



# Breeding in Mountain Tops: Water Velocity Determines Oviposition Site Selection of the Chinting Alpine Toad in Streams

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**Abstract** For oviparous species with little or no parental care, oviposition site choice may be a powerful agent of natural selection, playing an important role in the evolution of species fitness and life history. Identifying and clarifying the microhabitat characteristics of oviposition sites are key to understanding life history strategies and local adaptations for amphibians with complex biphasic life cycles. However, oviposition strategies for amphibians in alpine streams remain poorly understood. Here, we focused on oviposition site selection for the Chinting alpine toad (*Scutiger chintingensis*) on Mount Wawu on the eastern margin of the Qinghai-Xizang Plateau. We identified the microhabitat differences between oviposition sites and non-oviposition sites for this species, predicted oviposition site selection patterns, and evaluated the relative importance of each microhabitat variable as well as the effects of the variables on the model prediction probabilities. This study revealed that the microhabitat characteristics of oviposition sites in this species included high water velocity, shallow water, and high-water surface coverage. Water velocity and rock volume were the most important variables explaining the categories of oviposition sites. Additionally, we observed nearly half

the proportion of overlapping oviposition in this species, and the benefits of this behavior in terms of microhabitat characteristics need to be explored in the future. Our study revealed an oviposition site selection strategy for amphibians within alpine streams, and contributed to understanding the life history and environmental adaptation strategies of an endemic alpine stream species, and thereby providing guidance for effective conservation strategies for alpine stream amphibians under global change.

**Keywords** alpine streams, endemic amphibians, microhabitat characteristics, oviposition sites, *Scutiger chintingensis*

## 1. Introduction

Disentangling how the environment affects the survival and reproduction of organisms is one of the main endeavours of life history theory (Kuebbing *et al.*, 2018; Stearns, 2000). Numerous studies have revealed that species evolve life history (e.g., body size and movement activity) and reproductive fitness (e.g., egg size and clutch size) characteristics that allow them to adapt to environmental changes (Leung *et al.*, 2021; Mobley *et al.*, 2020; Wang *et al.*, 2019). The selection of oviposition sites also plays an important role in the evolution of life histories and reproductive strategies, as the specialization of species' life histories takes place in the habitats that are available for oviposition and offspring development (MacTavish and Anderson, 2020; Resetarits, 1996). In oviparous species with little or no parental care, oviposition site selection may be a powerful agent of natural selection, influencing embryo survival, juvenile performance, and progeny phenotype, and subsequently affecting population survival (Kloskowski, 2020; Refsnider and Janzen, 2010).

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Amphibians with a complex and biphasic life cycle, are considered the most threatened group of terrestrial vertebrates (Luedtke *et al.*, 2023; Wake and Vredenburg, 2008). The environmental characteristics of water bodies are important factors influencing their survival, development and reproduction due to the dependence of amphibians on water and oxygen during the embryonic and tadpole developmental stages (Lipinski *et al.*, 2020; Seymour, 1999; Wells, 2007). Additionally, most amphibians do not provide parental care or egg thermoregulation (Khatiwada *et al.*, 2019; Wells, 2007), and thus parents must assess environmental biotic and abiotic factors when selecting oviposition sites to ensure the local adaptation of offspring (Pintar and Resetarits, 2017; Resetarits, 2021). Identifying and clarifying the importance of specific conditions in the oviposition decisions of females is therefore key to understanding the evolution of amphibian's life histories and disentangling the causal agents of local adaptation (MacTavish and Anderson, 2020; Refsnider and Janzen, 2010; Resetarits, 1996). Studies have shown that rainfall and predation are often important factors influencing amphibian oviposition from ephemeral pools to large permanent lakes, respectively (Kloskowski, 2020; Touchon and Vonesh, 2016). In permanent water bodies, although fish represent a biotic threat, prolonged wetlands with more stable abiotic conditions may support more amphibian reproduction (Babbitt *et al.*, 2003; Gleason and Rooney, 2018). Amphibians may face greater environmental stress and extinction risks under extreme conditions such as high elevation, temperature extremes, and oxygen deficits (Alroy, 2015; Leung *et al.*, 2024; Yang *et al.*, 2022). At higher elevations, amphibians are subjected to lower air pressure, more ultraviolet radiation and lower oxygen availability (Elsen and Tingley, 2015; Schmeller *et al.*, 2022). As amphibians move into deeper bodies of water, they experience lower oxygen levels, higher pressure, and less solar radiation (Spence and Tingley, 2020). Thus, for most amphibians that reproduce in water, trade-offs in environmental conditions are critical to the survival, performance, and adaptability of individuals and offspring (MacTavish and Anderson, 2020; Resetarits, 2021). For example, as found in frogs endemic to alpine wetlands, the species lays its eggs in shallow water and disperses eggs, controlling the distance from the water surface at which the eggs are attached (Wang *et al.*, 2017). However, little is known about the influence of water systems (e.g., stream systems) on the survival and reproductive strategies of aquatic stages of amphibians when studying their adaptive strategies in extreme environments (Guirguis *et al.*, 2023; Wake and Vredenburg, 2008).

Alpine streams represent one of the most pristine freshwater ecosystems, and harbor unique species and

genetic diversity due to their remarkable habitat isolation and environmental heterogeneity (Hotaling *et al.*, 2017). There is growing evidence that climate change and anthropogenic activities have led to dramatic changes in freshwater ecosystems and threatened alpine amphibian taxa (Guirguis *et al.*, 2023; Mi *et al.*, 2022; Schmeller *et al.*, 2022). For example, changes in precipitation and temperature have altered habitat microhabitat characteristics, and habitat loss and fragmentation due to anthropogenic activities pose a significant threat to alpine amphibian reproduction (Manenti and Pennati, 2016; Mi *et al.*, 2022; Yang *et al.*, 2022). However, as with many aspects of alpine stream biology, the life histories of alpine amphibians, particularly their reproductive strategies, have not been extensively studied (Hotaling *et al.*, 2017). Reproductive adaptations of populations in the context of environmental change may depend on specific microhabitat preferences and the potential of life history traits to respond rapidly to changes in environmental conditions (Bennett *et al.*, 2015; Laspiur *et al.*, 2021).

The Qinghai-Xizang Plateau (QXP), known as the "Third Pole of the Earth" and the "Water Tower of Asia", is the source of major Asian rivers such as the Yangtze River, Yellow River, Yarlung Tsangpo River, Indus River and Ganges River (Immerzeel *et al.*, 2010; Qiu, 2008). Meanwhile, the QXP is a biodiversity hotspot and the amphibian taxa are endemic and exhibit unique environmental adaptations (Chen and Bi, 2007; Hu *et al.*, 2019). The genus *Scutiger* commonly known as "lazy toads", is notable for its high-elevation distribution, relatively poor dispersal ability, and complex local and regional diversity among the fauna of the QXP (AmphibiaChina, 2024; Fei *et al.*, 2009). This genus likely originated in the "Paleo-Tibet" region, and adapted to the alpine stream environment with the uplift of the QXP and associated drastic changes in the ecological environment (Fei and Ye, 1990; Hofmann *et al.*, 2017). Thus, species of the genus *Scutiger* provide excellent models for studying the extreme environmental adaptations of alpine amphibians.

In this study, we focused on the oviposition site selection strategies of the Chinting alpine toad (*Scutiger chintingensis*), which is endemic to alpine streams of the eastern margin of the QXP. This species is categorised as endangered (EN) by China's Red List of Biodiversity (Jiang *et al.*, 2021) and as vulnerable (VU) by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2024). The known distribution range of this species restricted to mountaintop streams at elevations of 2 500–3 050 m (Fei *et al.*, 2009; Fei *et al.*, 2012). Only four known populations have been recorded (Emeishan City, Hongya County, Wenchuan County and Yingjing County in Sichuan Province, China), and the main populations of the species are on Mount Emei and

Mount Wawu, with the largest occurring on Mount Wawu (Fan *et al.*, 2024; Fei *et al.*, 2009; Fei *et al.*, 2012). The reproductive mode of this species involves oviposition on the bottom of the rocks in alpine streams (Fei *et al.*, 2009; Li *et al.*, 2005; Xie *et al.*, 2000). Because it is difficult to observe the presence of eggs directly in streams, the microhabitat preferences of this species remain ambiguous. Through field surveys, we aimed to address the following questions: (1) whether the microhabitat characteristics of this species differ between oviposition sites and non-oviposition sites, and (2) what are the main microhabitat characteristics that influence the oviposition site selection of this species? By addressing these questions, we will unravel the patterns of this cryptic oviposition strategy, providing new insights into how alpine stream amphibians adapt to challenging environmental conditions. Meanwhile, it provides a practical data base for designing effective protection strategies.

## 2. Materials and Methods

**2.1. Study area** This study was conducted in the alpine region of Wawu Mountain National Forest Park, Sichuan Province, China (Figure 1A). The Wawu Mountain National Forest Park, is located on the eastern margin of the QXP, which is the transition zone from the western Sichuan Basin to the western Sichuan alpine valleys in the QXP. Mount Wawu is characterized by a typical montane climate with an elevation range of 2 300–2 830 m (Jiang *et al.*, 2000; Xie *et al.*, 2000). The annual mean temperature is approximately 16.8 °C. The annual precipitation is 1 385 mm, of which 1 101.8 mm occurs from May to September during the rainy season, accounting for 80% of the annual precipitation (The State-owned Forest Farm of Hongya County, 2022). Notably, Mount Wawu is the largest table mountain in Asia and the second largest table mountain in the world, with a flat summit area that has very little elevation change (The State-owned Forest Farm of Hongya County, 2022). The unique topography and adequate water resources maintain the habitat of *S. chintingensis* on Mount Wawu (Fan *et al.*, 2024). Its primary habitat is the three main streams on Mount Wawu: Yuanxi, Yangxi and Lanxi (Figure 1B). As a famous scenic spot in China, Mount Wawu is facing anthropogenic habitat modification (Jiang *et al.*, 2000). Sewage and waste discharge in streams are the main anthropogenic factors affecting the habitat and the survival of this species (Fan *et al.*, 2024).

**2.2. Oviposition site surveys and assessment of microhabitat** Based on known life history information, adults live overland and populations congregate near alpine streams for breeding from the end of May to the end of June (Fei *et al.*, 2012; Xie *et al.*, 2000). We conducted field surveys

during the breeding season of 2023 (from April to July) on Mount Wawu. We determined the breeding season based on the number of breeding pairs in the streams (Figure 1C). When we found increased numbers of breeding pairs in streams at the end of May, we assumed that oviposition had also occurred and conducted concentrated oviposition site surveys in June. There were four species, one Caudata (*Batrachuperus pinchonii*) and three Anuran species (*Zhangixalus dugritei*, *Oreolalax schmidtii* and *S. chintingensis*), with a sympatric distribution on the summit, but only the *S. chintingensis* exhibits a typical breeding habitat in alpine streams (Fei *et al.*, 2012; Jiang *et al.*, 2000; Xie *et al.*, 2000). We were therefore able to distinguish species by the location of oviposition and the morphological characteristics of the eggs (Fei *et al.*, 2009).

We adopted the transect method to survey the three streams on the Mount Wawu and recorded the locations of each oviposition and non-oviposition site as well as their microhabitat variables. The length and number of transects were determined based on creek habitat conditions (Fan *et al.*, 2024). Because the eggs were attached to the underside of rocks, we were not able to observe them directly. We checked the bottoms of the rocks one by one where the eggs might be attached. Two observers walked along the streams from downstream to upstream during the day on days without rainfall, from 12:00 to 17:00. When attached eggs were found on the underside of the rocks, we measured microhabitat variables that might influence oviposition site selection in a plot (1 m × 1 m) with the eggs in the centre. We quantified microhabitat variables, including vegetation coverage, water surface coverage, distance from bank, water velocity, water depth, water width, pH, conductivity and rock volume (for more details, refer to Table S1). Once an oviposition site plot was identified, we randomly selected a control plot (a non-oviposition site, 1 m × 1 m) adjacent to it, and the same microhabitat variables were measured. At the landscape scale, factors such as elevation, temperature, and precipitation often play an important role in amphibian distribution (Leung *et al.*, 2024; Wang *et al.*, 2022). However, the summit area of Mount Wawu is flat, and the maximum difference in elevation between the sites in our study was approximately 70 m (Table S3). Therefore, at the microhabitat scale, we excluded elevation and temperature from the microhabitat variables.

**2.3. Data analysis** We first identified microhabitat differences between oviposition and non-oviposition sites in stream habitats. Next, we resolved oviposition site microhabitat patterns for this species based on the type of oviposition sites and relevant microhabitat variables. We then evaluated the relative importance and effect of each microhabitat variable.

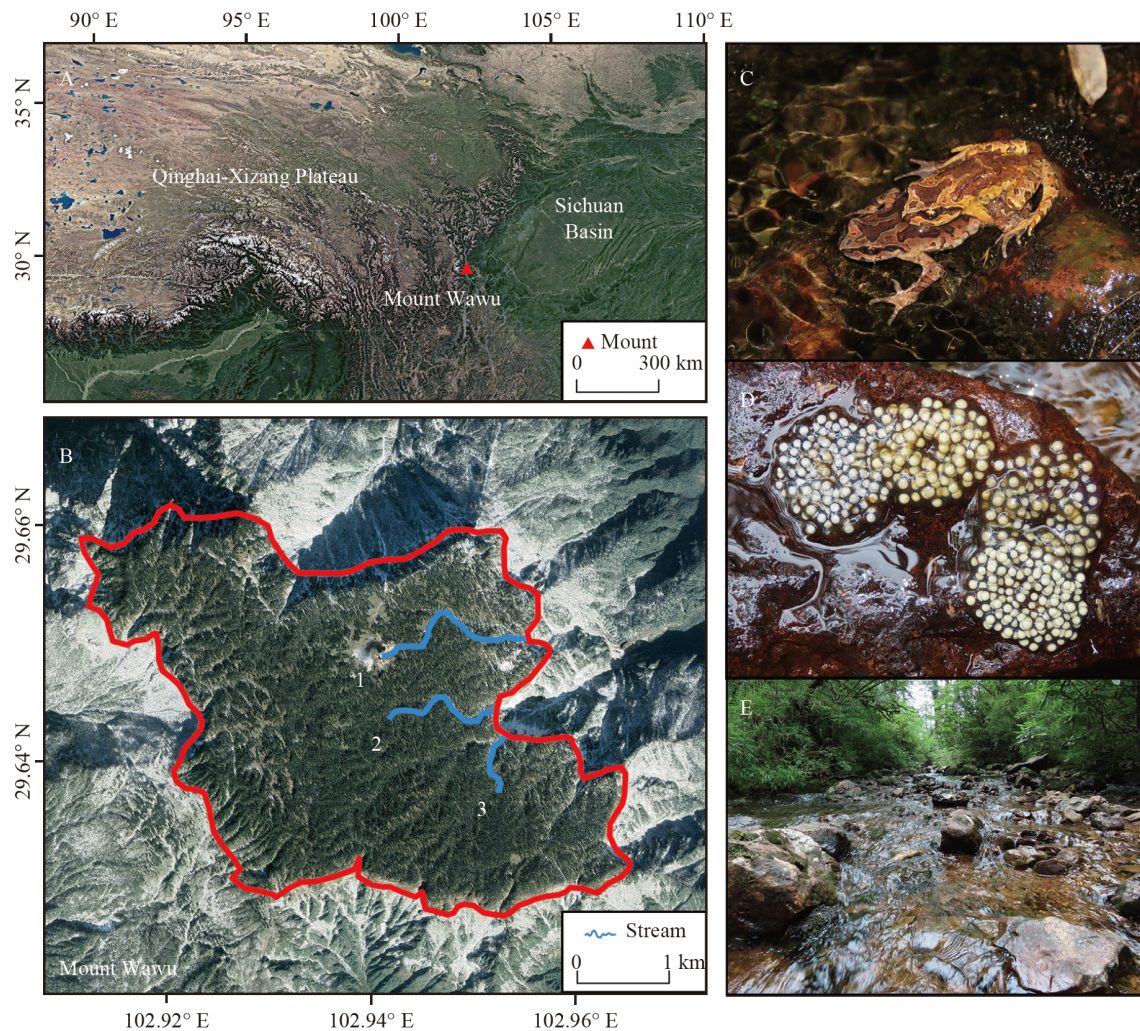


**Figure 1** Study area and study species. A: Geographical location of the study area. B: Three main streams on Mount Wawu (1: Yuanxi; 2: Yangxi; and 3: Lanxi). C: Breeding pair of the *Scutigera chintingsensis*. D: The overlapping oviposition site. E: Typical oviposition site habitat.

We divided the survey sample into three categories based on the number of clutches: non-oviposition site (0, number of clutches = 0), single oviposition site (1, number of clutches = 1) and overlapping oviposition site (2, number of clutches  $\geq 2$ ). The Shapiro-Wilk test revealed that the frequency distributions of the microhabitat variables were mostly skewed (except water width, Table S2). We therefore used Kruskal-Wallis tests, within the “*PMCMRplus*” R package (Pohlert, 2018), to detect microhabitat differences between oviposition sites and non-oviposition sites and calculate pairwise multiple comparisons.

The Spearman test results indicated a high correlation among microhabitat variables (Figure S1); therefore, we used the Random Forest (RF) classification algorithm (Breiman, 2001) to discriminate among the three categories of oviposition sites using the “*randomForest*” R package (Liaw

and Wiener, 2002). RF is a powerful statistical classifier that achieves a dramatic increase in classification and regression accuracy by using an ensemble of “trees”, where each tree in the ensemble grows according to a random parameter (Biau, 2012; Cutler *et al.*, 2007). RF uses the ensemble of all trees to predict new data and estimate the overall out-of-bag (OOB) error rate (Biau, 2012; Breiman, 2001); therefore, it is thought to resist collinearity of covariates, without assuming sample independence and can better model nonlinear relationships (Breiman, 2001; Cutler *et al.*, 2007; Severson *et al.*, 2016). The OOB error rate is affected by two parameters of the random forest model: the number of trees in the forest (*ntree*) and the number of randomly selected variables to use in each split of the tree (*mtry*) (Şen *et al.*, 2017). In this analysis, we used 70% of the data for training and 30% for testing, setting a random seed 333 to make the model results repeatable. After screening

all possible values of *mtry*, we selected *mtry* = 8 for the final RF model construction, in which the OOB error rate was lowest (9.41%, Figure S2A). The default *ntree* = 1 000 was selected for model construction, and the relationship between the RF error and *ntree* showed that the RF error stabilized at approximately 400 trees, indicating that 1 000 trees was an adequate value (Figure S2B). In the final RF model, the lowest OOB error rate was 10.34%.

In the final model, we calculated the precision (the rate of true positives among all predicted positives) and recall (the rate of all true positives predicted to be positive) for each category via the confusion matrix and assessed the overall predictive power of the model based on accuracy (the rate of all correct classifications). We calculated the relative importance of each microhabitat variable using the “*rfPermute*” R package (Archer, 2016) and tested the importance scores based on the principle of the permutation test, which determined the significance of each variable. The importance of a variable was indicated by the mean decrease accuracy (MDA), which was calculated by measuring the degree of decline in the accuracy of the model classification. We also used the “*forestFloor*” R package (Welling *et al.*, 2016) to visualize the effect of variables on model prediction probabilities based on cross-validated feature contributions. All data analyses were performed in R version 4.3.1 (R Core Team, 2023).

### 3. Results

**3.1. Microhabitat characteristics of oviposition sites** From June 9 to July 2, we set up 10 transects with an average transect length of 280 m (for more details, refer to Table S3). We found 42 clutches of *S. chintingensis* eggs; 24 were in single oviposition sites and 18 were in overlapping oviposition sites. We randomly selected 42 non-oviposition sites as controls.

Without distinguishing between the two categories of oviposition sites, oviposition sites exhibited significantly higher water velocity ( $H = 62.42, p < 0.001$ ), lower water depth ( $H = 51.77, p < 0.001$ ), higher water surface coverage ( $H = 39.51, p < 0.001$ ), higher pH ( $H = 20.07, p < 0.001$ ), and higher rock volume ( $H = 12.40, p = 0.002$ ) than non-oviposition sites (Table S2). The results of pairwise multiple comparisons showed that significantly higher water velocity ( $H = 9.22, p < 0.001$ ), lower water depth ( $H = 9.51, p < 0.001$ ), higher water surface coverage ( $H = 6.79, p < 0.001$ ), and higher pH ( $H = 5.40, p < 0.001$ ) at single oviposition sites compared to non-oviposition sites (Figure 2). Meanwhile, overlapping oviposition sites exhibited significantly higher water velocity ( $H = 8.97, p < 0.001$ ), lower water depth ( $H = 6.47, p < 0.001$ ), higher water surface coverage ( $H = 7.64, p < 0.001$ ), higher pH

( $H = 4.91, p < 0.01$ ), and higher rock volume ( $H = 3.65, p < 0.05$ ) than non-oviposition sites (Figure 2). Only overlapping oviposition sites showed significantly higher rock volume than single oviposition sites ( $H = 4.79, p = 0.002$ ) (Figure 2). However, vegetation coverage, distance from bank, water depth, and conductivity did not show significant differences between single and overlapping oviposition sites compared to non-oviposition sites.

**3.2. The relative importance of individual microhabitat variables in oviposition site selection** Predicting the test set using the final model, the results of the output confusion matrix showed that the model was able to correctly classify 12 non-oviposition sites, three single oviposition sites and four overlapping oviposition sites while misclassifying one non-oviposition site to single oviposition site, one single oviposition site to overlapping oviposition site and five overlapping oviposition sites to single oviposition sites (Figure 3A). For the non-oviposition site, both model precision (92.3%) and recall (100%) were high. For single oviposition site, model precision (75%) was better than recall (33.3%). And for overlapping oviposition site, model recall precision (80%) was better than precision (44.4%). The overall model prediction accuracy was 73.1% (Figure 3A).

Water velocity was the most important variable (MDA = 88.12%) explaining oviposition sites followed by rock volume (MDA = 34.26%), water surface coverage (MDA = 10.81%) and water depth (MDA = 9.76%); the permutation tests also yielded significant importance scores (Figure 3B). Vegetation coverage, pH, water width, conductivity and distance from bank were the lowest ranked variables (Figure 3B). The results of cross-validation revealed the feature contribution of each feature for each category. The feature contribution can be interpreted as the change in the predicted class probability attributed to a given feature (Welling *et al.*, 2016). Water velocity was the most important feature, strongly predicting (probability change up to  $\pm 40\%$ ) that oviposition sites had a velocity above approximately 0.2 m/s and non-oviposition sites had a velocity below that value (Figure 3C). However, single oviposition sites and overlapping oviposition sites were not distinguishable based on water velocity feature. Notably, rock volume as the second most important variable, distinguished single oviposition sites and overlapping oviposition sites, where the lowest level predicted up to  $\pm 20\%$  probability change. As rock volume increased, there was a tendency for single oviposition sites to transform into overlapping oviposition sites. The magnitude of the effect of water surface coverage and water depth on the model was approximately  $\pm 10\%$  probability change. More than 60% water surface coverage and water depths below 10 cm tended

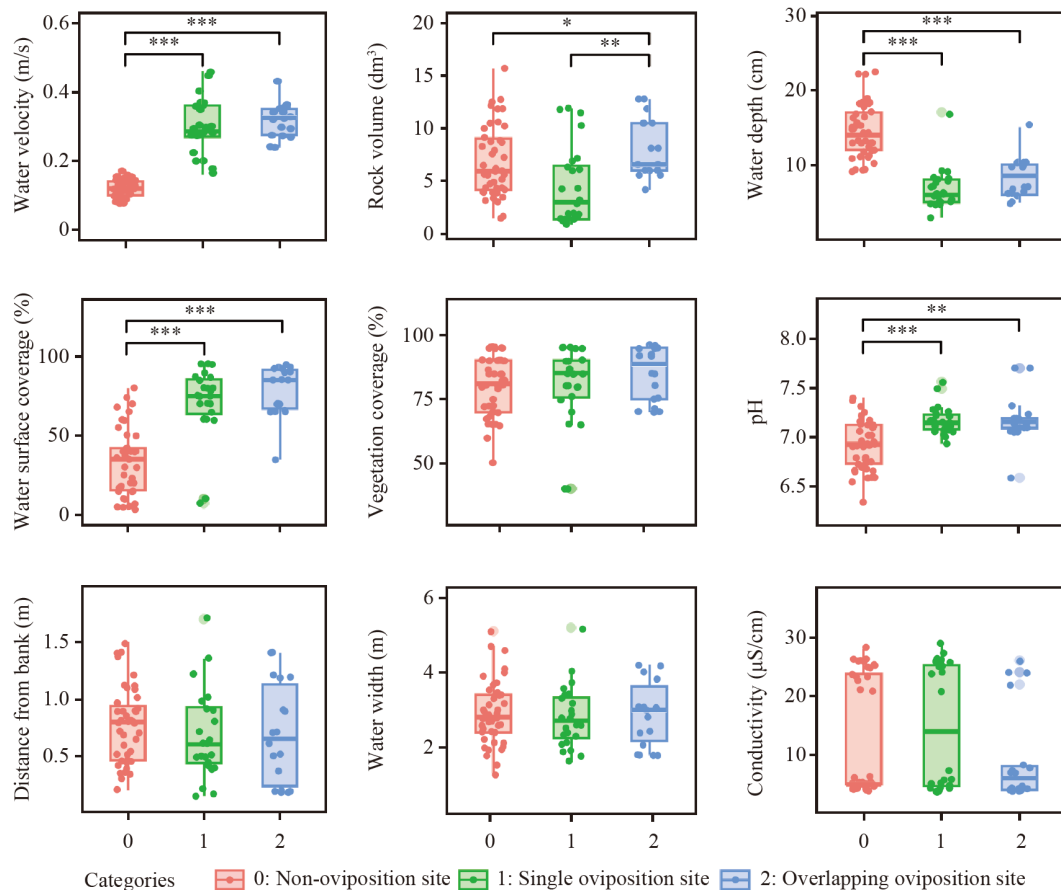


**Figure 2** Microhabitat differences among different oviposition sites of *S. chintingensis* (0: Non-oviposition site; 1: Single oviposition site; and 2: Overlapping oviposition site). The levels of significance are as follows: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

to be oviposition sites. However, neither variable could distinguish between single oviposition sites and overlapping oviposition sites (Figure 3C).

#### 4. Discussion

Understanding the adaptive strategies of species to their habitats is important for guiding species conservation in the context of increased anthropogenic activities (Leung *et al.*, 2021; MacTavish and Anderson, 2020; Mi *et al.*, 2022). In this study, we found that water velocity was significantly greater at oviposition sites than at non-oviposition sites for *S. chintingensis*, which is endemic to alpine streams. The microhabitat characteristics of *S. chintingensis* oviposition sites included high water velocity, shallow water, and high-water surface coverage. Water velocity and rock volume were the most important variables explaining oviposition site categories. Water velocity at oviposition sites was almost 0.2 m/s or more, while at non-oviposition sites was below 0.2 m/s. Additionally, we found that for this species changing

behavior to habitat preferences and nearly half of the clutches had overlapping oviposition.

Alpine streams are often isolated and represent some of the most primitive freshwater ecosystems in the world, with a high degree of environmental heterogeneity at very small spatial scales (Hotelling *et al.*, 2017). In this study, we found that the significant differences in microhabitat characteristics between oviposition and non-oviposition sites in terms of water velocity, water depth, water surface coverage, pH and rock volume, which ultimately influenced the model predictions of the oviposition sites (Figure 2). In particular, with respect to water velocity, the eggs were almost found on the bottom of rocks that were located in the rapid currents produced by small drop-offs in the streams (Figure 1E), whereas we never found eggs in deeper and gently flowing water. Due to the adhesive clutches, this contrasts with the strategy of other amphibians without adhesive clutches, which select more stable oviposition sites to reduce egg displacement during high-flow events (Guy *et al.*, 2004; Semeniuk *et al.*, 2007). However, the location of *S. chintingensis* eggs

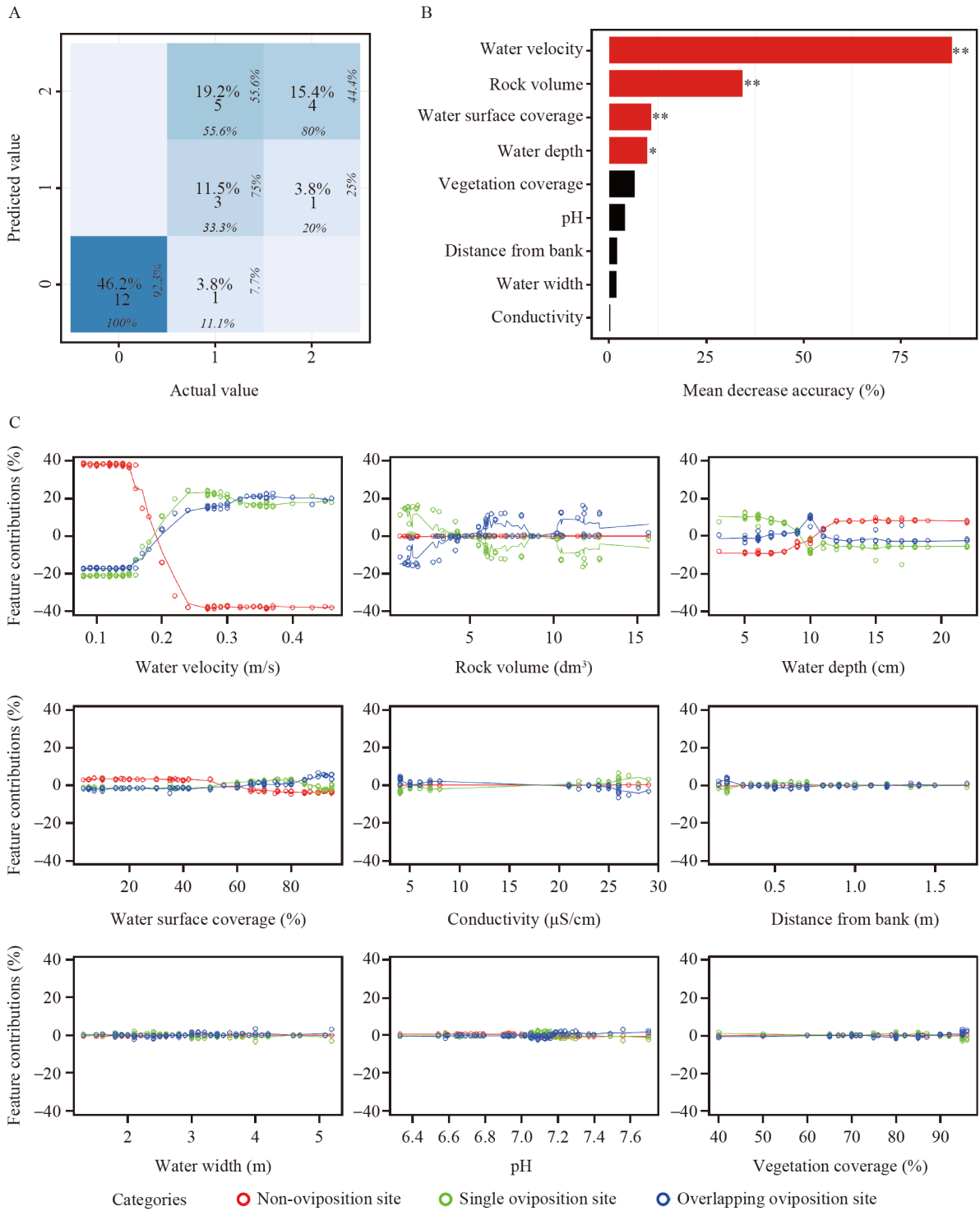


**Figure 3** Predictive power of the oviposition site selection model and relative importance of variables. A: The confusion matrix; B: The relative importance and the significance for each microhabitat variable; and C: Feature contributions of each variable for each category. In Panel A, the number and proportion in the center of each box represent the number and percentage of results, respectively, for that classification. In Panel B, levels of significance are as follows: \* $p < 0.05$  and \*\* $p < 0.01$ . In Panel C, the actual feature value for each variable is depicted on the x-axis, and the y-axis describes the feature contributions for each value for each class. The feature contribution can be interpreted as the change in the predicted class probability attributed to a given feature.

coincides with that of certain stream-dwelling fish. Shallow water, high dissolved oxygen, and high-water velocity increase mixing during oviposition, prevent siltation or suffocation of eggs, and facilitate the transport of hatchlings to suitable developmental environments (Bilkovic *et al.*, 2002; Hightower *et al.*, 2012). Additionally, rock volume was the second most important variable for oviposition site selection (Figure 3B). Previous research has indicated that attributes of the substrate, such as suitable vegetation and particle size, provide anchor sites for the eggs, thus preventing them from being scoured or washed away by water currents or waves (Refsnider and Janzen, 2010; Shirotori *et al.*, 2006). In contrast, for amphibians in ephemeral streams, the quantity of rocks does not affect their oviposition preferences and drought remains the most important habitat factor (Oh *et al.*, 2024). Oviposition site preferences like *S. chintingensis* have been reported in studies of lake sturgeon (*Acipenser fulvescens*), where the composition of the oviposition substrate and the presence of turbulence favorably reduce the probability of predation and ensure the exchange of oxygen with eggs, which are important for the reproductive success of the species (Chiotti *et al.*, 2008; Fischer *et al.*, 2018). Thus, our results suggest that *S. chintingensis* has oviposition site preferences like those of stream-dwelling fish, which indicates an adaptive strategy for this species to maintain the oxygen requirements of eggs in alpine stream habitats. However, our study species is distributed only in alpine streams, and whether this adaptive strategy is variable along elevational gradients needs to be tested in stream amphibians with a broader elevational range.

For the categories of oviposition sites, approximately 43% of the eggs overlapped, which is much greater than the percentage reported by Xie *et al.* (2000). These findings suggest that the overlapping oviposition behavior may be underestimated. Indeed, at least five members of the genus *Scutiger* have been recorded as exhibiting overlapping oviposition behavior (Fei *et al.*, 2012), but the prevalence of this behavior in amphibians has not been comprehensively reviewed to date. In our study, cross-validation of feature contributions revealed a gradual shift in model predictions from single oviposition sites to overlapping oviposition sites as rock volume increased (Figure 3C). This may occur because only a larger volume of rock can provide a larger attachment area to support more eggs as well as a more stable anchor point during high-flow events. Whatever, the results of the present study suggest that the mechanisms driving the evolution and maintenance of overlapping oviposition behaviors may differ across taxa. In terms of reproductive patterns, a study of *Leptobranchium boringii* revealed that the species was both polygynous and polyandrous, with males creating nests and providing parental care to protect multiple

clutches (Zheng *et al.*, 2010). In addition, Wang *et al.* (2014) reported on the group-spawning behavior and simultaneous polyandry of the *Feirana kangxianensis*. Thus, the evolution of overlapping oviposition behaviors may be related to nonmonogamous mating systems and associated reproductive behaviors. In terms of habitat factors, Rudolf and Rodel (2005) reported that whether a site was used for oviposition was determined by abiotic factors, whereas repeated use of oviposition sites was determined by responses to conspecifics. Conspecific offspring may serve as cues to habitat quality during oviposition site selection, suggesting a lower risk of predation and thus leading to overlapping oviposition (Doligez *et al.*, 2002; Doody *et al.*, 2009; Rudolf and Rodel, 2005). In our study, we acknowledged that some control sites may be used in the future but assumed that during the same survey period, the oviposition sites meant that they were better suited than the control sites (Grossman and Ratajczak, 2006; Lantyer-Silva *et al.*, 2018). Owing to the nocturnal nature of breeding activity in this species, our daytime oviposition site surveys failed to document the abundance of adult frogs. The abundance of adult frogs may influence the frequency of overlapping oviposition sites. Additionally, we did not include a predation factor because there were no predatory fish in the habitat, and there was insufficient evidence to support the suggestion that *Batrachuperus pinchonii* prey on the clutches. The patterns, causes, and ecological and evolutionary consequences of overlapping oviposition remain unresolved, and it is necessary to determine in the future whether this behavior provides specific adaptive benefits to offspring.

Alpine biota is particularly vulnerable to rapid environmental warming and anthropogenic activities (Hotaling *et al.*, 2017; Rubidge *et al.*, 2012). In terms of the properties of the species itself, high ecological specialization creates difficulties for species in adapting to rapidly changing environments and imposes a relatively high risk of extinction (Clavel *et al.*, 2010; Williams *et al.*, 2009). At high elevations, amphibians must weigh the environmental stresses of severe cold, low oxygen and more ultraviolet radiation caused by rising altitudes (Hotaling *et al.*, 2017; Hu *et al.*, 2019; Zhou and Zhang, 2005). In this study, we emphasized a unique adaptive strategy by which *S. chintingensis* resists extreme environments by investigating oviposition site selection in alpine streams. As a taxon of amphibians adapted to high elevation, *S. chintingensis* oviposits in fast, shallow streams to ensure that the eggs have abundant oxygen in high-elevation, hypoxic habitats. Therefore, microhabitat characteristics in streams, such as streamflow monitoring, substrate composition, and vegetative cover, are critical to protecting the breeding habitat of a species and ensuring population survival. Additionally, we

assessed the high proportion of overlapping oviposition behavior in this species. However, our study was unable to distinguish the benefits of overlapping oviposition behavior in terms of microhabitat characteristics, and the evolutionary history of this behavior in the adaptive evolution of species in the genus *Scutigera* and the adaptive significance of this behavior for offspring need to be further investigated. Our studies of oviposition site preferences for this amphibian can be generalized to the ecological needs of other taxa in alpine streams and help to understand the adaptive strategies of other endemic taxa in alpine streams, thereby providing guidance for more effective conservation strategies for alpine stream biota under climate change and anthropogenic activities.

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**Supplementary information** Supplementary information is available at <https://doi.org/10.3724/ahr.2095-0357.2024.0031>

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