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Posted Date: 13 December 2023

doi: 10.20944/preprints202312.0946.v1

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Measurement of Fitness and Predatory Ability of 4 Predatory Mite Species on Tibetan Plateau

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Simple Summary: Two-spotted mites (*Tetranychus urticae*) greatly harm the vegetables and other crops in Tibet, while predatory mites are vital biocontrol factors to control spider mites. Herein, we clarified the fitness and predatory abilities of 4 predatory mite species in Tibetan plateau, and confirmed that the predatory mite *Amblyseius swirskii* had the highest fecundity (Mean ± SEM=11.60 ± 0.34) and the highest pre-adult survival rate (Mean ± SEM = 83.33 ± 3.33%), and *A. swirskii* also had the highest predation capacity on *T. urticae* adult mites at 15 d post-release (Mean ± SEM =14.28 ± 2.24). Taken together, *A. swirskii* was considered as the most effective predatory mite species to control the two-spotted mites in Tibet.

Abstract: Predatory mites are a type of biological control agent used in many countries against various vegetable pests, particularly spider mites. Despite the significant presence of predatory mites in the Tibetan plateau, there is limited research on their fitness and prey capacity in the area. Herein, four predatory mite species, including one species *Neoseiulus californicus* and three species from genus *Amblyseius* (*Amblyseius swirskii*, *Amblyseius barkeri,* and *Amblyseius cucumeris*) were investigated. Specifically, their fitness and prey capacity on two-spotted mites (*Tetranychus urticae*) at highland conditions were evaluated. Compared to the other three predatory species, *A. swirskii* had the highest fecundity (Mean ± SEM=11.60 ± 0.34) and the highest pre-adult survival rate (Mean ± SEM = 83.33 ± 3.33%). Since their juvenile Survival Rate (SR) was extremely low (Mean ± SEM =13.33% ± 5.77%), most *A. barkeri* nymphs died before emergence. Compared to the other three predatory mites, *A. swirskii* had the highest predation capacity on *T. urticae* adult mites at 15 d post-release (Mean ± SEM =14.28 ± 2.24). Collectively, these findings imply notable advantages in employing *A. swirskii* for controlling two-spotted mites in the Tibetan Plateau. Furthermore, based on the results, *A. swirskii* was the most effective, and *A. barkeri* was the least effective in controlling two-spotted mites in the Tibetan Plateau. These findings could inform the development of a feasible biological control method based on suitable predatory mite species to manage *T. urticae* in the Tibetan Plateau, and would be helpful in delaying the rise or emergence of chemical pesticide resistance in two-spotted mites.

Keywords: fitness; predatory mites; biological control; spider mite; Tibetan Plateau doi:10.20944/preprints202312.0946.v1

1. Introduction

In all mite families, the Tetranychidae family, which encompasses > 1300 species of spider mites (Acari: Tetranychidae), holds a significant position regarding its impact on agricultural production [1]. Specifically, more than 100 pest spider mite species could harm agricultural and forestry crops [2]. The two-spotted mite (*Tetranychus urticae*), a generalist herbivore that could feed on > 1150 host plant species (including a multitude of important crops such as corn, soybean, tomato, cucumber, and so on) [1,3] is the most widely distributed and destructive spider mite species worldwide. Furthermore, multiple *T. urticae* populations could strongly resist major acaricides and insecticides in various regions [4]. Since 1983, *T. urticae* has been widespread in most regions in China and is currently the dominant spider mite species [5,6]. Specifically, in the Tibetan Autonomous Region of China, *T. urticae* was reported to harm numerous vegetable crops and was also difficult to control [7]. However, few studies on the biological management of spider mites have been conducted in Tibet, the world's highest plateau.

Based on the drawbacks of insecticide or pesticide resistance and pollution, biological control is considered one of the most environmentally friendly and economical methods for managing insect pests [8]. Predatory mites are one of the most important natural biological control agents. Specifically, phytoseiid mites are vital biological control agents, with *Amblyseius swirskii Athias-Henriot* (Acari: Phytoseiidae) as the most successful predatory mite, having been employed in > 50 countries globally [9,10]. Other biological factors used for controlling the disastrous harm caused by two-spotted mites include predatory mite species such as *Neoseiulus californicus* and many other mites of the genus *Amblyseius* [11–13].

With an average elevation of >4000 m above sea level, the Tibetan Plateau is the highest of China's three major landform ladders. It is characterized by low temperatures and hypoxia, the most substantial threats to the lives of arthropods and other animals in the area [14]. Invertebrates often develop various useful strategies to combat adverse highland conditions. For example, to combat hypoxia conditions (2% O_2), the bean weevil increases the synthesis of metabolites, including carbohydrates, amino acids, and organic acids [15]. Furthermore, since the cytochrome oxidase activity is higher in Tibetan locusts, they show a higher hypoxia tolerance than lowland locusts [16]. Additionally, as a means of surviving the high-latitude environment, the enemy insect *Bombus pyrosoma* would upregulate energy metabolism genes [17]. Despite these useful insights, there are no recent explorations on the effects of highland conditions on the fitness and prey capacity of predatory mites.

The predator *N. californicus* was first reported by McGregor in 1954 in California, United States [18,19]. As a polyphagous predator, *N. californicus* mainly fed on Tetranychid spider mites (especially *T. urticae*, and other spider mites such as *T. evansi*) [20–22]. It could also consume various small insects, including other mite species or thrips; hence, McMurtry classified it as a type II specialist predatory mite (selective predators of Tetranychid mites) [10].

On the other hand, predatory mites of the genus *Amblyseius*, popular for feeding on various prey including a multitude of mites from many families (such as Tetranychidae, Eriophyidae, Pyroglyphidae, and so on) and many other arthropods including enormous pest insects (such as whiteflies, thrips, nematodes, and so on) [10,23], were always considered as generalist predators, hence were listed as subtype III-b in type III lifestyle predators by McMurtry [23]. However, although the species *N. californicus* and other predators also belong to the genus *Amblyseius*, the impact of highland conditions on them has received little research attention, and their predatory capacity remains largely unknown.

Herein, four predatory mite species, including species *N. californicus* and three species from the genus *Amblyseius* (*A. swirskii*, *A. barkeri*, and *A. cucumeris*) were examined to determine the most suitable predatory mite species for controlling *T. urticae* in the Tibetan Plateau, the world's highest plateau. The fitness and predatory capacities of the four species under highland conditions were assessed. The results showed that in the Tibetan Plateau, *A. swirskii* and *A. barkeri* were the most effective and least effective predatory mites in preying on *T. urticae*, respectively.

2. Materials and Methods

2.1. Establishment of the T. urticae and predatory mite populations

The *T. urticae* population was collected from soybean leaves in the solar greenhouse of the Lhasa Agricultural Science and Technology Park (roughly E91°07′, N29°38′). Spider mite species identification was confirmed by COI sequencing [24]. After introducing the spider mites into the laboratory, the isofemale line of *T. urticae* mites was established and grown on the leaves of kidney beans (*Phaseolus vulgaris* L.) in controlled conditions. The *T. urticae* population was used as the target or objective pest mite to determine the prey capacity of the predatory mites.

Herein, four predatory mite species, including *A. swirskii*, *Amblyseius barkeri* (Hughes), *Amblyseius cucumeris* (Oudemans), and *N. californicus* were examined. All predatory mite species were purchased from Fujian Yanxuan Biological Control Technology Company (Fuzhou, China) and were categorized based on three characteristics: spermatheca, abdominal shield, and dorsal shield [25].

2.2. Fitness experiment for the four predatory mite species

The fitness of all four predatory species was assessed through a life-table experiment after rearing in highland conditions in Lhasa, Tibet Autonomous Region, China (roughly E91°07′, N29°38′) for more than ten generations (approximately six months). The rearing conditions were as follows: 1. Temperatures of 25.5 ± 1°C; 2. Relative Humidity (RH) of 80 ± 5%; 3. A photoperiod of 16:8 h (L: D); and 4. A mean altitude of 3642 m above sea level. First, thirty new 1-day-old emerged female adults and thirty new 1-day-old emerged male adults were selected for each mite species to oviposit for 4 h in one dish (9 cm). Following that, 30 eggs of each species were randomly selected to conduct the subsequent test, and one egg was placed in one dish with a kidney bean leaf (about 7 cm) as a replicate, with each mite species having 30 replicates. In each dish, five *T. urticae* eggs and five *T. urticae* nymphs (mixed age stages) were added every five days as a diet. To calculate the juvenile survival and developmental rate of different predatory mite species, the stages of all predatory mites were monitored and recorded every 12 h. Ten predatory mites were regarded as a replicate for juvenile survival calculation. Each mite species experiment was performed in triplicate.

Once a female predatory mite emerged, one newly emerged male adult was introduced to mate with one female adult. Ten female adults of each mite species were randomly selected each day to record fecundity. Given that the pre-adult Survival Rate (SR) of *A. barkeri* was extremely low, ten 1-day-old female *A. barkeri* adults were selected from other leaf dishes. Fecundity and hatchability data were first tested for normality (Kolmogorov–Smirnov test) and then homogeneity of group variances (Levene's test). Subsequently, one-way ANOVA analysis (SPSS 21.0) was employed to determine the variance of fecundity and the developmental rate among all four predatory mites. Given that the SRs did not follow a normal distribution, they were analyzed by a Kruskal–Wallis test, and multiple comparisons were performed using Dunn's test with Bonferroni correction (SPSS v21.0). The Log-rank (Mantel-Cox) test was used to compare the survival curves of each predatory mite's female adults (GraphPad Prism v9.0.2).

2.3. Functional response experiment

The functional response experiments on the ability of the four predatory mite species to prey on *T. urticae* (different stages) were conducted under 25°C conditions. First, an equivalent amount of *T. urticae* at three variant stages (egg, nymph, and adult) was selected at six density gradients (1, 3, 5, 7, 9, and 11), and the same number of *T. urticae* eggs, nymphs, and adults were all placed in a dish, while one 1-day-old female adult predatory mite was placed in the same dish after 24 h of starvation. After 24 h, the predation activities of the predatory mites on different stages of *T. urticae* were recorded. The preying of predatory mites on different stages of *T. urticae* was measured using a predator-prey model, specifically the Holling type II functional response [26]:

$$N_a = \frac{\mathrm{a} * T * N}{1 + T_h * \mathrm{a} * N}$$

doi:10.20944/preprints202312.0946.v1

Where N_a = the number of *T. urticae* mites preyed on, a = the attack rate (proportion of prey captured by each predator per unit of searching time), T_h = the handling time (the time it takes for predatory mites to identify, kill, and consume *T. urticae mites*), N=the prey density, and T = the time it takes for predators to find the prey (in this test, T is 1 d).

2.4. Predator interference by different densities of predatory mites

The predatory abilities of different mites at various densities were measured. Herein, six predatory mite density gradients were used (1, 3, 5, 7, 9, and 11). Each mite species was prepared per each density, and these combinations were replicated three times. For every replicate, forty *T. urticae* female adults were first put in a leaf dish. One selected density of 1-day-old female adults from a single predatory species was then placed in the same dish, and the predation situation was recorded after 24 h. Predator interference analysis was performed using Watt's model of the effect of the densities of attacked and attacking species on the number of the attacked (Watt, 1959):

$$A = \alpha X^b$$

Where A = the number of *T. urticae* female adults preyed on, a = the attack rate without competition, X = the density of predators per leaf dish, and b = the intraspecific competition parameter.

Data on the predatory capacity, handling time, attack coefficient, and max predatory amount were tested for normality (Kolmogorov–Smirnov test) and homogeneity of group variances (Levene's test) if they followed a normal distribution and One-way ANOVA analysis (SPSS 21.0) was performed to determine the variance among all four predatory mites. On the other hand, the data were subjected to a Kruskal–Wallis test, and multiple comparisons were performed using Dunn's test with Bonferroni correction if they followed a non-normal distribution (SPSS 21.0).

3. Results

3.1. Compared to other mites in the highland area N. californicus had the fastest developmental rate

One-way ANOVA was used in this part of the results as all the mites' developmental time data followed a normal distribution. When the development times of the four predatory mites from eggs to emergence were compared, *A. barkeri* (Mean ± SEM =14.50 ± 0.61) and *A. cucumeris* (Mean ± SEM = 13.54 ± 0.15) had a significantly longer juvenile period in the Tibet area. On the other hand, *N. californicus* had the fastest developmental rate (Mean ± SEM = 9.50 ± 0.16) ($P < 0.05$, One-way ANOVA, SPSS v21.0), implying that it had an applicability advantage over the remaining three predatory mite species grown in Tibet (Figure 1).

In terms of the specific developmental stages, *A. swirskii* and *N. californicus* had similar developmental rates for the egg period and larva stage, showing that they could spend non-significantly different periods to complete both growth stages. On the other hand, *A. barkeri* and *A. cucumeris* took significantly longer periods to complete the two growth stages ($P < 0.05$, One-way ANOVA, SPSS v21.0) (Figure S1A and S1B). Additionally, compared to the other three mite species, *N. californicus* had the fastest developmental rate for the protonymph stage. On the other hand, although the growth time of *N. californicus* during the deutonymph stage was significantly shorter than that of *A. barkeri* and *A. cucumeris*, it was comparable to that of *A. swirskii* (Figure S1C and S1D).

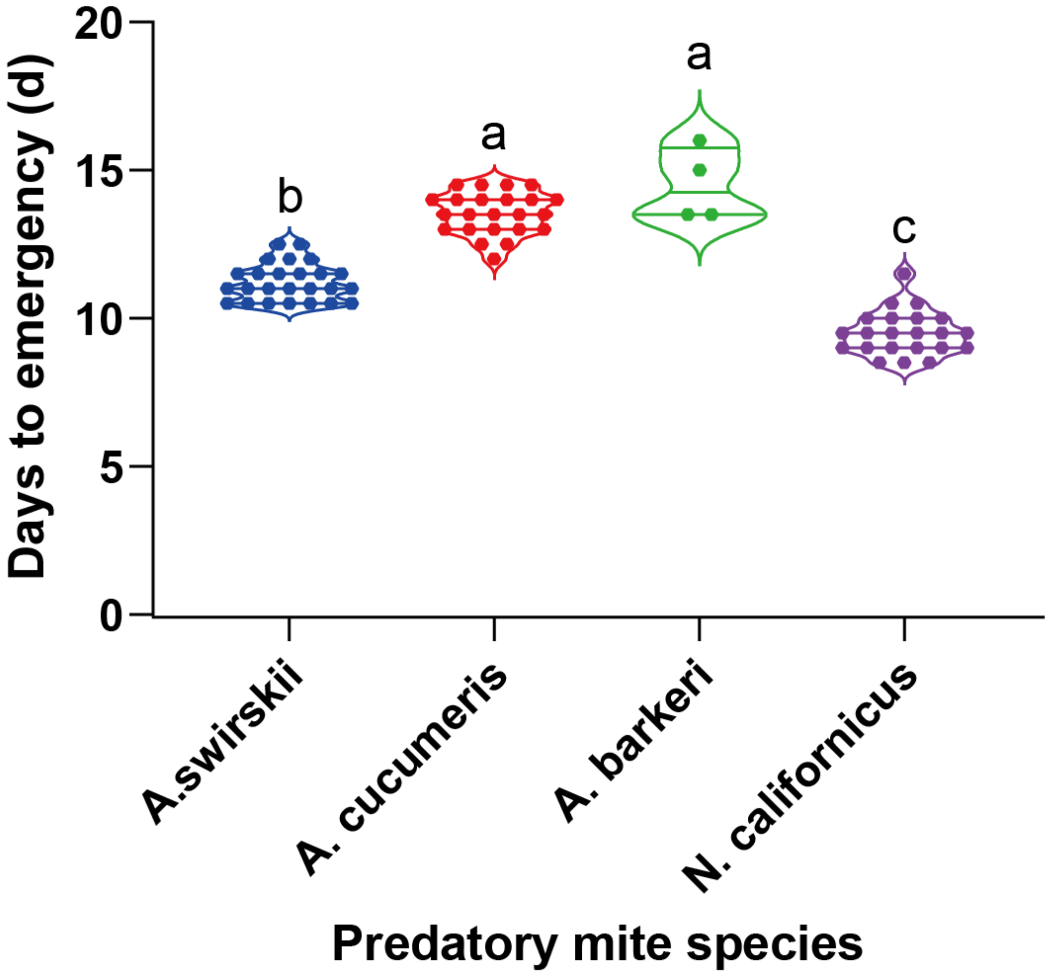

Figure 1. Developmental time of four predatory mite species in Tibetan Plateau. The same letters above columns represent homogeneous groups in post-hoc tests ($P > 0.05$) following an ANOVA.

3.2. A. barkeri had relatively less fecundity than A. swirskii

Egg counts of all predatory mites were determined per female animal. Consistent with the developmental time data, all fecundity data followed a normal distribution. According to the results, *A. swirskii* (Mean ± SEM =11.60 ± 0.65) had a significantly higher fecundity compared to the other four predatory species, followed by *A. barberi* (Mean ± SEM = 9.60 ± 0.34) ($P < 0.05$, One-way ANOVA). Compared to those of *A. swirskii* or *A. barberi,* the fecundities of *A. cucumeris* (Mean ± SEM = 10.30 ± 0.633) and *N. californicus* (Mean ± SEM =10.10 ± 0.43) were non-significantly different (Figure 2).

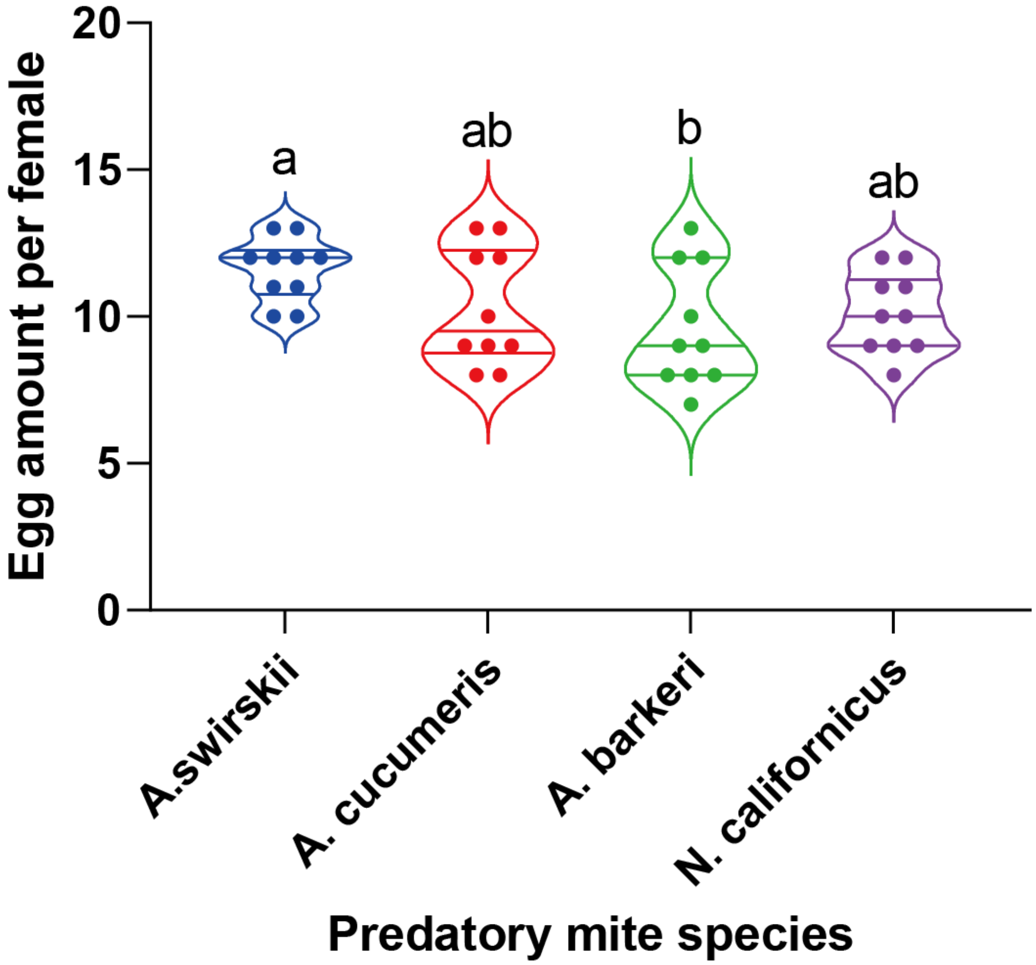

Figure 2. Fecundity of four predatory mite species in Tibetan Plateau. The same letters above columns represent homogeneous groups in post-hoc tests (*P* > 0.05) following an ANOVA.

3.3. Most A. barkeri mites died before emergence in Tibet

During rearing in the Tibet plateau, *A. swirskii* had the highest pre-adult SR (Mean ± SEM =83.33% ± 5.77%), while *A. barberi* had the lowest SR (Mean ± SEM =13.33% ± 5.77%), significantly lower than that of the other three predatory species (P < 0.05, Kruskal–Wallis test and Dunn's test). At the same time, the pre-adult SRs of *A. cucumeris* and *N. californicus* were not significantly different compared to that of *A. swirskii* (Figure 3).

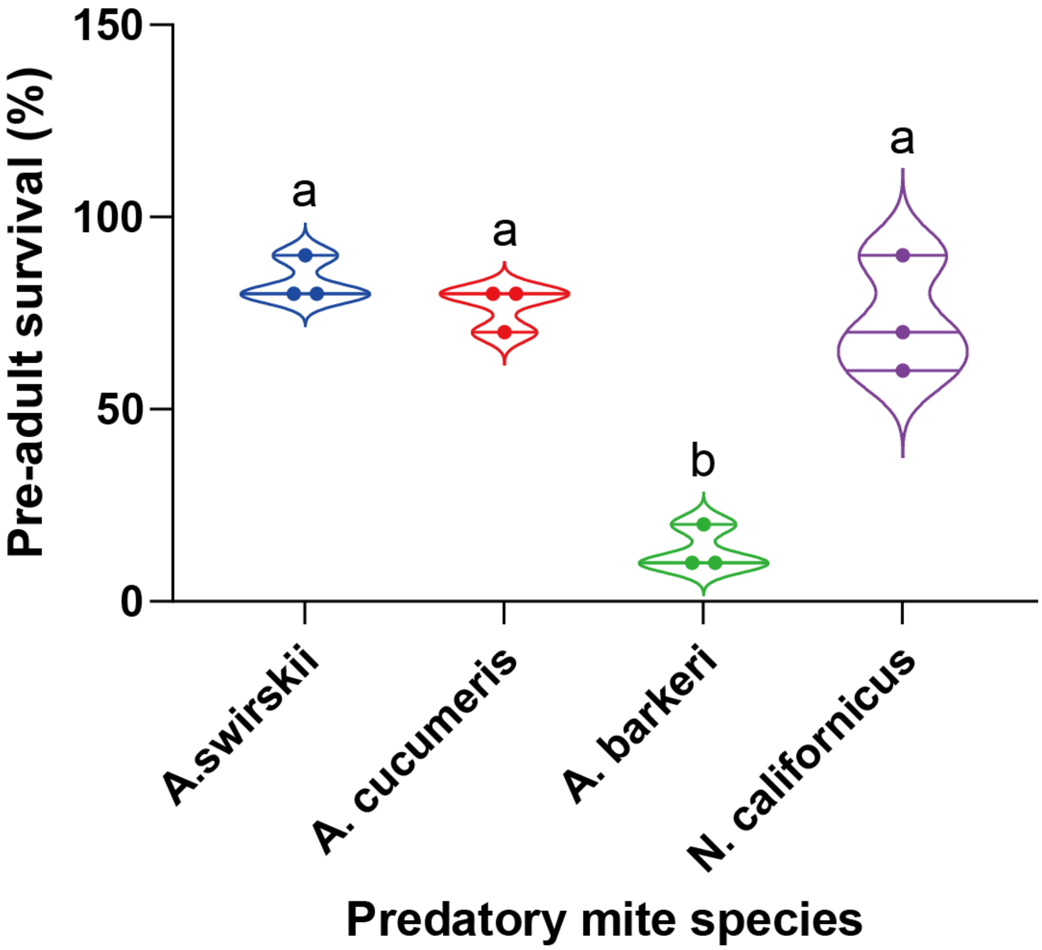

Figure 3. Pre-adult survival rate of four predatory mite species in Tibetan Plateau. The same letters above columns represent homogeneous groups in post-hoc tests ($P > 0.05$) following by Kruskal–Wallis tests and Dunn's tests with Bonferroni correction for multiple comparisons.

3.4. All four predatory mite species shared similar longevities in high altitudes

Each species' longevity from mite emergence to death was measured to further clarify the influence of highland conditions on the life span of the four predatory species. According to the results, there were no significant differences among all four predatory species ($P = 0.13$, Log-rank (Mantel-Cox) test), but *A. barberi* had the greatest longevity (Median survival = 38 d). (Figure 4).

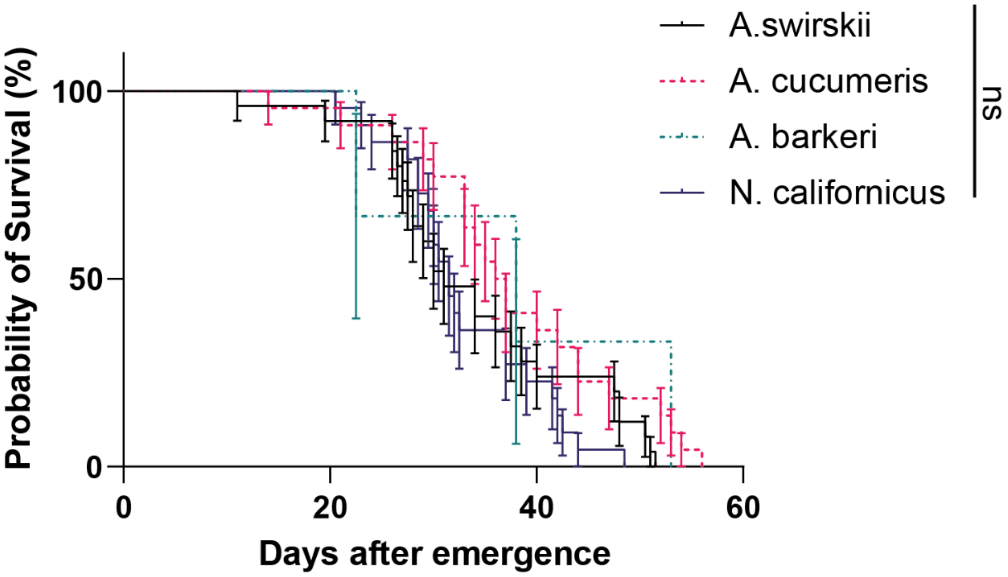

Figure 4. Longevity of four predatory mite species in Tibetan Plateau. Survival curves for individual hosts were compared using the Kaplan–Meier method and log-rank test.

3.5. The predatory capacity of predatory mites varied at different densities under different release times

The prey capacities of the four predatory mite species with different predator densities were measured at 1 d and 15 d post-release. At 1 d post-release, most species exhibited the highest predatory capacity on *T. urticae* mites at density 5. Specifically, *A. swirskii* had the highest predatory capacity at density 5 (Mean ± SD = 11.20 ± 1.30), and other two species; *A. barkeri* (Mean ± SD=10.20 ± 1.30) and *N. californicus* (Mean ± SD = 8.20 ± 0.84,) also had a high predatory capacity at the same density. On the other hand, *A. cucumeris* had the highest predatory capacity on spider mites at density 1 (Mean ± SD = 9.40 ± 2.07) (Figure 5).

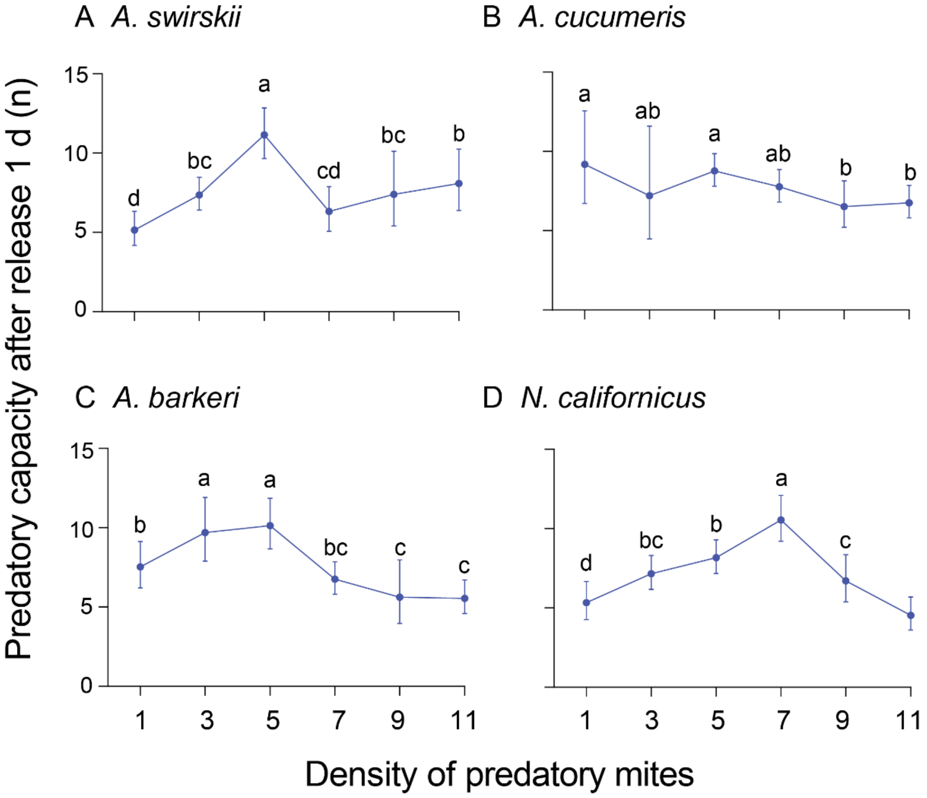

Figure 5. Predatory capacity (preyed spider mites per predator) after release for 1 d of four predatory mite species in Tibetan Plateau. The same letters above columns represent homogeneous groups in post-hoc tests ($P > 0.05$) following an ANOVA.

At 15 d post-release, the highest predatory capacity was observed at density 9 for all mites except *A. swirskii*, which had the highest capacity at density 7 (Mean ± SD = 23.00 ± 1.87). Compared to *A. cucumeris* and *A. barkeri*, *N. californicus* had a higher predatory capacity at density 9 (Mean ± SD = 22.60 ± 1.82) (Figure 6). At the same time, data in Figures 5 and 6 successfully fitted into the Holling II disc equation and the Attack coefficient (α), Handling time (Th (d)), and Maximum predation capacity (1/ Th) results are all summarized in Table 1 (1 d post-release) and Table 2 (15 d post-release). Notably, the predatory mites' prey capacity was always significantly higher at 15 d post-release than at 1d post-release (Figure 7).

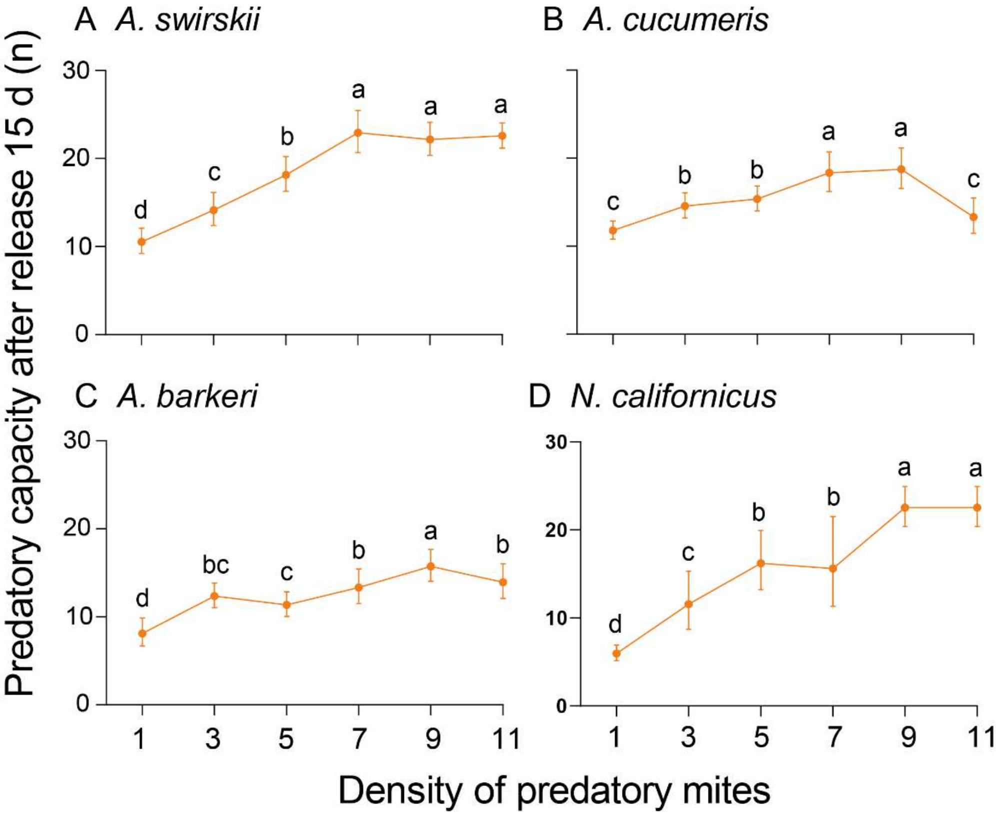

Figure 6. Predatory capacity (preyed spider mites per predator) after release for 15 d of four predatory mite species in Tibetan Plateau. The same letters above columns represent homogeneous groups in post-hoc tests ($P > 0.05$) following an ANOVA.

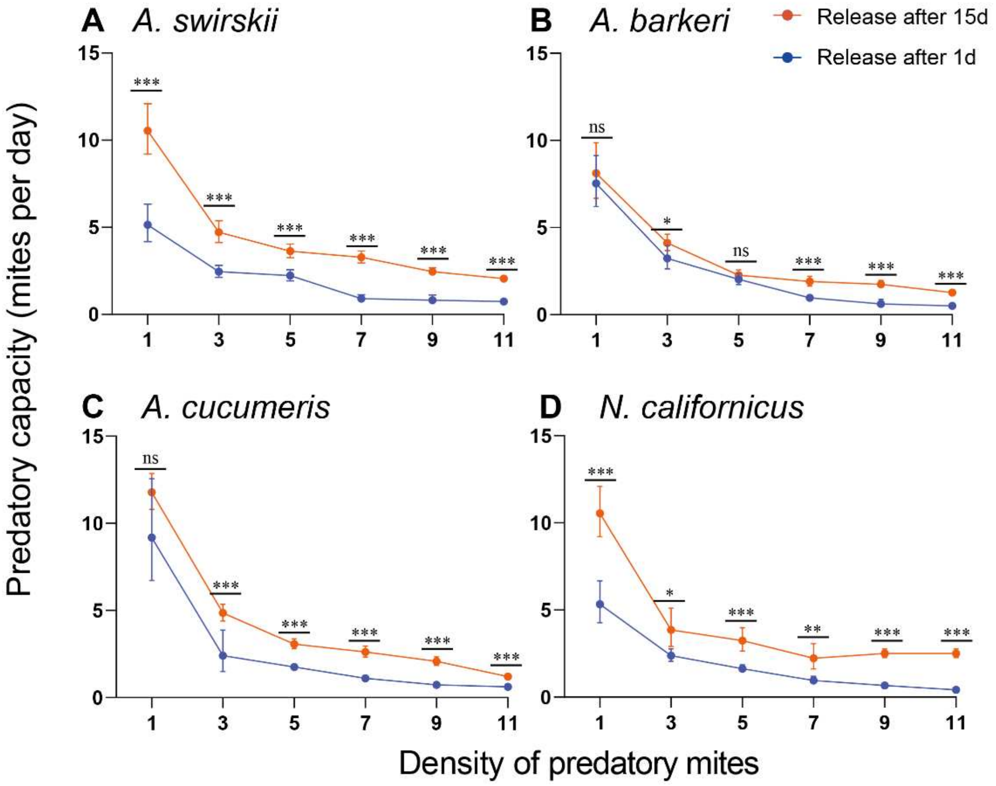

Figure 7. Predatory capacity (preyed spider mites per predator) between release for 1 d and 15 d of four predatory mite species in Tibetan Plateau. Asterisks above points indicate statistically significant differences (Mann–Whitney U-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant).

Table 1. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 1d), the 1st repeat.

Predatory mite species	*Tetranychus urticae* (green) stage	Functional response model (N_a=)	*R*2	Chi-sqaure value
Amblyseius swirskii	adult	$0.238N_0/(1+0.206\ N_0)$	0.992	28.547
	nymph	$1.346\ N_0/(1+0.751\ N_0)$	0.751	16.336
	egg	$1.178\ N_0/(1+0.506\ N_0)$	0.767	17.043
Amblyseius cucumeris	adult	$0.238\ N_0/(1+0.329\ N_0)$	0.783	17.794
	nymph	$0.759\ N_0/(1+0.289\ N_0)$	0.925	24.793
	egg	$0.928\ N_0/(1+0.186\ N_0)$	0.922	24.672
Amblyseius barkeri	adult	$0.246\ N_0/(1+0.241\ N_0)$	0.988	28.335
	nymph	$0.925\ N_0/(1+0.561\ N_0)$	0.873	22.091
	egg	$0.808\ N_0/(1+0.343\ N_0)$	0.879	22.382
Neoseiulus californicus	adult	$0.246\ N_0/(1+0.241\ N_0)$	0.985	28.145
	nymph	$0.925\ N_0/(1+0.561\ N_0)$	0.965	27.032
	egg	$0.808\ N_0/(1+0.343\ N_0)$	0.826	19.776

Table 2. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 1d), the 2nd repeat.

Predatory mite species	*Tetranychus urticae* (green) stage	Functional response model (N_a=)	*R2*	Chi-sqaure value
Amblyseius swirskii	adult	0.204N/(1+0.048N)	0.977	27.677
	nymph	0.894N/(1+0.132N)	0.893	23.111
	egg	0.605N/(1+0.027N)	0.965	27.033
Amblyseius cucumeris	adult	0.430N/(1+0.079N)	0.963	26.894
	nymph	0.629N/(1+0.063N)	0.963	26.887
	egg	0.835N/(1+0.043N)	0.986	28.169
Amblyseius barkeri	adult	0.235N/(1+0.160N)	0.978	27.745
	nymph	0.453N/(1+0.117N)	0.949	26.092
	egg	0.973N/(1+0.244N)	0.875	22.189
Neoseiulus californicus	adult	0.199N/(1+0.026N)	0.973	27.444
	nymph	1.020N/(1+0.352N)	0.760	16.744
	egg	1.098N/(1+0.146N)	0.912	24.111

3.6. Four predatory species showed various functional responses at different developmental stages of two-spotted spider mites, whether at 1 d or 15 d after release

The functional response experimental results were subjected to the Holling type II functional response analysis to obtain the functional response models of each predatory mite species' prey capacities on different stages of *T. urticae*. All experiments were performed in triplicate (Tables 1–6). At 1 d post-release, data on the predatory capacity of all four predatory mite species did not follow a normal distribution. Consequently, nonparametric analysis was employed, showing that the predatory capacities of all four predatory mite species were significantly different ($P < 0.05$, Kruskal–Wallis tests). However, multiple comparisons through Dunn's tests with Bonferroni correction showed no significant difference between any two groups, with *A. cucumeris* (Mean ± SEM = 10.14 ± 3.41) having the highest predatory capacity on adult *T. urticae* mites, and *A. swirskii* (Mean ± SEM = 7.58 ± 3.25) also having a high predatory capacity on adult *T. urticae mites* (Figure 8A). Additionally, the four predatory mite species' data on handling time and attack coefficient did not follow a normal distribution. Although no statistically significant difference was detected among the four species in handling time ($P = 0.293$, Kruskal–Wallis tests) (Figure 8B), there was a statistically significant difference between them in the attack coefficient data ($P < 0.01$, Kruskal–Wallis tests). On the other hand, Dunn's tests with Bonferroni correction for multiple comparisons revealed no statistically significant difference in the attack coefficient data (Figure 8C). The Max predatory amount data of all four predatory mite species followed a normal distribution, but with no significant difference [$F_{(11, 24)} = 1.272$, $P = 0.298$, one-way ANOVA] (Figure 8D).

Table 3. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 1d), the 3rd repeat.

Predatory mite species	*Tetranychus urticae* (green) stage	Functional response model (N_a=)	*R2*	Chi-sqaure value
Amblyseius swirskii	adult	0.431N/(1+0.064N)	0.961	26.774
	nymph	0.935N/(1+0.180N)	0.941	25.690
	egg	0.885N/(1+0.126N)	0.946	25.925
Amblyseius cucumeris	adult	0.426N/(1+0.117N)	0.916	24.335
	nymph	0.662N/(1+0.118N)	0.907	23.869
	egg	1.028N/(1+0.112N)	0.920	24.533
	adult	0.529N/(1+0.385N)	0.886	22.573

Amblyseius barkeri	nymph	0.703N/(1+0.172N)	0.914	24.212
	egg	0.882N/(1+0.122N)	0.940	25.616
Neoseiulus californicus	adult	0.445N/(1+0.196N)	0.919	24.501
	nymph	0.657N/(1+0.083N)	0.934	25.321
	egg	1.059N/(1+0.133N)	0.917	24.369

Table 4. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 15d), the 1st repeat.

Predatory mite species	*Tetranychus urticae* (green)	Functional response equation	R2	Chi-sqaure value
Amblyseius swirskii	adult	0.209N/(1+0.075N)	0.982	27.993
	nymph	0.631N/(1+0.091N)	0.939	25.573
	egg	1.064N/(1+0.097N)	0.929	25.036
Amblyseius cucumeris	adult	0.214N/(1+0.063N)	0.995	28.684
	nymph	0.645N/(1+0.096N)	0.915	24.256
	egg	0.867N/(1+0.075N)	0.979	27.810
Amblyseius barkeri	adult	0.240N/(1+0.340N)	0.784	17.827
	nymph	0.466N/(1+0.234N)	0.899	23.455
	adult	0.701N/(1+0.225N)	0.914	24.222
Neoseiulus californicus	adult	0.853N/(1+0.491N)	0.844	20.699
	nymph	1.093N/(1+0.131N)	0.921	24.613
	adult	1.170N/(1+0.503N)	0.820	19.477

Table 5. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 15d), the 2nd repeat.

Predatory mite species	*Tetranychus urticae* (green)	Functional response equation	R2	Chi-sqaure value
Amblyseius swirskii	adult	0.459N/(1+0.018N)	0.913	24.517
	nymph	0.919N/(1+0.154N)	0.934	25.315
	egg	0.731N/(1+0.029N)	0.930	25.067
Amblyseius cucumeris	adult	0.196N/(1+0.010N)	0.968	27.169
	nymph	1.171N/(1+0.308N)	0.821	19.563
	egg	1.099N/(1+0.100N)	0.977	27.654
Amblyseius barkeri	adult	0.428N/(1+0.092N)	0.956	26.504
	nymph	0.663N/(1+0.105N)	0.930	25.101
	adult	0.644N/(1+0.062N)	0.939	25.545
Neoseiulus californicus	adult	0.197N/(1+0.010N)	0.963	26.912
	nymph	0.861N/(1+0.076N)	0.988	28.329
	adult	1.104N/(1+0.123N)	0.898	23.367

Table 6. Parameter estimates of four predatory species' functional response when feeding on different stages of *T. urticae* (green) by Holling II functional response model (after release for 15d), the 3rd repeat.

Predatory mite species	*Tetranychus urticae* (green)	Functional response equation	R2	Chi-sqaure value
Amblyseius swirskii	adult	0.940N/(1+0.195N)	0.918	24.449
	nymph	0.903N/(1+0.153N)	0.917	24.385
	egg	0.608N/(1+0.030N)	0.947	26.031
Amblyseius cucumeris	adult	0.455N/(1+0.195N)	0.910	24.001
	nymph	1.046N/(1+0.228N)	0.852	21.062
	egg	0.833N/(1+0.054N)	0.950	26.173

Amblyseius barkeri	adult	0.432N/(1+0.126N)	0.927	24.914
	nymph	0.657N/(1+0.112N)	0.959	26.676
	adult	0.653N/(1+0.076N)	0.907	23.858
Neoseiulus californicus	adult	0.438N/(1+0.164N)	0.913	24.186
	nymph	0.830N/(1+0.078N)	0.903	23.628
	adult	0.605N/(1+0.051N)	0.864	21.650

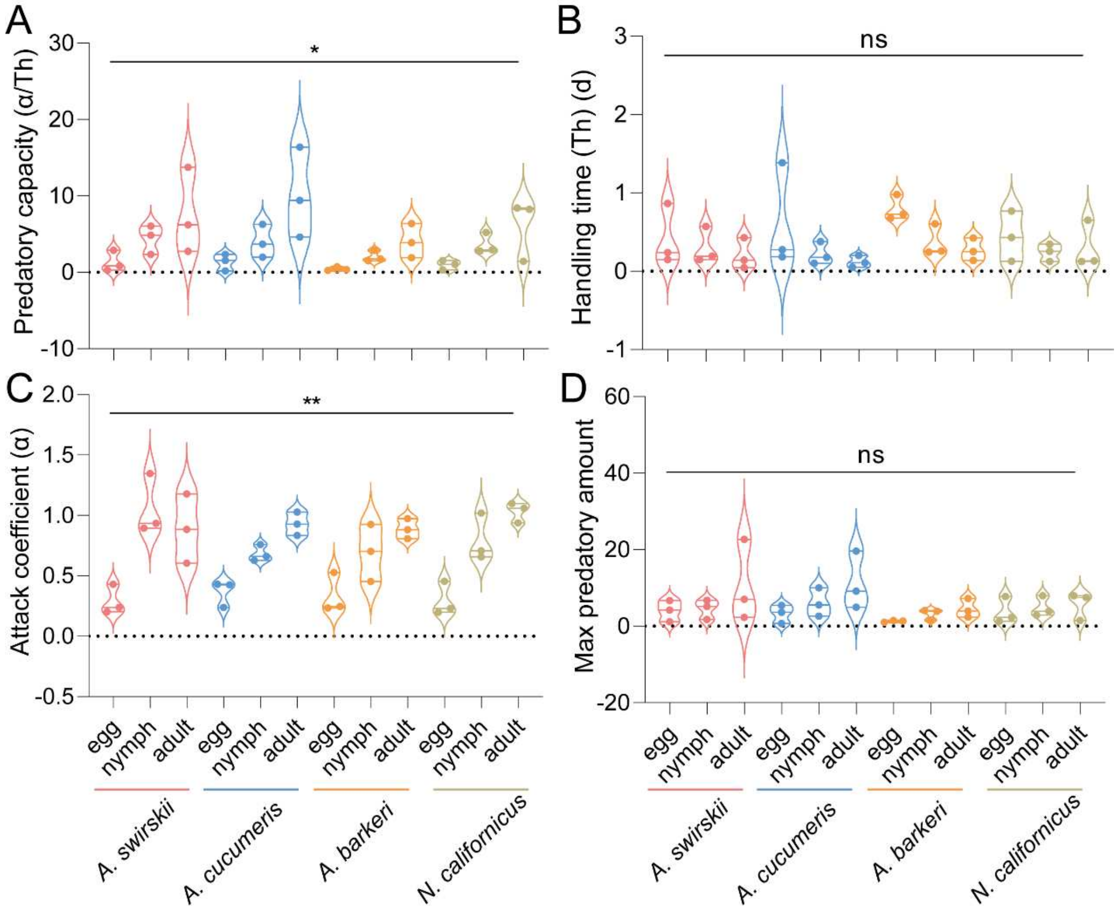

Figure 8. Predatory capacity (**A**), handling time (**B**), attack coefficient (**C**) and max predatory amount (**D**) after release for 1 d of four predatory mite species in Tibetan Plateau. The max predatory amount data are analyzed by one-way ANOVA, SPSS 21.0, while the data of predatory capacity, handling time and attack coefficient are analyzed by Kruskal–Wallis tests, SPSS 21.0 (**P* < 0.05, ***P* < 0.01, ns, not significant).

At 15 d post-release, the predatory mites' data on predatory capacity, handling time, and max predatory amount all followed a normal distribution. A statistically significant difference was found in all mites' predatory capacity [$F_{(11, 24)} = 7.473$, $P < 0.001$, one-way ANOVA]. Specifically, *A. swirskii* (Mean ± SEM = 14.28 ± 2.24) had the highest predatory capacity on adult *T. urticae mites, with A. cucumeris* (Mean ± SEM =11.62 ± 1.48) also having a relatively high predatory capacity on adult *T. urticae mites*. On the other hand, *A. barkeri* had the lowest predatory capacity on various stages of *T. urticae* mites (Figure 9A). No statistically significant difference was found in handling time [$F_{(11, 24)} = 1.245$, $P = 0.312$, one-way ANOVA] (Figure 9B). The attack coefficient data did not follow a normal distribution, and a significant difference was found in the attack coefficients among the four predatory mite species ($P < 0.05$, Kruskal–Wallis tests). On the other hand, Dunn's tests with Bonferroni correction for multiple comparisons found no statistically significant difference in the attack coefficient data (Figure 9C). There was no significant difference detected in mite species' max predatory amount data [$F_{(11, 24)} = 1.385$, $P = 0.242$, one-way ANOVA] (Figure 9D).

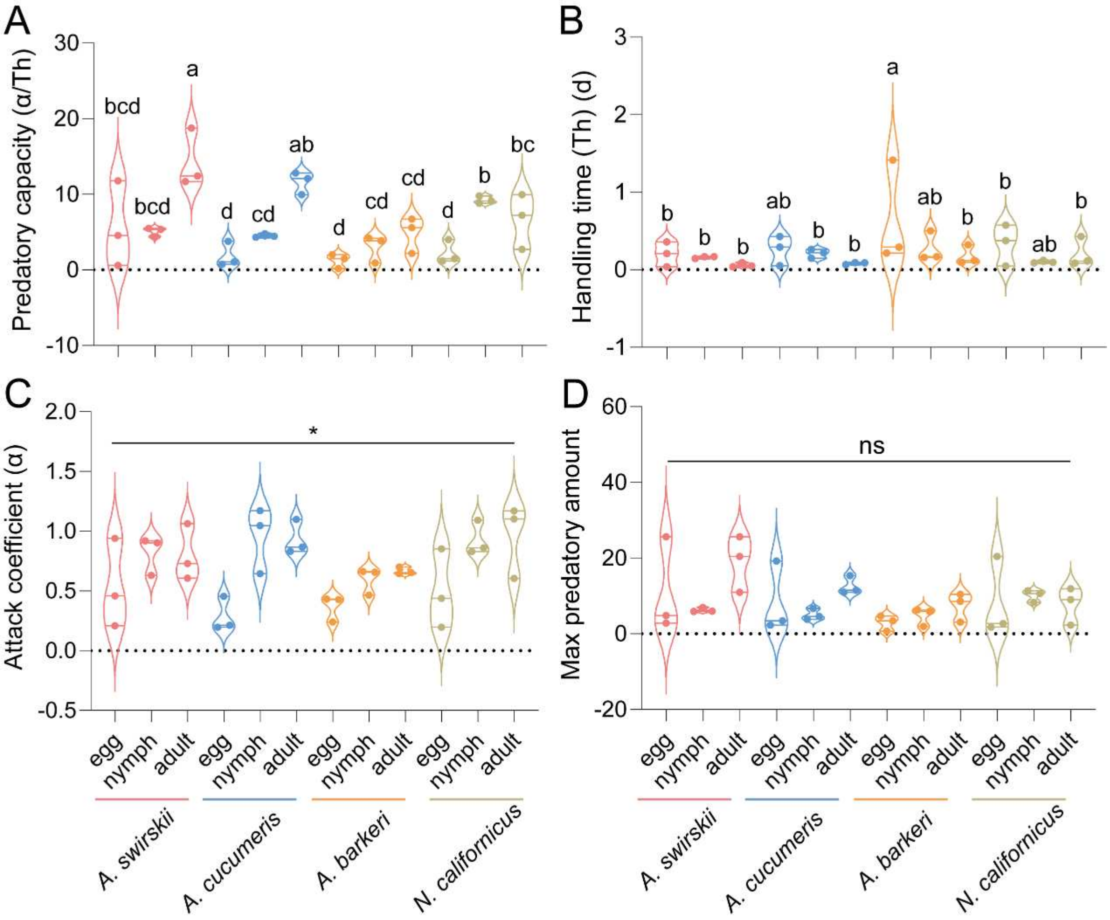

Figure 9. Predatory capacity (**A**), handling time (**B**), attack coefficient (**C**) and max predatory amount (**D**) after release for 15 d of four predatory mite species in Tibetan Plateau. Results are shown as box and whiskers; the same letters above columns represent homogeneous groups in post-hoc tests ($P > 0.05$) following an ANOVA, except for attack coefficient, which is analyzed by Kruskal–Wallis tests, SPSS 21.0 (*$P < 0.05$).

4. Discussion

Herein, the fitness and prey capacity of four predatory mite species (*A. swirskii*, *A. barkeri*, *A. cucumeris,* and *N. californicus*) were assessed using the life-table and functional response experiments. According to the results, *A. swirskii* had the highest fecundity and pre-adult SR, *N. californicus* took the least time in transitioning from the egg stage to emergence, and *A. barkeri* could not survive in highland conditions, as most of its nymphs died before emergence. Furthermore, based on the functional response results, *A. swirskii* had the highest prey capacity on *T. urticae* mites compared to the other three predatory species on many parameters. Our findings collectively implied that among the four tested groups, *A. swirskii* could be the best predatory mite species to biologically control two-spotted mites in the Tibetan Plateau.

The two-spotted mite (*T. urticae)* is one of the world's most disastrous agricultural arthropod pests, damaging a wide range of host plants, including many vital crops such as vegetables, cotton, maize, and so on [27,28]. In China, chemical acaricides and insecticides were the primary methods for controlling *T. urticae*. However, over the past few decades, *T. urticae* rapidly developed a global resistance to these chemical agents, especially in China [29–32]. In Tibet, the world's largest highland, chemical pesticides were widely applied to combat pests, and the resulting significant residues became an issue of concern [33]. Interestingly, no treatments other than chemical control were used in Tibet to effectively prevent the harm caused by two-spotted mites. Despite being important biological control agents against many pest species, including spider mites, predatory mites were rarely used in Tibet, and pertinent research was also extremely limited even in our studies; hence, the fitness and predatory capacity of many predatory mites on *T. urticae* in this region remained unclear [33–35]. Adding to the literature, this study comprehensively explored the fitness and predatory capacity of the four widely used predatory mite species in the Tibetan area.

Multiple studies have reported that high-attitude conditions could significantly influence arthropods' development, morphological characteristics, and body size, among many other aspects [36–40]. For example, a significant link was found between insects' body size and altitude in a study on wing-reduced stonefly in New Zealand [40]. Furthermore, insects' fitness was found to decrease with increasing elevation, as demonstrated by the reduced fecundity of the willow leaf beetle (*Chrysomela aeneicollis*) under high altitude conditions [41]. Herein, we confirmed that a high attitude significantly adversely affected *A. barkeri*, resulting in an extremely low SR before emergence (13.33% ± 5.77%), whereas the other three predator species had a better SR as they all had a relatively better fitness, especially the *A. swirskii* female adult mites, which had the highest fecundity and pre-adult SR (Figures 2 and 3).

It has been reported that *A. swirskii* is one of the most successful biocontrol agents globally, as it can prey on many severe pests and is easy to rear [9]. Furthermore, another study in China indicated that *A. swirskii* had an advantage in the predation of whiteflies over other two predatory mite species; *Amblyseius orientalis* and *Neoseiulus californicus* [42]. These results are consistent with our findings, as *A. swirskii* showed a significantly higher predatory capacity on *T. urticae* than *N. californicus* at 15 d post-release (Figure 9A), and its fitness in Tibet was comparable to that of similar strains reared under normal conditions [43]. As for *N. californicus*, our results indicated that although most fitness parameters including developmental time, pre-adult SR, and longevity in Tibet were comparable to those of mites reared in low altitude conditions [44–46], its fecundity in Tibet (Mean ± SEM = 10.10 ± 0.43) was significantly lower compared to that under relatively lower elevation laboratory conditions (Mean ± SEM =38.31±2.52) [44]. This finding was unsurprising as, just like other arthropods, mites' fitness could be influenced by many abiotic and biotic factors [44,47,48].

Phytoseiid mites, including *N. californicus* are widely distributed biological agents for controlling spider mites. They have been used for decades and have attracted substantial attention given their satisfactory capacity to control the harm caused by *T. urticae* [44,49–51]. Notably, the prey capacity of Phytoseiid mites on spider mites varies under various conditions, including different temperature ranges [47,51], biotic factors such as host plants and prey quality and prey densities [23]. Here, we elucidated the influence of high altitudes on the prey capacities of four predatory mite species and deduced that compared to the other three predators, *A. swirskii* has the highest predation capacity on adult *T. urticae* mites at 15 d post-release (Mean ± SEM = 14.28 ± 2.24), indicating an applicability advantage over the other three species in controlling two-spotted mites in the Tibetan Plateau.

Overall, four predatory species, including one species *N. californicus,* and three species from the genus *Amblyseius* (*A. swirskii*, *A. barkeri,* and *A. cucumeris*) were investigated herein to compare their fitness and prey capacities on *T. urticae* in the Tibetan Plateau. We found that *A. swirskii* had a relatively higher fitness and better predatory capacity than the other predatory mites for controlling *T. urticae* in a highland area. Furthermore, we discovered that the density of nine predatory mites per plant was the most effective in controlling spider mites and that *A. barkeri* was an unsuitable biological control agent in highland conditions. Our findings could inform the development of feasible biological control methods for managing *T. urticae* in the Tibetan Plateau using suitable predatory mite species. They could also be useful in delaying the emergence or increase of resistance to chemical pesticides in two-spotted mites.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, D.X. and K.Y.; methodology, D.X., K.Y. and H.-H.Z.; software, W.Z. and K.Y.; validation, K.Y.; formal analysis, L.X. and K.Y.; data curation, K.Y.; writing-original draft preparation, D.X.; writing-review and editing, K.Y. and Y.-C.W.; visualization, K.Y.; supervision, D.X. and K.Y.; funding acquisition, D.X. and K.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Qingdao Agricultural University High-level Talent Fund (663-1121025), Lhasa Comprehensive Experimental Station of National Technical System of Bulk Vegetable Industry of China, CARS-23-G39, The Central Guide Local Projects of China (XZ202301YD0042C), The Central Guide Local Projects of China (XZ202001YD0014C), ZUNSHI BS [2023] 5

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article and Supplementary Materials.

Acknowledgments: The authors would like to thank all the editors and reviewers for the modifications and suggestions regarding the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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