18.30 ± 17.27	64
			P = 0.6049	

P-values show the result of Wilcoxon rank sum test between sexes in each Site and year

C. s. akitanus phenology in our study area is summarized as follows. The adults emerged from late June to July and decreased after peaking in early to mid-August (see Fig. 3). Although most adults were dead in late-September, some individuals, especially females, survived until mid-October.

Adult mobility

Our MRR showed that most adults were recaptured within the study sites. In 2017 at Site A where is very narrow, two males moved approximately 40 m outside of the site. The maximum distances in 6.3% of males and 66.7% of females was ≦ 5 m in Site A (Fig. 4a). The maximum distances in 42.9% of males and 44.7% of females were ≦ 5 m in Site B (Fig. 4b). At Site A, adults moved 5 to 15 m was 50% and at Site B, adults moved 15 to 30 m was less than 10%. In both years, some individuals moved 40 to 60 m were recorded (Fig. 4a, b). The mean distance between successive captures was significant difference between the sexes at Site A in 2016 (Wilcoxon rank sum test, $P < 0.0001$) and at Site B in 2017 (Wilcoxon rank sum test, $P < 0.05$), showing that females moved short distance, whereas males moved to relatively long distance per capture (Table 5). No individuals dispersed between Site A and B in both years.

Table 5
The movement distance of *Celes skalozubovi akitanus*

Site	Year	Sex	Mean distance between captures (m) (mean ± SE)	Longest distance between captures (m)
A	2016	Male	4.7 ± 1.0	25.0
		Female	0.4 ± 0.3	9.4
			$P < 0.0001$	
	2017	Male	14.0 ± 3.0	40.0
		Female	7.8 ± 2.5	28.6
			$P = 0.59$	
B	2016	Male	4.9 ± 1.4	86.2
		Female	2.9 ± 0.6	20.0
			$P = 0.97$	
	2017	Male	9.3 ± 1.3	57.9
		Female	5.2 ± 0.7	28.9
			$P < 0.05$	

P-values show the result of Wilcoxon rank sum test between sexes in each Site and year

DISCUSSION

Habitat and behavior

The result of the transect counts survey clearly demonstrated that the red wing grasshopper, *C. s. akitanus* in the studied area, inhabits only a limited narrow space such the ridge of semi-natural grassland (including Site A and B) but not forest environment, indicating the grasshopper can be considered as true grassland specialist species. Although previous literatures noted that *C. s. akitanus* inhabits undergrowth and edges in sparse pine forests (Furukawa 1939, 1965), these environments are more likely to correspond to a part of the vast semi-natural grassland that used to be military training areas (Nagahata et al. 2023). In our study grassland area, *C. s. akitanus* adults were not observed at forest–grassland edges and sparse forests composed of pine and/or deciduous trees. These suggest that the differences in the grassland condition such as plant communities, topographic features and human activities to maintain grasslands (e.g. mowing frequency) between the semi-natural grassland in our study and the military training areas seem to affect the occurrence and the behavior of *C. s. akitanus*.

Our study revealed that *C. s. akitanus* adults strongly prefer a bare ground within the semi-natural grassland since most individuals occurred in the mown area (the study route and the mown plot) and no individuals were found in the unmown area. Such habitat preference is more likely to be closely related to raising grasshopper's body temperature and engaging in courtship (reproductive) behavior. Temperature is an important factor for the several behaviors of grasshoppers belonging to the family Acrididae (Lactin and Johnson 1995), and thermophilic grasshoppers are known to require warming and high temperature on ground surface for their hatching and development (Rada et al. 2015). Fisher (1992) have reported that the Acrididae species, *Aulocara elliotti* preferring bare ground, selects specific oviposition sites such as the south slopes, where surface and soil temperature are higher than the slopes of different directions. *C. s. akitanus* in the studied grassland, also occurred only in the south-facing slopes exposed to direct sunlight, and consequently, seem to be thermophilous and xerophilous species. In other words, the bare ground on the south-facing slope within the grassland is a critical habitat requirement for the population of *C. s. akitanus*.

Field observation and cage experiment in the present study showed that *C. s. akitanus* adults have a wide variety of host plant preference, which is consistent with the result of previous study (Yamamoto and Uchida 2018), and tend to prefer the leaves of the plant species belonging to families Asteraceae and Fabaceae. Among them, *Artemisia japonica* and *Pueraria lobate* were the preferred plants of *C. s. akitanus*, indicating these species are important feeding plants of the grasshopper in the studied habitat. Furthermore, we observed feeding marks of dry (dead) leaves of *A. japonica* fed by *C. s. akitanus* adults. This means the grasshopper may feed on ground litter (dead leaves) as well as on fresh leaves. In contrast, we didn't observe *C. s. akitanus* adults and larvae feeding on the dominant species, *Miscanthus sinensis* and *Pteridium aquilinum* in the semi-natural grassland and the cage experiment. These suggest that grasslands vegetation with diverse plants growing are more important for the habitat of *C. s. akitanus* than grasslands dominated by specific plant species such as *M. sinensis* and *P. aquilinum*.

In the present study, there were few observations of the feeding, mating and oviposition behavior of *C. s. akitanus*, despite of many field observations (61 times, combined with transect counts survey and MRR survey) with multiple researchers. This may be due to those behaviors in limited time such as morning and evening (except for the time of our field study) with avoiding high temperature on the ground during the day, and/or at the dense vegetation within grassland far from bare ground because of avoiding detection by predators. Joen et al (1986) showed that more than 80% of the behavior of some Acrididae grasshoppers were "quiescence" during the day. Given that *C. s. akitanus* has also exhibited quiescent activity during certain portions of the day, the details of behavior such as feeding, mating and oviposition can be difficult to observe. Further research will be needed to determine diurnal activities of *C. s. akitanus*.

Our study showed that an escaping behavior of *C. s. akitanus* in the studied grassland depend mainly on jumping but not flying and the difference in escape pattern between sex. Jumping with spreading hindwings can be considered to stabilize posture and to perform a deimatic display against predators. We observed that when females jumped repeatedly (with spreading hindwings infrequently), it could not

move from the same spot. In contrast, males can fly with spreading hindwings relatively long distances (about 3 ~ 5 m) and hide in the grass. Thus, *C. s. akitanus* adults probably use the following strategies for escaping: the first is a matching color (crypsis) on ground surface in the grassland aiming to prevent detection by predators. Secondly, if predators attack resident grasshoppers, females jump repeatedly (with spreading hindwings infrequently) and hide in the grass, and males surprise predators with spreading brightly red-colored hindwings and then fly away into the grass. Such flying habits seems to be associated with the description “escape in the grass without flying” in the literature (Kano et al. 2016).

Population demographic and movement

The MRR survey revealed that the population size of *C. s. akitanus* in the study area is extremely small. The seasonal population size of Site B was estimated about 129 individuals in 2016 and 92 individuals in 2017, and combined with it in Site A, the total population size in the study area made about 120 to 140 individuals. The estimated population size had difference between studied years (2016 and 2017). Although this cause was not identified, it is presumed the following possibilities. First, the number of *C. s. akitanus* individuals is temporarily increased or decreased by being affected by the annual change of climate, such as temperature and precipitation and the number of predators. Second, the population fluctuations are produced by demographic stochasticity without external (environmental) factors (Den Boar 1998; Pullin 2002; Primack 2010). Third, increasing the number of marked individuals in 2017 was driven by the establishment of some bare grounds (including study route) which is preferred microhabitat of the species. Concerning the orthopteran grasshoppers’ population size, there are studies of *Eusphingonotus japonicus* (Nomura and Kuramoto 2005) and *Epacromius japonicus* (Hasegawa et al. 2011, 2012) in Japan. These authors showed that the estimated population size of studied species were a few hundreds to thousands. Moreover, in well-studied endangered butterfly which the habitat is limited to small area like *C. s. akitanus*, the estimated population size was a hundred to thousands (Nowicki et al. 2005; Hernandez-Roldan et al. 2009; Nowicki et al. 2009), these population sizes were much larger than our results. Thus, the current population of *C. s. akitanus* in the studied habitat is in an immediate danger of extinction.

Our MRR data provided important insights into the mobility behavior of *C. s. akitanus*. The result of the present study showed that this species has the short movement distances and the high recapture rates (67.3–100%). This indicates the grasshopper tends to be behaviorally sedentary and has the low dispersal ability. In general, the grasshopper having a developed hindwings moves longer distance than our results, for instance, it is known that *Eu. japonicus* moved 162 to 1831 m (average 428 m) and *Ep. japonicus* moved 500 m at a maximum (Nomura and Kuramoto 2005; Hasegawa et al. 2012). In contrast, the flightless grasshopper in small population and limited habitat, moves relatively shorter distance (e.g. López et al. 2007; Weyer et al. 2012). *C. s. akitanus* adults have the developed hindwings, nonetheless, the maximum distance of most individuals was fairly low (≦ 5 m, Fig. 4). Therefore, it is more likely that the species normally moves using walking behavior instead of flying and jumping.

Interestingly, some adult males moved 50 to 60 m in the study site, which was much larger than most individuals (Fig. 4 and Table 5). This result is consistent with the result of flying behavior. Adults usually jumps short distance with spreading hind legs. Consequently, these results support that some males may move longer distance with flexing hind legs. Comparing the maximum movement distance between the males and the females, the distance in males was significantly longer than females. The male grasshopper is generally more mobile than females (Maes et al. 2006), and our results also showed the similar pattern. In our study, no adults moved between patches (Site A and B) separated by about 150 m, which supports indirectly the low mobility of *C. s. akitanus*. Therefore, the exchange between two studied populations (gene flow) may not have occurred currently. This may be caused by the paved road and square between both study sites and the small-isolated population (see Fig. 1).

Conservation implications

The present *C. s. akitanus* populations in the study area are in a crisis that cannot survive until the near future. The results of our study can contribute to conservation practices of the *C. s. akitanus* population and its habitat environments. Our study showed mainly the following three points. First, from our MRR results, *C. s. akitanus* population size is extremely small (total about 120 to 140 individuals in the study area) at present. Second, the studied populations became fragmented and isolated, and from the result of the transect counts survey, other populations have not been remained within the study area. Third, a meta-population was not established in the studied habitat because no individuals moved between Site A and B. For an insect species with a very small population and low mobility such as *C. s. akitanus*, environmental changes such as habitat abandonment and development will cause local extinction. To preserve the *C. s. akitanus* population in the studied grassland, it is crucial to maintain the current grassland managements such as prescribed burning in spring and mowing in summer. Annual prescribed burning plays a much more important role in maintaining grassland environments because Japanese grasslands belonging to the Asian monsoon region usually change quickly to a forest environment (due to the progression of succession) without human managements. Most of remaining habitats of *C. s. akitanus* in Japan, including that of this study, are grasslands that had been burned by human for a long time (Nagahata et al. 2023). This indicates that the grasshopper depends strongly on burnt grasslands. This may be related to the hatching of the grasshopper, which is a thermophilous and xerophilous species, as the prescribed burning in spring (after the snow melts) burns the dead grass in the grassland and exposes the ground surface to direct sunlight.

Mowing of grass in the habitat of *C. s. akitanus* within the grassland is also essential for the recovery of this species population. To improve the present population of *C. s. akitanus*, the habitat patch enlargement and increasing by creating more bare ground that the species adults preferred, within the study sites and surrounding area are needed. Mowing and removing mowed grass in the studied grassland in summer (especially in July or August when *C. s. akitanus* adults are begin to active) had a positive effect on the habitat of the species because of creating non-grass area (i.e. bare ground). Furthermore, to establishment meta-population, a swath bare ground is installed as habitat corridors between patches. In the study area, the bare ground temperature was considerably higher (about 60℃ at

maximum) than surrounding atmospheric temperature (about 40℃), and we recorded upper tolerance temperature limit of *C. s. akitanus* was about 53 to 57℃ (Nagahata and Okatsu unpublished). Therefore, the vegetated area is regarded as a role of a shadow area in which the species uses for thermoregulation and predator avoidance. In addition, the results of our study revealed that *C. s. akitanus* does not feed on the dominant plant species, *M. sinensis* and *P. aquilinum*, but prefers a variety of plants, especially those of the Fabaceae and Asteraceae families. In other words, diverse plant communities within the grassland are very important in ensuring preferred food resources for *C. s. akitanus*. To increase such a favorable environment, selective mowing of *M. sinensis* and *P. aquilinum*, which are dominant in the habitat of this grasshopper, is considered to be an effective way.

Declarations

Author Contribution

Y.O. wrote the main manuscript text and Y.N. prepared figure 2. All authors reviewed the manuscript.

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Figures

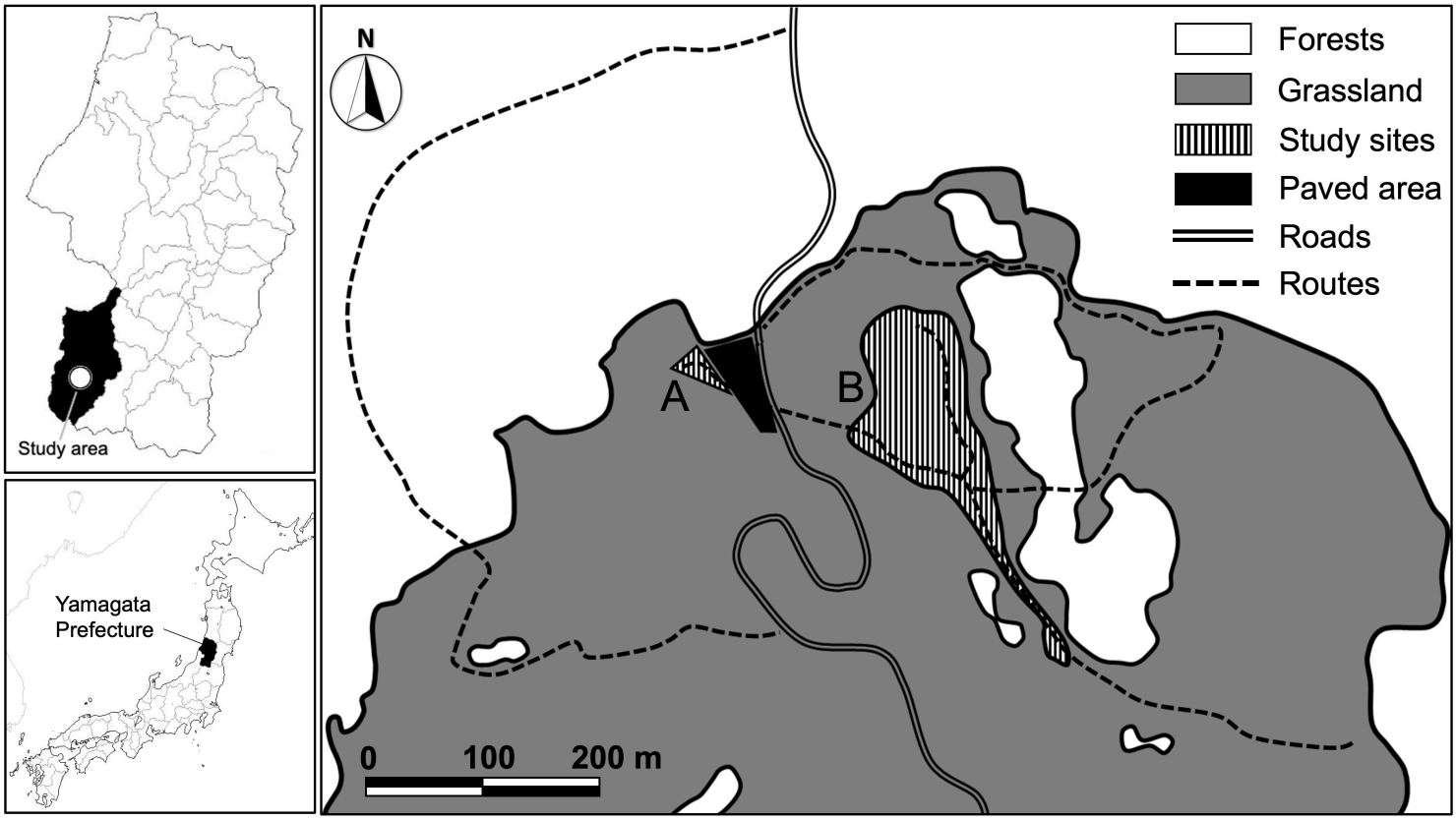

Figure 1

Map of the study area, Yamagata Prefecture in Japan. Study sites A and B show that the occurrence area of *Celes skalozubovi akitanus* and the area of mark release recapture study. Routes for the transect counts survey indicate dashed line on the map

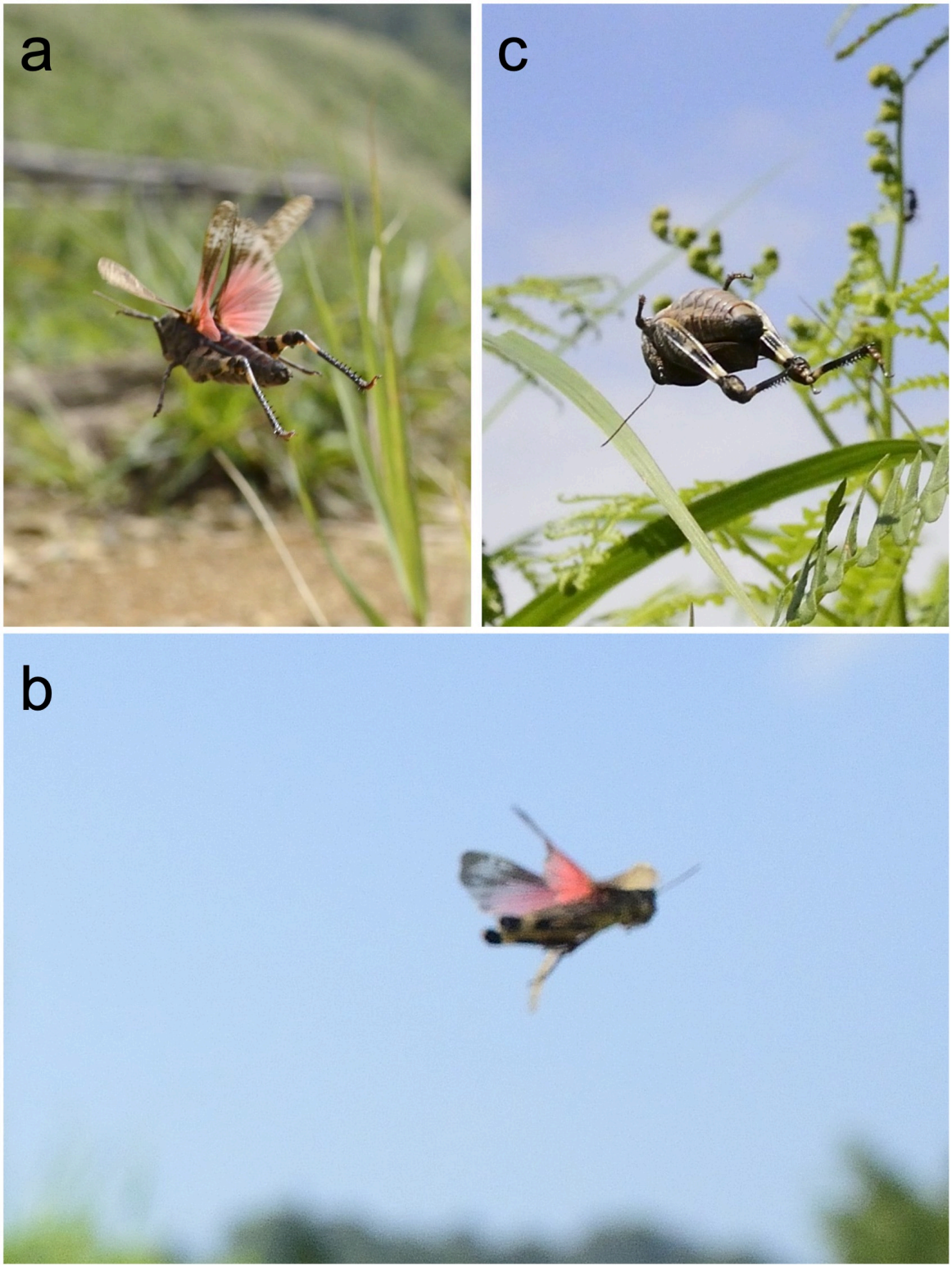

Figure 2

The form of flying and jumping behavior of *Celes skalozubovi akitanus*, (a) adult female flying with spreading hind legs, (b) adult male flying with flexing hind legs and (c) adult female jumping

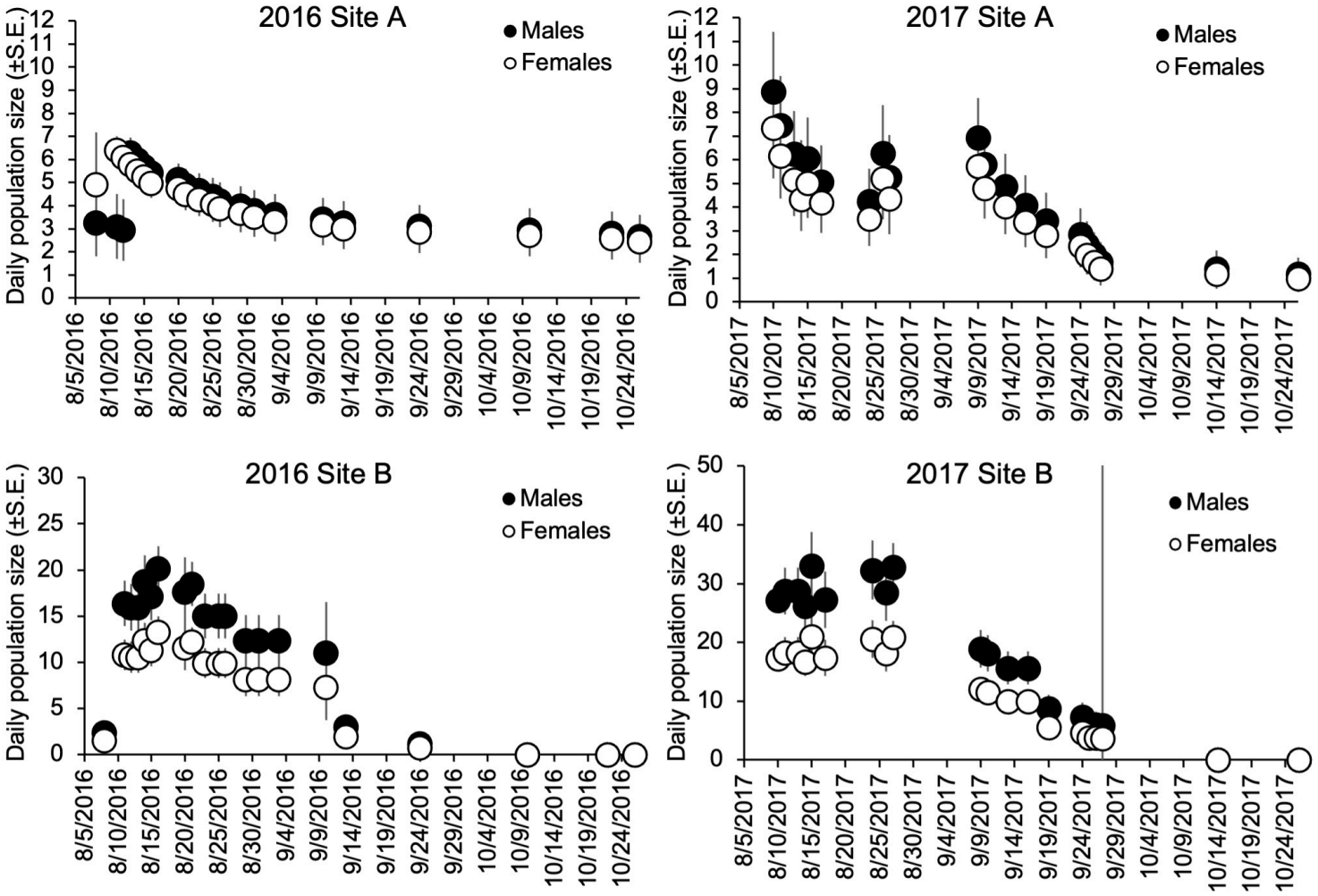

Figure 3

The daily population estimates of *Celes skalozubovi akitanus* adults across the survey period, at Site A and B in 2016 and 2017. Black circles represent the estimate with males, open circles females. Standard error bars are shown

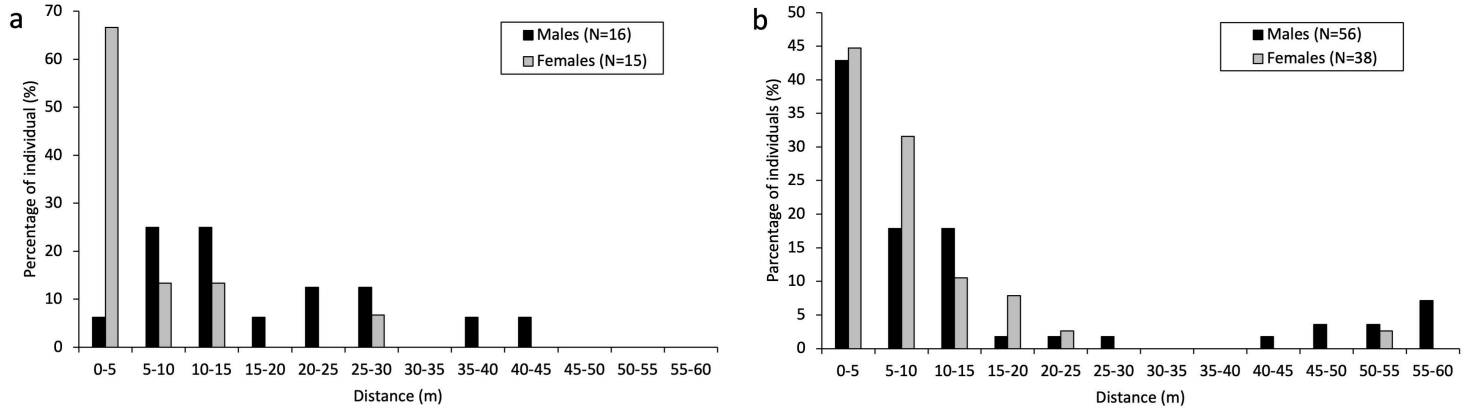

Figure 4

The maximum movement distance of *Celes skalozubovi akitanus* from first capture point to recapture point, at Site A (a) and B (b) in 2016 and 2017