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The effect of caste, mass and colony identity on rescue priorities in the red imported fire ant

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Abstract

1. Rescue behavior is observed across the animal kingdom. For example, many ant species engage in rescue activities.
2. This study used experimental approaches to understand how rescuers prioritized rescue targets in the fire ant *Solenopsis invicta*. This research also investigated the cues that *S. invicta* used to locate rescue targets.
3. We found that rescue teams likely used chemical signals to locate larval rescue targets.
4. Reproductive larvae were preferentially rescued over worker larvae in simple choice trials; however, caches of multiple worker larvae were given equal priority during rescue compared to single reproductive larvae.
5. Non-nestmate reproductive larvae were rescued at the same frequency as nestmate reproductive larvae.
6. Our results provide information on how ants locate distressed conspecifics, supporting the use of chemical signalling. Our study also offers insight into rescue priority, belying expectations of kin and reproductive prioritization by demonstrating that mass can override caste preferences.
7. Overall, this study provides evidence that olfactory cues contribute to the coordination of rescue behaviour and suggests that large groups of workers may actually hold equal value to a eusocial insect colony as a single reproductive.

KEYWORDS
alarm signal, collective behaviour, eusocial, prioritization, red imported fire ant, rescue

INTRODUCTION

Rescue behaviour occurs when one individual puts themselves at risk to aid another individual in distress without receiving any inherent benefit (Nowbahari & Hollis, 2010). Many mammals (Kamdar et al., 2025; Masilkova et al., 2021; Rood, 1983; Teixeira et al., 2016; Vogel & Fuentes-Jiménez, 2006), birds (Hammers & Brouwer, 2017) and invertebrates (Frank & Linsenmair, 2017; Kwapich & Hölldobler, 2019; Nowbahari et al., 2009; Wojciech et al., 2002) engage in rescue behaviour. For example, male elephants will attempt to defend sedated females from humans (Kamdar et al., 2025), wild boar will free one another from traps (Masilkova et al., 2021) and birds will risk entanglement in sticky "bird-catcher" seed pods to free conspecifics (Hammers & Brouwer, 2017).

Rescue behaviour is particularly common in ants (Miler & Turza, 2021). Ants are eusocial insects that live in colonies that may

contain dozens to millions of nestmates (Hölldobler & Wilson, 1990). Ants cooperate in almost all tasks and are particularly successful because of their group activities (Gordon, 2019). Ants, like all eusocial insects, are characterized by a reproductive division of labor (Hölldobler & Wilson, 1990; Wilson, 1953). Only a few individuals, the queens and males, are capable of reproduction, while the majority of individuals, the workers and soldiers, are largely sterile but perform colony tasks such as foraging, nest construction, defense and nursing (Hölldobler & Wilson, 1990). Ants are also holometabolous. That is, individuals begin development as more or less helpless larvae, then undergo metamorphosis, and eclose as adults to take on their roles within the colony.

Rescue activities are expected in ants because many helping behaviours in insect societies have evolved through kin selection (Bourke & Franks, 1995; Hamilton, 1963). Moreover, ants often live in conditions where rescue may be needed, such as in underground nests subject to collapse (Hollis & Nowbahari, 2013b; Miler, Yahya, & Czarnoleski, 2017). Additionally, larval ants need continued monitoring and protection, and brood is often retrieved when relocated outside the nest (Lenoir, 1981; Szczuka et al., 2014). Thus, rescue behaviour is expected because ants are eusocial, and both larvae and adults may frequently experience peril.

Previous foundational studies of rescue have found that ants will rescue nestmates from spiderwebs (Kwapich & Hölldobler, 2019; Silva-Júnior et al., 2026; Uy et al., 2019), artificial snares (Nowbahari et al., 2009; Taylor et al., 2013), or violent encounters with dangerous prey (Frank & Linsenmair, 2017). In some cases, ants have even been shown to rescue mutualists (Schifani et al., 2023). Rescue in ants is often a collective behavior (Caine, Bellisimo, et al., 2026; Kwapich & Hölldobler, 2019), with multiple workers acting together to free trapped individuals. Moreover, rescue can be directed towards adults (Nowbahari et al., 2022), newly eclosed callows (Nowbahari et al., 2016), or brood (Bar et al., 2023; Caine, Nejad, et al., 2026).

The first goal of this study was to provide insight into how rescue targets are identified. Imperilled individuals typically must alert rescuers to their distress with some sort of signal (Blum, 1969; Hollén & Radford, 2009). Animals can make various alarm calls; vertebrates, including capuchin monkeys threatened by conspecifics (Vogel & Fuentes-Jiménez, 2006) and warbler birds caught in sticky "bird-catcher" seeds (Hammers & Brouwer, 2017), make auditory alarm calls. Ants may also employ auditory signals such as stridulation (Dai et al., 2025; Rauth & Vinson, 2006; Roces et al., 1993); however, other investigations suggest they might rely more on visual (Lagos-Oviedo et al., 2025) or chemical (Frank & Linsenmair, 2017) alarm signals. Thus, the cues used in rescue activities in ants remain unclear. In this study, we investigated cues that rescue teams may use to find rescue targets.

Our second and main goal was to untangle rescue priority. Rescue is a complex process and likely to be one of several tasks that a colony needs to undertake. Moreover, in a large eusocial insect colony, multiple individuals may become trapped at once, all simultaneously requiring rescue. Thus, ant colonies may need to make group decisions about task allocation and how to coordinate rescue behaviours. It remains unclear how ants decide on rescue targets and rescue priorities. Different types of individuals within the colony may hold different values to a colony; for example, ants with shortened life expectancies receive fewer rescue attempts (Turza & Miler, 2023a). Reproductive individuals are typically larger than sterile workers (Tschinkel, 2013) and thus represent a larger prior resource investment. Moreover, the reproductive function of a queen or male may be more valued than the functions of a sterile worker. As such, reproductive individuals represent a different kind of resource than sterile workers and could be valued differently in rescue scenarios. In scenarios with multiple targets requiring simultaneous rescue, rescuers may thus decide on a prioritization such that one individual is rescued before the other. In collective rescues, these decisions may even be complicated by the fact that different individual rescuers within a colony vary in their rescue propensity and specific behaviours, based on factors including the individual rescuer's size (Turza & Miler, 2023b), life expectancy (Miler, Symonowicz, & Godzińska, 2017; Turza & Miler, 2023a), age, behavioural caste (Nowbahari et al., 2012), or genotype (Caine, Bellisimo, et al., 2026; Nowbahari et al., 2022).

In this study, we explored the effect of three variables on target prioritization decisions. We specifically considered if the rescue target caste, mass, or colony-of-origin affected rescue behaviors. We were interested in determining which rescue targets are chosen for rescue and whether there was any consistent underlying system to these decisions. We found that rescue teams do make decisions about rescue priority based on the rescue targets. These group decisions enhance individual and colony survival and demonstrate collective choices in insect societies.

METHODS

Rescue experimental system

This study investigated alarm signals and rescue prioritization in the red imported fire ant, *Solenopsis invicta*. *S. invicta* forms large colonies consisting of hundreds of thousands of individuals (Tschinkel, 2013). Invasive *S. invicta* in the United States sometimes forms polygyne (multiple-queen) colonies (Porter, 1992; Porter et al., 1991). These polygyne colonies can contain many matrilines, and relatedness among nestmate workers may approach zero in some populations (DeHeer & Ross, 1997; Goodisman et al., 2007; Ross et al., 1996). *S. invicta* nests in a variety of soil types (Tschinkel, 2013). Such subterranean nests may be subject to frequent environmental insults leading to situations where adults and brood are trapped. These situations could require rescue by nestmates.

S. invicta colonies were collected from natural, polygyne populations in and around Atlanta, Georgia in the fall of 2021 and 2022, the spring of 2022 and 2024 and the summer of 2024. Collected colonies were confirmed as *S. invicta* according to Pitts et al. (2018) and were identified as polygyne by the presence of more than one dealate queen. For the purposes of this study, nests were considered as being from distinct colonies if they were separated by greater than

100 metres. Individuals from distinct nests were housed separately in lab and treated as distinct entities in experimental manipulation and analysis. Colonies were housed at ambient temperature in plastic bins coated with fluon and baby powder and provided ad libitum food and water (Ross, 1988).

Rescue trials were staged with colony fragments consisting of ~2500 adult workers (determined by mass) in 32 cm × 18 cm plastic bins. The mass of 2500 adult workers was calculated on a colony-by-colony basis by averaging three weights of 100 manually counted ants. Colony fragments did not include any queens or brood. The sides of bins were coated with baby powder to prevent ants from escaping. Each bin also contained a tube of water, a dish of food and a single nest box consisting of a petri dish with a plaster base (Figure 1). Colony fragments were allowed to acclimate to containers for 24 h prior to the initiation of rescue trials. Each colony fragment was considered a single experimental unit and each fragment was used for only a single trial.

The colony fragment was provided with a weigh boat containing 40 g of Potter's glass bead substrate (d = 0.24 ± 0.03 mm) (Potters Industries LLC, Malvern, PA, U.S.) moistened with 4 mL water, in which one or multiple rescue targets were buried. The location of the rescue target in the weigh boat was denoted with Sharpie tick marks on the edges of the weigh boat in order to aid in tracking of the subsequent rescue. A small ramp was placed against the weigh boat, which provided ants access into the rescue arena (Figure 1a–c).

A camera (Logitech International S.A., Lausanne, Switzerland) was used to record the rescue trials at 30 fps; rescue trials lasted up to 24 h. Videos were observed to determine the time at which various key events occurred. In particular, the observer recorded whether the rescue target was successfully rescued, as well as the time at which (a) the target was first located and (b) the target was carried out of the weigh boat to be returned to the nest. Targets were considered located if rescuers dug at the direct intersection of the sharpie tick marks. Targets were considered successfully rescued if they were carried out of the weigh boat; rescues were not considered successful if the target was cannibalized in the dish but remnants were subsequently carried out.

We note that the methods employed in this study differ from previous laboratory investigations of ant rescue behaviour. For one, trapped targets were not artificially ensnared in our study (Duhoo et al., 2017; Hollis & Nowbahari, 2013b; Miler, Yahya, & Czarnoleski, 2017; Nowbahari et al., 2009, 2012, 2016; Taylor et al., 2013), but rather buried. Additionally, this study enabled multiple rescuers to participate in rescue attempts; both of these methods would mimic natural rescue in *S. invicta*, but have the limitation of potentially introducing noise into the experimental system. Finally, this study allowed rescuers significantly more time to locate and rescue targets as compared to previous investigations (Nowbahari et al., 2012); this was necessary and allowed rescuers time to locate targets and had the advantage of enabling comparisons of latency to rescue in addition to just success or failure.

No-choice rescue trials

No-choice rescue assays were conducted to investigate if *S. invicta* workers responded differently to single rescue targets. We were

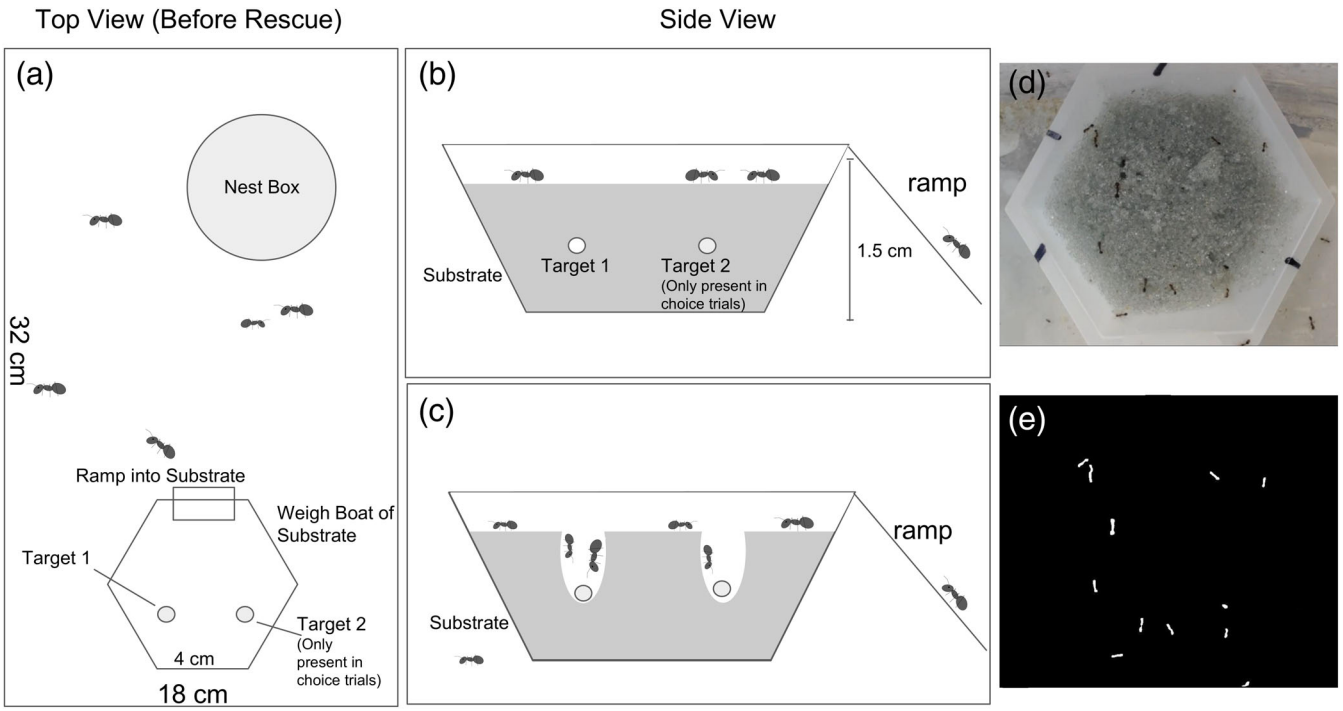

FIGURE 1 Rescue arena: (a) Top view of a rescue trial before ants have begun rescue. (b) Side view of a trial before ants have begun rescue illustrating buried rescue targets. (c) Side view of a trial during rescue showing rescue team extracting larvae. (d) Top view of example still image from rescue video. (e) Example still image of same frame as panel D from binarized rescue video.

interested in how often different types of targets were rescued and how quickly rescue occurred. In no-choice trials, a single rescue target was buried 0.75 cm deep into the substrate (Figure 1a–c). Rescue targets included (a) a plastic dummy roughly the size of a reproductive larva (hereafter control dummy) (Video S1), (b) a plastic dummy rubbed for 30 s on either side with a reproductive larva (hereafter experimental dummy) (Video S2), (c) a live worker larva (Video S3), (d) a dead worker larva killed by freezing at −20°C for 24 h (Video S4), (e) a live reproductive larva (Video S5), or (f) a dead reproductive larva killed by freezing at −20°C for 24 h (Video S6) (Table 1).

Statistical analyses for no-choice trial outcomes

For all single target trials, the likelihood of target rescue and location over time was modelled in R with Cox Frailty models using the survival, coxme and survminer packages (Kassambara et al., 2026; Therneau, 2024, 2026). Initial analyses revealed that data structure in rescue success frequency prevented reliable estimates for hazard ratios. Therefore, the Cox frailty model for rescue successes was repeated without the control dummy data, for which no rescues were observed.

Because failed rescue trials may be informative, we complemented these Cox Frailty models with Generalized Linear Mixed Models (GLMMs) and Linear Mixed Models (LMMs) in order to more fully explore the data, as these latter analyses would not treat failed trials as randomly censored. Specifically, we modelled target location and rescue success using a GLMM with binomial error distribution and a logit link function using the packages lme4 and lmerTest (Bates et al., 2015; Kuznetsova et al., 2017). Because frozen reproductive larvae were located in all cases and this complete separation prevented the estimation of accurate pairwise comparisons, target location rate was subsequently modelled without the inclusion of frozen reproductive larval data. Similarly, rescue success was also subsequently modelled without the inclusion of control dummies, which were never rescued. Log-transformed time until target location and log-transformed time until target rescue were also separately modelled with a LMM. Date and Colony ID were initially included as random effects in all models; however, due to contributing zero variance, Date and/or Colony ID were subsequently dropped from several models (Table S1).

The overall effect of target type on survival, as well as on location and rescue likelihood and time, was determined with a log likelihood test comparing each full model to a null model containing only the random effects. Following significant omnibus tests, pairwise contrasts between all target types were conducted using the emmeans package with Holm's correction for multiple comparisons (Lenth & Piaskowski, 2026). These analyses were repeated for the subset of trials with live reproductive and live worker larvae to assess the effect of caste. Formulas and Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) statistics for all models can be found in Table S1, along with comparisons to null models. Pairwise comparisons, fixed effects and random effects can be found in Tables S2–S4.

Choice rescue trials

Choice trials were conducted to investigate whether *S. invicta* workers would prioritize rescue of certain individuals if two different rescue targets were provided at the same time. Colony fragments were established as in the no-choice experiments. However, in choice trials, the weigh boat contained two potential rescue targets, buried in separate sectors of the hexagonal weigh boat. The two burial locations were denoted with Sharpie tick marks in two unique colors.

Target pairings included (a) one reproductive larva versus one worker larva (48 trials); (b) one reproductive larva versus a cache of multiple worker larvae, the total mass of which matched the mass of the single reproductive larva (47 trials); (c) one nestmate reproductive larva versus one non-nestmate reproductive larva (24 trials) (Table 1); (d) one big worker larva versus one small worker larva. One trial between a single reproductive larva and cache of worker larvae had to be excluded from analysis due to poor video quality. Excepting the 24 trials comparing nestmate to non-nestmate larvae, all choice trials were performed using nestmate larvae. Targets were buried in either the left or right sectors of the dish at random (Table 1). We recorded whether one, both, or neither of the rescue targets in choice trials were successfully retrieved. We also recorded time to rescue for each target.

Statistical analyses for choice trial outcomes

We investigated the effects of caste and mass on target location order by comparing choice rescue trials between a single reproductive larva and a single worker larva, between a single reproductive larva and a mass-matched cache of multiple worker larvae and between two single worker larvae of varying sizes. Resulting data were fit to a GLMM with binomial error distribution and a logit link function with a response variable of "LocatedFirst", which described which one of the two rescue targets was located first. The difference between target masses and castes was included as fixed effects in the model. For seven choice trials between a single reproductive larva and a single worker larva, mass was not recorded; for the purpose of these analyses, mass difference was estimated to be the average mass difference between a reproductive and worker larva across the other choice trials (12.89 mg). Analyses were also performed with these trials removed entirely; this did not change any statistical conclusions. The interaction between terms was initially included in models but was removed for final models if not significant. The same approach was used to determine the effects of caste and mass on target rescue success order.

A LMM was fit to the difference between the time target one was located and the time target two was located in order to determine the effects of caste and mass on target location time. The same approach was used to determine the effects of caste and mass on the difference between the time target one was rescued and the time target two was rescued.

TABLE 1 Description of all choice (C) and no-choice (NC) trials used in this study, including target abbreviations which appear in figures and target characteristics including caste, status and number.

		Target 1					Target 2					*n*	*N*	Question			
Rescue target	Trial type	Abbr.	Caste	Status	No.	Colony	Abbr.	Caste	Status	No.	Colony			Rescue cues	Rescue priority and caste	Rescue of non-nestmates	Rescue priority and mass
Control plastic dummy	NC	D_C		Plastic	1							4	10	X			
Experimental plastic dummy	NC	D_E	R	Plastic	1	NM						4	24	X			
Frozen reproductive Larva	NC	R_F	R	Frozen	1	NM						5	9	X			
Frozen worker larva	NC	W_F	W	Frozen	1	NM						5	11	X			
Reproductive larva versus 1 worker larva	C	R_L	R	Live	1	NM	W_L	W	Live	1	NM	10	48		X		
Worker larva	NC	W_L	W	Live	1	NM						8	43	X	X		
Reproductive larva	NC	R_L	R	Live	1	NM						12	56	X	X	X	
Non-nestmate reproductive larva	NC	$R_{L,DN}$	R	Live	1	Non NM						5	20			X	
Nestmate versus non-nestmate reproductive larva	C	$R_{L,SN}$	R	Live	1	NM	$R_{L,DN}$	R	Live	1	NM	4	24			X	
Reproductive larva versus multiple worker larva	C	R_L	R	Live	1	NM	$W_{L,M}$	W	Live	Many	NM	8	47				X
Big versus small worker larvae	C	$W_{L,B}$	W	Live	1	NM	$W_{L,S}$	W	Live	1	NM	7	35				X

Note: Castes include reproductive (R) and worker (W) larvae. Individuals were either nestmates (NM) or non-nestmates (Non NM). The number of colonies tested (n) and the total number of trials (N) are also provided. The final 4 columns highlight which trials were used to answer particular study questions.

We next investigated whether polygyne *S. invicta* workers would prioritize rescue of their own nestmates over non-nestmates using choice trials between a nestmate and non-nestmate reproductive larva. The preference to locate a nestmate larva before a non-nestmate larva was fit using a GLMM with abinomial error distribution and a logit link function. Similarly, the preference to actually rescue a nestmate before a non-nestmate was modelled using a GLMM. The time difference between locating the nestmate and non-nestmate larva was modelled with a LMM. The time difference between actually rescuing the nestmate and non-nestmate was modelled in a similar fashion. For all models, date, target colony identity, rescuer colony identity and the interaction between target colony identity and rescuer colony identity were initially included as random effects. However, in some models, these random effects contributed zero variance and were subsequently dropped. The mass difference between targets was included as a fixed effect. The intercept was used to determine whether rescuers preferred one target to the other for reasons other than mass and random effects.

The overall significance of effects was determined with a Type III Wald Chi-Square test using the Anova function in the car package (Fox & Weisberg, 2019). Formulas and AIC and BIC statistics for all models can be found in Table S1, along with comparisons to null models. Pairwise comparisons, fixed effects and random effects can be found in Tables S2–S4.

Video analysis

Videos of rescues were analysed to determine the maximum number of rescuing ants within the weigh boat at one time. Only successful rescues were included in this analysis and the quality of several videos precluded inclusion in this analysis. Ultimately, 28 trials with a single reproductive larva and 15 with a single worker larva were included in this analysis. Videos were downsampled to 1 fps and analysed in MATLAB. Videos were first binarized using a colour thresholder which could distinguish the dark ants from the white substrate (Figure 1e). The colour thresholder occasionally also detected small holes and shadows; therefore, a size and eccentricity filter was used to eliminate shapes too small or perfectly circular to be ants. Logical masks were also applied when needed in order to disregard conspicuous dark holes visible at the end of the video, typically including the hole dug directly at the rescue site.

Binarized videos were used to calculate the number of ants in the dish. The total number of ant-pixels was summed and divided by the average number of pixels that made up a single ant to calculate the approximate number of ants in the dish. This conversion was manually checked using three randomly selected frames per video. Maximum ant counts per video were compared between no-choice trials with live worker larvae and no-choice trials with live reproductive larvae using a Mann–Whitney test in Graphpad Prism.

RESULTS

What cues does *S. invicta* use to locate rescue targets?

We conducted no-choice experimental trials to discern what signals or cues *S. invicta* rescue teams used to find larval rescue targets. Trials included live and frozen larvae, experimental plastic dummies that came into physical contact with live reproductive larvae and thus hypothetically carried chemical cues of larvae, and control plastic dummies that never came into contact with any larvae.

Cues sufficient for location of rescue targets

We first investigated if different rescue targets were located with different probabilities. We found that live and frozen larval targets and experimental dummies were frequently located by rescue teams. In contrast, control dummies were located only once in 10 trials (Table 2, Figure 2). A Cox frailty model suggested that target types significantly varied in their likelihood of being located over time ($\chi^2 = 41.7$, df = 6, $p < 0.0001$) (Table S1). A GLMM confirmed that target type significantly affected location likelihood ($\chi^2 = 38.3$, df = 5, $p < 0.001$) (Table S1), with live reproductive larvae being located significantly more often than control dummies ($p = 0.026$), but not experimental dummies ($p = 1$) (Table S2). We also found that experimental dummies were located significantly more often than control dummies ($p = 0.035$) (Table S2). However, live worker larvae were not located more frequently than frozen worker larvae ($p = 1$) (Table S2). A LMM further confirmed that target type also had a significant effect on time until target location ($\chi^2 = 15.0$, df = 6, $p = 0.02$) (Table S1). Specifically, time until location was significantly shorter for experimental dummies than live reproductive larvae (ratio = 2.83 ± 0.743, df = 12.2, $p = 0.039$) (Table S2).

In summary, target type had a significant effect on both location likelihood and latency. Location likelihood and speed to location did not differ significantly between live and dead rescue targets. However, experimental dummies that had come into contact with a reproductive larva were located more often than control dummies that did not.

Cues sufficient for full rescue response

We next investigated if different rescue targets had different likelihoods of being rescued. A Cox frailty model describing time until rescue for all no-choice trials suggested that target types significantly varied in their time until rescue ($\chi^2 = 32.8$, df = 6, $p < 0.001$) (Table S1); this result was confirmed when the model was rerun without control dummies ($\chi^2 = 21.8$, df = 5, $p < 0.001$). A GLMM confirmed that rescue success likelihood was significantly affected by target type ($\chi^2 = 38.8$, df = 6, $p < 0.001$); this was further supported when control dummies were removed from analysis ($\chi^2 = 28.1$, df = 5, $p < 0.001$) (Table S1). Specifically, the rescue likelihood of

TABLE 2 Results of no-choice rescue trials, including number of successful rescues (Rescue), number of targets being located but not rescued (Locate) and number of targets that rescuers never located (Fail), followed by mean elapsed time until target location and successful rescue in minutes (m) with standard error.

Target	*N*	Rescue	Locate	Fail	Time to locate (m)	Time to rescue (m)
Reproductive Larva	56	36	16	4	190.3 ± 18.5	526.6 ± 45.2
Worker Larva	43	15	16	12	238.3 ± 45.5	285.3 ± 84.1
Reproductive Larva (non-nestmate)	20	11	7	2	219.8 ± 60	331.8 ± 49.6
Frozen reproductive Larva	9	1	8	0	170.6 ± 62.6	254.1
Frozen worker Larva	11	2	5	4	234.4 ± 80.8	104.9 ± 18.4
Plastic dummy (control)	10	0	1	9	493.5	N/A
Plastic dummy (experimental)	24	3	20	1	72.6 ± 12.4	1146.1 ± 25.3

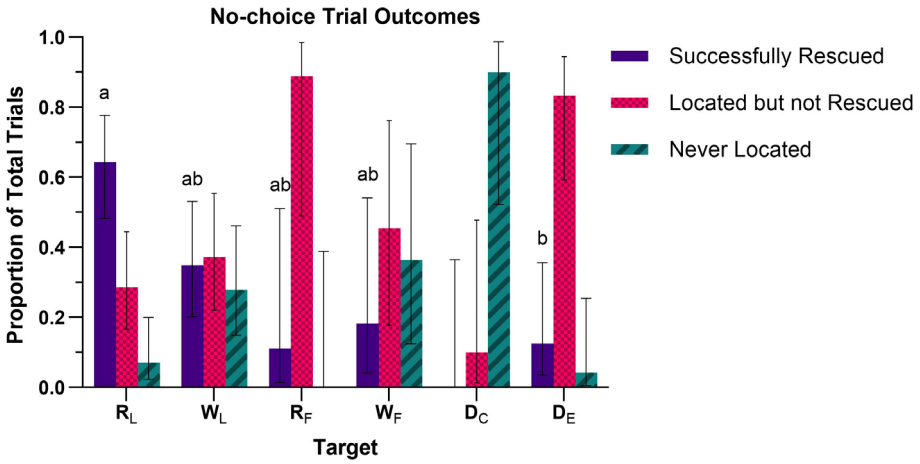

FIGURE 2 No-choice trials of larval ant rescue comparing rescue success frequency of different rescue targets. Error bars show 95% confidence intervals, calculated using Goodman's method. R_L, live reproductive larva; W_L, live worker larva; R_F, frozen reproductive larva; W_F, frozen worker larva; D_C, control plastic dummy; D_E, experimental plastic dummy. Letters are used to denote significant differences in rescue success among groups.

reproductive larva was found to significantly vary from the rescue likelihood of experimental dummies ($p = 0.014$) (Table S2). Target type also had a significant effect on time until rescue ($\chi^2 = 30.0$, df = 5, $p < 0.001$) (Table S1), which differed between live reproductive larvae and live worker larvae ($p = 0.002$) (Table S2). In summary, target type had a significant effect on both rescue success likelihood and latency.

Does *S. invicta* prioritize rescue of reproductive larvae over rescue of worker larvae?

Effect of caste on target location

A Cox frailty model suggested that target caste had a marginally significant impact on rescue target location likelihood over time ($\chi^2 = 6.8$ df = 3, $p = 0.080$) (Table S1). The GLMM suggested a stronger effect of caste on likelihood of target location ($\chi^2 = 7.8$, df = 1, $p = 0.005$), with worker larvae less likely to be located than reproductive larvae ($p = 0.048$) (Table S2). However, caste was not found to significantly contribute to variation in time until target location ($\chi^2 = 0.2$, df = 1, $p = 0.668$) (Table S1).

Effect of caste on rescue success

We conducted no-choice trials with live worker or live reproductive larvae in order to gain insight into which larval caste elicited a more robust rescue response. Reproductive larvae were rescued in 36 out of 56 trials (64%) (Table 2, Figure 3a). In contrast, worker larvae were rescued in only 15 out of 43 trials (35%) (Table 2, Figure 3a). A Cox Frailty model suggested that caste did not contribute significantly to variation in rescue success ($\chi^2 = 6.0$, df = 3, $p = 0.112$) (Table S1). However, a GLMM suggested that caste did significantly contribute to rescue success likelihood ($\chi^2 = 8.6$, df = 1, $p = 0.003$) such that workers were significantly less likely to be rescued compared to reproductive larvae ($p = 0.006$) (Tables S1 and S2). The effect of caste on time until rescue success was confirmed with a LMM ($\chi^2 = 13.6$, df = 1, $p < 0.001$) such that workers were rescued significantly faster than reproductive larvae ($p < 0.001$) (Tables S1 and S2). The direction of this effect, with workers being rescued faster than reproductives, which are rescued more frequently, explains the discrepancy with the Cox Frailty model result because the Cox Frailty model right-censors all failures. Overall, these results suggest that the caste of the rescue target does impact rescue success.

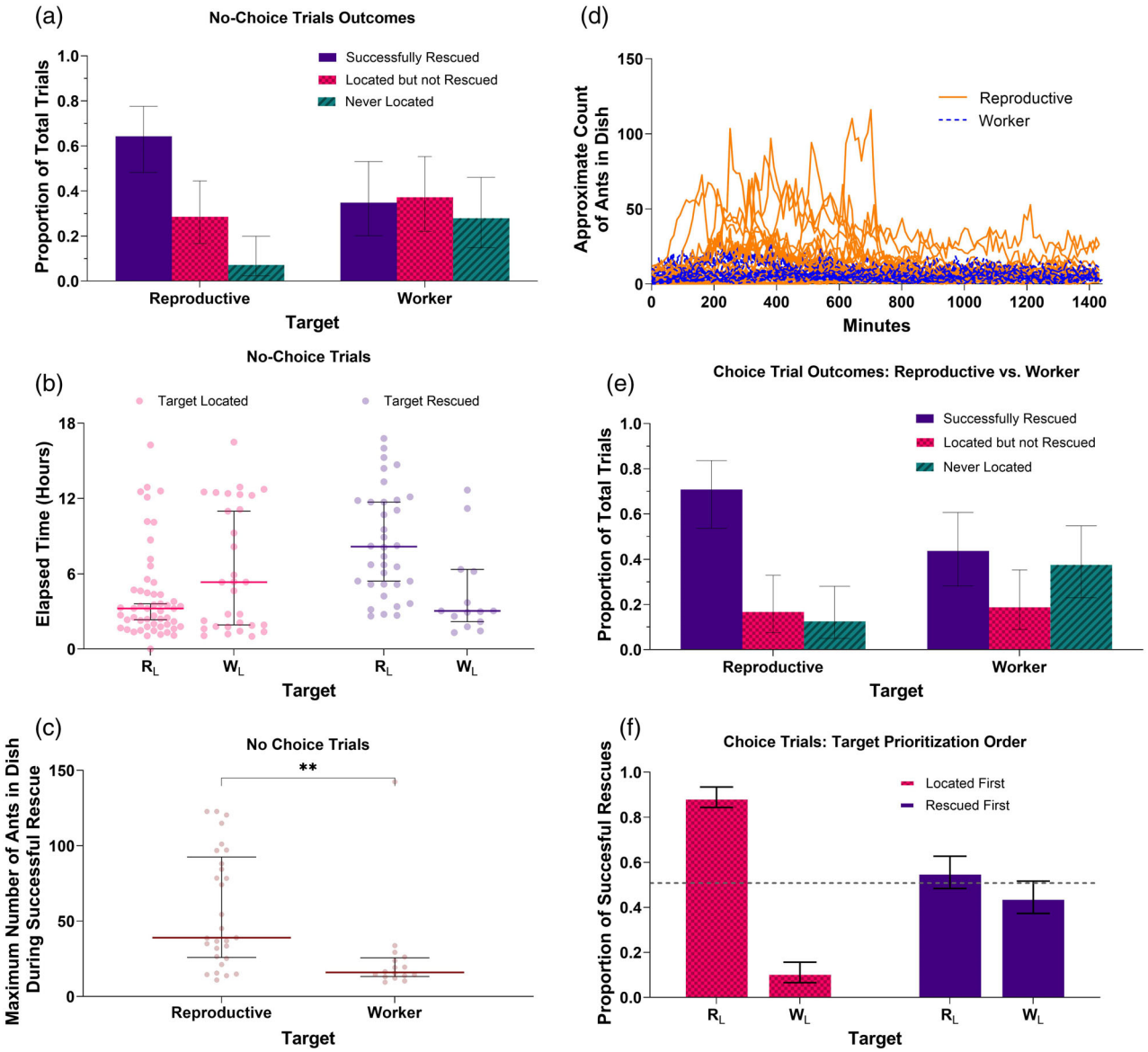

FIGURE 3 Rescuers prioritize rescue of reproductive larvae over worker larvae: (a) Trial outcomes for no-choice rescue trials with either one reproductive larva or one worker larva. Error bars show 95% confidence intervals. (b) Elapsed time until successful rescue for no-choice trials with one reproductive larva and one worker larva. Bars denote median and 95% confidence intervals. (c) Maximum ant count in rescue arena for successful no-choice trials. Bars denote median and 95% confidence intervals. (d) Estimated ant count over time for no-choice trials with one reproductive larva (yellow, solid line) and one worker larva (blue, dashed line). (e) Outcomes for choice trials with one reproductive and one worker larva. Error bars show 95% confidence intervals. (f) Percentage of time the reproductive larva was located (pink) and rescued (purple) first relative to the worker larva for choice trials with one reproductive larva and one worker larva in which both targets were rescued. Grey dashed line represents 50%. Error bars show 95% confidence intervals. R_L, live reproductive larva; W_L, live worker larva.

Effect of caste on rescuer participation

We compared rescuer participation in rescue activities. Specifically, we determined the maximum number of rescuing ants in the rescue arena in no-choice trials conducted with reproductive and worker larvae. We found that no-choice trials with reproductive larvae attracted significantly more rescuer participation than no-choice trials with worker larvae ($p < 0.001$) (Figure 3d). In particular, after a reproductive target was located, activity would typically sharply spike and would remain high during the rescue. Then, activity would decline after the target was successfully retrieved (Figure 3c, Video S5). This spike of activity was not seen in worker rescues, where activity remained at a baseline level before, during and after rescue (Figure 3c, Video S3). Given that all trials consisted of the same number of workers, this higher rescuer engagement further supports that reproductive larvae are prioritized over worker larvae in rescue scenarios.

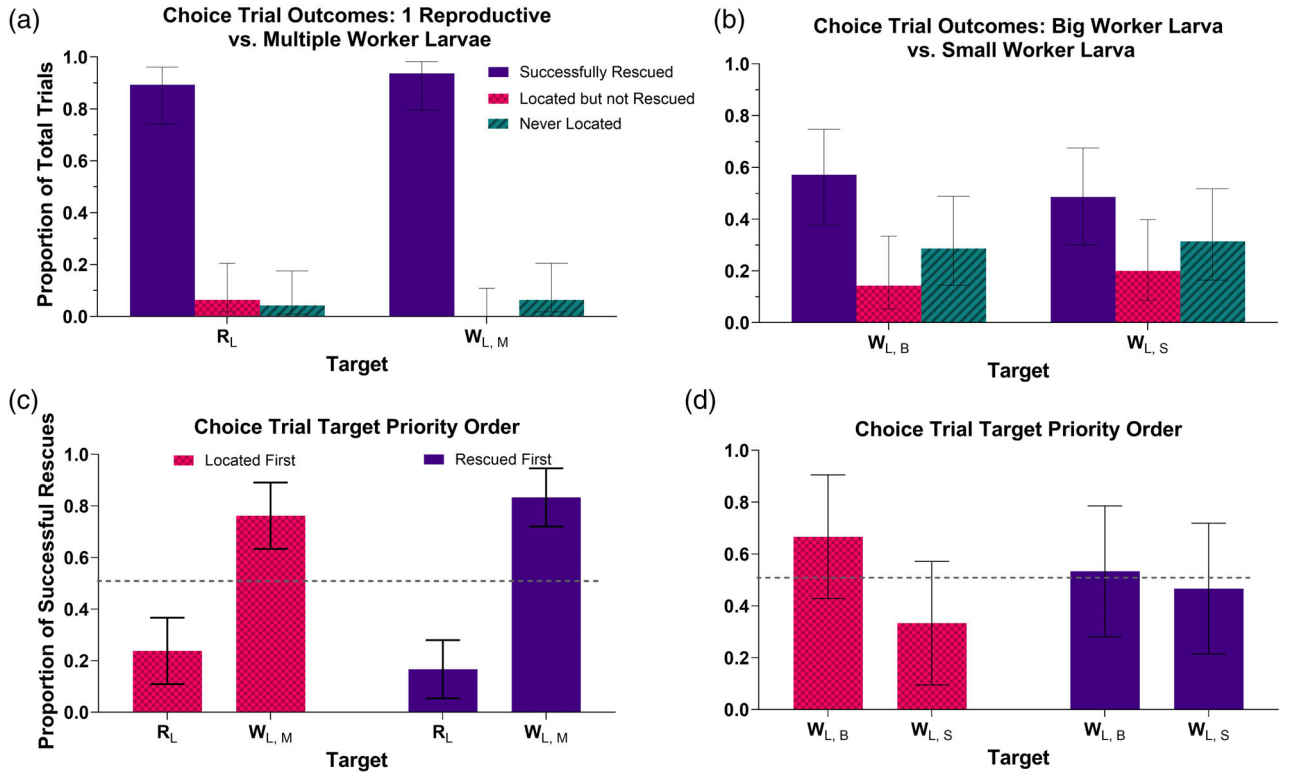

FIGURE 4 Rescue priority varies with target caste and aggregate brood mass: (a) Trial outcomes for choice trials with one reproductive and multiple worker larvae. Error bars show 95% confidence intervals. (b) Trial outcomes for choice trials with one big worker larva and one small worker larva. Error bars show 95% confidence intervals. (c) Proportion of time each target was located and rescued first for choice trials with one reproductive larva and multiple worker larvae in which both targets were rescued. Grey dashed line represents 50%. (d) Proportion of time each target was located and rescued first for choice trials with one big larva and one small worker larva. R_L, live reproductive larva; $W_{L,M}$, multiple live worker larvae; $W_{L,B}$, big live worker larva; $W_{L,S}$, small live worker larva.

Effect of caste on rescue priority in choice trials

We were interested in determining whether reproductive larvae would be preferred to worker larvae by rescue teams in choice trials. We conducted 48 choice rescue trials between one reproductive and one worker larva. In these choice trials, reproductive larvae were rescued more frequently than worker larvae and were usually located first (Figure 3e,f). These results show that *S. invicta* workers prioritize reproductive larval rescue targets in choice trials with single reproductive and worker larval targets.

Is preferential rescue of reproductive larvae over worker larvae driven by caste or mass?

To further understand the drivers of rescue target prioritization, we compared rescue of worker larvae and reproductive larvae, but this time we corrected for mass differences between the castes. Specifically, we buried a cache of worker larvae equivalent to the mass of one reproductive larva and monitored rescue success of the individual versus the cache. Because mass was kept consistent between the targets, while caste alone varied, these tests helped determine if rescue target caste or rescue target mass influenced rescue priority. Overall, we found that the reproductive larva and cache of worker larvae were both rescued often (Figure 4a). However, the cache of worker larvae was located and rescued first more often (Figure 4c).

We further investigated the question of mass versus caste priority by studying if *S. invicta* preferentially rescued a large worker larva or a small worker larva; these tests isolated the influence of mass on rescue priority controlling for caste. For clarity in distinguishing between trial parameters, the large worker larva will hereafter be referred to as "big." In these trials, we note that the big worker larvae had a mean mass ~1.7 mg larger than the small worker larvae in our experiments; this equated to a mass ratio of ~3:1. In contrast, reproductive larvae had a mean mass ~12.9 mg larger than worker larvae; this equated to a ratio of ~13:1.

We found that both the big and small worker larvae were rescued about half the time (Figure 4b). Out of the 15 trials in which both targets were successfully rescued, the big worker larva was located first 10 times (67%) (Figure 4d). Out of these same 15 trials, the big worker larva was rescued first eight times (53%) (Figure 4d).

Comparing the choice trials with 1 reproductive and 1 worker larva, with 1 reproductive and multiple worker larvae, and with 2 worker larvae of varying masses, we found that both caste ($\chi^2 = 4.5$, df = 1, $p = 0.033$) and the difference in target mass ($\chi^2 = 14.3$, df = 1, $p < 0.001$) significantly contributed to variation in which target was located first. However, thedifference in target mass did not contribute significantly to the variation in the elapsed time

between the location of the first target and the location of the second target ($\chi^2 = 1.4$, df = 1, $p = 0.241$) while target caste did ($\chi^2 = 7.4$, df = 1, $p = 0.007$); the interaction between target caste and difference in mass was marginally significant ($\chi^2 = 3.6$, df = 1, $p = 0.059$). Likewise, both target caste ($\chi^2 = 5.3$, df = 1, $p = 0.021$) and the difference in target mass ($\chi^2 = 8.1$, df = 1, $p = 0.004$) significantly contributed to variation in which target was rescued first. However, again, neither target caste ($\chi^2 = 0.4$, df = 1, $p = 0.542$) nor difference in target mass ($\chi^2 = 0.02$, df = 1, $p = 0.893$) contributed significantly to variation in the elapsed time between the rescue of the first target and the rescue of the second target.

Overall, these results suggest that both caste and mass contribute to target prioritization. Larger targets and reproductive targets are located and rescued before smaller targets and worker targets. However, neither caste nor mass contributes significantly to the variation in how long passes between the location and rescue of two targets.

Does *S. invicta* prioritize rescue of nestmate larvae over non-nestmate larvae?

We investigated if workers from one colony would rescue larvae from another colony. We found that non-nestmate reproductive larvae were rescued in 11 out of 20 trials in no-choice assays (55%) (Table 2, Figure 5a). This rescue success rate was not significantly different from the observed rescue success rate of no-choice trials with live nestmate reproductive larvae (36/56, 64%; $p = 1$) (Table S2). No-choice trials with live nestmate reproductive larvae also did not differ from no-choice trials with live non-nestmate reproductive larvae in time until rescue ($p = 0.358$), target location likelihood ($p = 1$), or time until target location ($p = 1$). This suggests that workers will rescue reproductive larvae from another colony with the same probability as larvae from their own colony.

We further tested if rescuing workers showed a preference towards rescuing their own nestmates if given the choice between a nestmate or a non-nestmate. We performed 24 choice trials with one nestmate reproductive larva and one non-nestmate reproductive larva. In these trials, each larva was rescued 23 out of 24 times (96%) (Figure 5b). Out of the 22 trials in which both targets were successfully rescued, the nestmate reproductive larva was located first 12 times (55%) (Figure 5c). Out of the same 22 trials, the nestmate reproductive larva was rescued first 14 times (64%) (Figure 5c). A GLMM suggested that colony identity match did not have a significant effect on either which target was located first ($z = 0.248$, $p = 0.804$) (Table S3) or which target was rescued first ($z = 0.772$, $p = 0.44$) (Table S3). Therefore, we found no evidence for significant differences in rescue success of nestmate versus non-nestmate larvae.

DISCUSSION

Rescue behaviour represents a remarkable activity undertaken by many animals. We studied rescue behaviour in the fire ant *Solenopsis invicta* to gain greater insight into the decisions that eusocial insects make during rescue. We staged choice and no-choice rescue trials in order to test how rescue targets were located and understand the decision-making process involved in group rescue responses. We found that olfaction likely plays a role in finding buried rescue targets. Moreover, mass rather than caste alone drives rescue priority, belying expectations about the "expendability" of eusocial insect workers. The results of these experiments provide insight both into what cues may be useful to rescuers in locating targets and into which targets are considered a high priority to the colony.

What cues does *S. invicta* use to locate rescue targets?

This study investigated the kinds of cues ants use in locating and rescuing imperilled rescue targets and found that chemical cues are likely

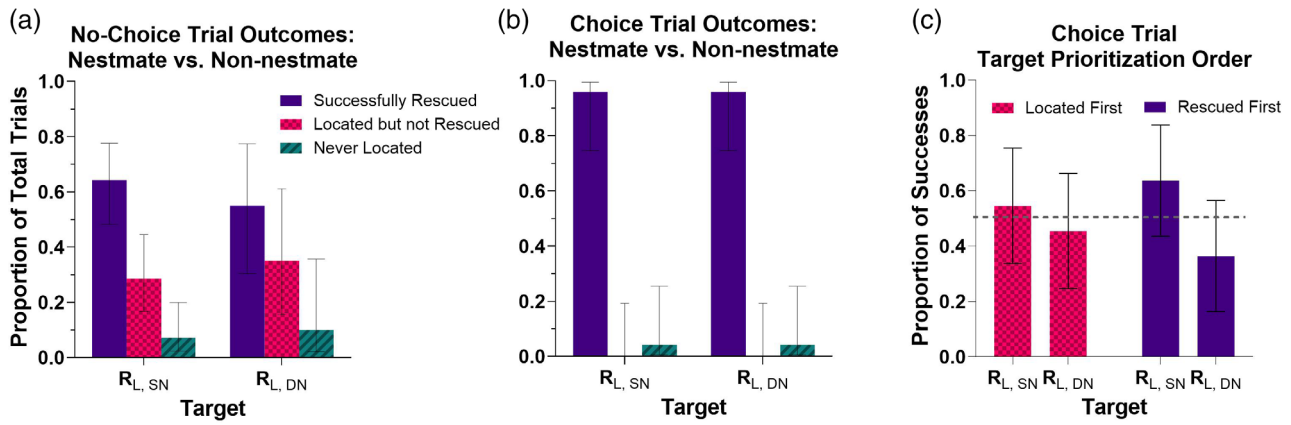

FIGURE 5 Workers rescue non-nestmate larvae: (a) Trial outcomes for no-choice trials with one nestmate or one non-nestmate reproductive larva. Error bars show 95% confidence intervals. (b) Trial outcomes for choice trials with one nestmate and one non-nestmate reproductive larva. Error bars show 95% confidence intervals. (c) Proportion of time each target was located and rescued first for choice trials with one nestmate and one non-nestmate reproductive larva. Grey dashed line represents 50%. $R_{L,\ SN}$, live reproductive larva from the same nest; $R_{L,\ DN}$, live reproductive larva from a different nest.

involved. We found that *S. invicta* workers located both live and frozen larval rescue targets; however, rescue teams only rescued live targets. Moreover, *S. invicta* workers located a plastic dummy that had come into contact with live larvae, but also typically left this target behind. Overall, these results are consistent with a role for chemical cues in the rescue of larvae in *S. invicta*. Our experiments suggest that auditory cues may not be necessary for locating larval rescue targets because frozen targets and plastic dummies putatively carrying surface chemicals were successfully located by rescue teams (Figure 2). However, further investigations manipulating acoustic or vibrational cues directly would be necessary to fully rule out auditory cues as being involved in rescue activities.

These results contrast with the vast majority of alarm calls in animals, which have been found to be auditory (Brudzynski, 2001; Hammers & Brouwer, 2017; Vogel & Fuentes-Jiménez, 2006). Auditory signals are particularly suited to rescue scenarios because they can be carried over long distances and are capable of both quick initiation and quick cessation. Indeed, stridulation has long been posited as the signal given by trapped adult ants (Golden & Hill, 2016; Roces et al., 1993), including *S. invicta* (Dai et al., 2025; Rauth & Vinson, 2006). For example, anaesthetised adult *Cataglyphis cursor* ants were not rescued in experimental trials (Nowbahari et al., 2012), suggesting that auditory cues are important to rescue activities of adult ants. In contrast, our data suggest that chemical cues are important to the location and rescue of ant larvae.

There are several reasons why larvae may rely on chemical cues during rescue. Proximately, it is possible that larvae are unable to use auditory cues. We note that, like adults, the pupae of many eusocial insect species can produce vibratory signals (Bagheri et al., 2024; Casacci et al., 2013). However, *Myrmica* ant larvae are unsclerotized and thus 'mute' (Casacci et al., 2013), rendering them unable to produce auditory cues. Similarly, it is possible that our larval rescue targets are also mute. In contrast, *S. invicta* brood have long been suggested to contain pheromones which induce tending behavior (Bigley & Vinson, 1975; Walsh & Tschinkel, 1974).

Ultimately, chemical signalling may result in more robust and consistent rescue behaviour, particularly for immature individuals. For example, in mammals, younger individuals tend to produce different alarm calls than older individuals (Blumstein & Munos, 2005; Randall et al., 2005), which are often more attractive to rescuers (Hollén & Manser, 2007). In ants in particular, calls for help have been suggested to decline with life expectancy (Miler, 2017). Chemical signals last longer than auditory signals (Chivers et al., 2013) and may therefore result in a more robust response. Moreover, chemical cues are able to persist after the individual has died (Figure 2), enabling cannibalism in the case of failed rescue, potentially as a means to re-acquire lost resources. Thus, while the quick cessation of auditory cues is likely an advantage for adult targets (Turza & Miler, 2023b), the longer-lasting chemical cues may be more appropriate for larvae, which are both helpless and of high value.

We note that, in our trials, frozen larvae were located at similar rates to live larvae (Figure 2). Ant corpses are known to produce oleic and linoleic acids, which prompt removal from the nest (Diez et al., 2013; Haskins & Haskins, 1974). Moreover, olfactory cues are also used to drive hunting responses in many ant species (Manubay & Powell, 2020; Stevens, 2013; Yusuf et al., 2014). Thus, it is possible cues other than alarm calls are prompting the location of dead targets. Indeed, the response to dead targets differed from the response to live targets in our study. Dead targets were frequently located, but rescued significantly less often than live targets. Thus, other cues may be involved in locating dead conspecifics and mediating this different behavioral response.

In summary, our findings align with other prior evidence suggesting that ants may rely on chemical cues in locating targets in need of rescue (Blum, 1969; Frank & Linsenmair, 2017). Chemical rescue signals have been suggested in several ant species (Frank & Linsenmair, 2017; Hangartner, 1969; Miler & Kuszewska, 2017; Spangler, 1968). Moreover, pheromones have long been identified across ant species and are involved in a wide array of activities (Blum, 1969), including aggressive and defensive behaviors which could result in rescue from a predator.

Does *S. invicta* prioritize rescue of reproductives over workers?

In eusocial insect colonies, individuals of different castes may be prioritized during rescue (Nowbahari et al., 2012). Reproductive castes are the primary individuals capable of reproducing and passing on the colony's genetic material to the next generation. As such, they may be valued more than sterile workers and thus may be prioritized during rescue. We found evidence for this in our trials, with reproductive larvae being located and rescued more often than worker larvae, and attracting more rescuer participation (Figure 3). Thus, it appears that reproductive larvae are prioritized over worker larvae in simple rescue scenarios.

This prioritization of the reproductive caste is common in eusocial insects. Reproductive brood are typically fed more and higher quality food (Dietz & Lambremont, 1970; Smith et al., 2008). Adult reproductives are housed in specialized, safer nest compartments (Darlington, 1985; Takata et al., 2023), whereas the workers, responsible for foraging and nest defense, can be considered relatively "expendable" (Porter & Jorgensen, 1981). *S. invicta* workers have been shown to deliver higher venom doses when defending nests containing reproductive brood relative to worker brood (Haight, 2018), indicating that reproductive brood are more highly valued to the colony.

There are two main hypotheses why reproductive larvae might be prioritized during rescue. First, reproductive larvae and worker larvae have different roles in the colony; reproductive larvae are the predominant caste capable of reproduction and may be preferentially saved for this reason. Alternatively, reproductive larvae are merely larger than worker larvae and thus represent higher resource investment.

To test these hypotheses, we investigated the effect of mass on rescue target prioritization. We found that rescue teams would rescue

a cache of worker larvae as often and as quickly as a similarly sized reproductive larva (Figure 4), suggesting that aggregate brood amount can outweigh the preferential rescue of a single reproductive larva. These results support the idea that mass may contribute to the preferential rescue of reproductive larvae. The location of rescue targets of high mass may be in part due to a larger target mass resulting in more surface chemicals, leading to a higher concentration of relevant pheromones. Given that larvae are often housed together and thus may become trapped together and that colonies typically have more worker larvae than reproductive larvae, our results suggest that worker larval rescue may in fact be prioritized over rescue of reproductive larvae in natural rescue conditions.

Does *S. invicta* prioritize rescue of nestmates over non-nestmates?

We found that polygyne *S. invicta* rescued brood from other nests at the same frequency as they rescue brood from their own nests (Figure 5). This result might seem surprising. Helping behaviours, such as rescue, likely evolved through kin selection. As such, helpers would be expected to focus their helping behaviours on relatives and nestmates. Indeed, such nepotism is observed in the helping behaviours of many social mammals (Sherman, 1980; Wahaj et al., 2004) and birds (Griesser & Ekman, 2005).

However, among eusocial insects, nepotism is often not observed within a colony (Holzer et al., 2006; Kellner & Heinze, 2011; Rangel et al., 2009; Strassmann et al., 2000; Zinck et al., 2009). Instead, individuals treat nestmates equivalently regardless of relatedness and act aggressively towards individuals of other colonies (Abbot, 2022; Jelley & Moreau, 2023). Some ants display the highest aggression towards familiar conspecifics (Newey et al., 2010), while others are most aggressive towards unfamiliar conspecifics (Dimarco et al., 2010). But some degree of territoriality and non-nestmate aggression is common among ants (Abbot, 2022; Guerrieri et al., 2009).

Many invasive ants form large "supercolonies" lacking non-nestmate aggression (Eyer et al., 2018; Holway et al., 1998). Invasive *S. invicta*, in particular, can form polygyne colonies that have very porous colony boundaries and low relatedness among nestmates (Goodisman et al., 2007; Morel et al., 1990; Vargo & Porter, 1989; Weeks et al., 2004). Within a nest, workers are not highly related to the individual eggs they rear or queens they feed (DeHeer & Ross, 1997). In fact, polygyne *S. invicta* are even known to raid brood from other polygyne colonies (Tschinkel, 1992). Thus polygyne *S. invicta* workers may be expected to behave charitably towards non-nestmate individuals, in contrast to general expectations for eusocial insects. And yet, it is worth noting that polygyne *S. invicta* have been shown to preferentially feed related brood and to selectively cannibalize unrelated brood (Kjeldgaard et al., 2022). Our findings of non-preferential treatment towards nestmates in a rescue context therefore align with the aforementioned studies supporting a lack of colony boundaries in invasive *S. invicta* (DeHeer & Ross, 1997; Goodisman et al., 2007; Morel et al., 1990; Vargo & Porter, 1989; Weeks et al., 2004) and contrast with recent findings of nepotism (Kjeldgaard et al., 2022).

It remains unclear if the lack of nestmate priority in rescue behaviour is advantageous to fire ant colonies, or merely a result of an inability to distinguish nestmate from non-nestmate brood in rescue scenarios. Rescue of non-nestmate brood does not occur in some ant species (Hollis & Nowbahari, 2013a; Nowbahari et al., 2009), but does occur in others (Taylor et al., 2013; Uy et al., 2019). Ultimately, this study expands on previous work showing that *S. invicta* workers are not only willing to rescue non-nestmates, but actually show no preference towards nestmates even when given the choice between rescuing a nestmate and non-nestmate larva.

AUTHOR CONTRIBUTIONS

Paige B. Caine: Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; formal analysis; data curation; validation. **Natalie Nejad:** Methodology; investigation. **Daniel I. Goldman:** Conceptualization; funding acquisition; project administration; supervision. **Michael A. D. Goodisman:** Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in this manuscript are available from the following Dryad Digital Repository: https://doi.org/10.5061/dryad.fn2z34v9x.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Video S1. Representative no-choice trial for control dummy (plastic that did *not* contact live larva). Unsuccessful rescue. Video downsampled to 1 fps, played back at 100x speed. The target was never located or rescued.

Video S2. Representative no-choice trial for experimental dummy (plastic that came into physical contact with live reproductive larva). Unsuccessful rescue. Video downsampled to 1 fps, played back at 100x speed. Target located at 00:00:20, but never rescued.

Video S3. Representative no-choice trial for live worker larva. Successful rescue. Video downsampled to 1 fps, played back at 10x speed. Larva located at 00:10:37, lifted above ground at 00:13:37 and rescued at 00:18:13.

Video S4. Representative no-choice trial for frozen worker larva. Unsuccessful rescue. Video downsampled to 1 fps, played back at 100x speed. Larva located at 00:00:24, but never rescued.

Video S5. Representative no-choice trial with live reproductive larva. Successful Rescue. Video downsampled to 1fps, played back at 10x speed. Larva located at 00:03:32, lifted above ground at 00:14:28 and rescued at 00:16:07.

Video S6. Representative no-choice trial for frozen reproductive larva. Unsuccessful rescue. Video downsampled to 1 fps, played back at 100x speed. Larva located at 00:00:42, but never rescued and ultimately eaten in the dish.

Table S1. Descriptions, formulas and statistics for all models used. Date and colony were initially included as random effects, but dropped from models when they contributed zero variance. Generalized linear mixed models (GLMM) were fit with binomial distribution and logit link function. Linear mixed models were refit with REML for the purposes of ANOVA. Differences in mass and time were calculated by subtracting the value for target two from the value for target one. Frozen reproductive larvae were excluded from GLMMs for location likelihood for all no-choice trials due to failure to converge. Likewise, control dummies were excluded from the GLMMs for rescue success due to failure to converge. For no-choice trials, elapsed times were log-transformed. Null models are specified with "_null" following the letter designation. Numbers following letters (example: C2) indicate models that were rerun with subset data to enable reliable pairwise comparisons and estimates due to complete separation in the full dataset; this data subset is specified under the "formula" column. Column 2, "Question," refers to which scientific question the model is addressing. The final 3 columns represent a chi-squared test comparison of the model to its designated null model, with chi-square test statistic (χ^2), degrees of freedom (DF) and probability that the model and null model differ more than expected by chance (P).

Table S2. Pairwise contrasts for all single target trials. Model designation from Table S1 is provided, followed by specific elements being compared (contrasts), odds ratios, standard error (SE), degrees of freedom (DF), Z ratio (Z) and p value from post hoc Holm test (P). Abbreviations are as follows: R_L (live reproductive larva), W_L (live worker larva), R_F (frozen reproductive larva), W_F (frozen worker larva), D_C (control plastic dummy), D_E (experimental plastic dummy), $R_{L,DN}$ (live reproductive larva from a different nest).

Table S3. Hazard ratios for Cox models, and fixed effect estimates for all generalized linear mixed models and linear mixed models, with standard error (SE), degrees of freedom (DF, if applicable), test statistic (Z or t) and probability value (P). Abbreviations are as follows: R_L (live reproductive larva), W_L (live worker larva), R_F (frozen reproductive larva), W_F (frozen worker larva), D_C (control plastic dummy), D_E (experimental plastic dummy), $R_{L,DN}$ (live reproductive larva from a different nest). Model designation from Table S1 provided in first column.

Table S4. Random effect estimates (variance) for all random effects (Effect) for all models included in the study (Model), including variable type (Variable) and standard deviation (SD). Model designation from Table S1 provided in first column.

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