Short Note

Midwife toads (*Alytes obstetricans*) select their diurnal refuges based on hydric and thermal properties

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Abstract. Most amphibians use both aquatic and terrestrial habitats. While the aquatic phase attracted considerable interest, terrestrial habitat use is often less investigated. We studied diurnal refuge selection in the Midwife toad in Western central France. We used a factorial design and tested the effect of refuge type (wood *versus* rubber boards) and substrate (wet sawdust *versus* bare soil). Most animals were observed under refuges with sawdust substrate. An interaction between refuge type and temperature was detected with higher presence probability under rubber refuges at low temperature. Conflicting hydric and thermic requirements are likely determinants of the observed pattern.

Keywords: amphibians, artificial refuges, habitat selection, terrestrial.

Amphibians are currently facing a worldwide decline involving a diversity of proximate causes such as habitat loss and fragmentation (Beebee and Griffiths, 2005). Most amphibians have a dual life cycle combining terrestrial and aquatic phases (Cody and Smallwood, 1996). Aquatic sites are required for breeding, egg laying and larval development (Cody and Smallwood, 1996). During the aquatic stages, breeding adults usually gather in large numbers in ponds and are often easily detected (breeding call, conspicuous activity) (Dodd, 2010). Conversely, terrestrial habitat use is much less investigated because individuals are much more secretive (Dodd, 2010). Consequently, a majority of studies have focused on aquatic phases of the life cycle resulting in a large amount of knowledge on reproduction and aquatic stages (breeding, embryonic and tadpole development). In order to understand the threats that currently affect amphibian populations, it is crucial to collect data during terrestrial life stages and understand the microhabitat use during the terrestrial phase.

Because amphibians have a permeable skin, they are particularly sensitive to evaporative water loss (Lillywhite, 2006) and they require hygrometric conditions for activity (Osbourn, Connette and Semlitsch, 2014). Yet, they also need to actively thermoregulate to achieve optimal temperatures and performances (Köhler et al., 2011). A possible conflict can therefore exist between water and thermal needs; which in turn will influence habitat use and specific microclimatic conditions to thermo- and hydroregulate (Heard, Robertson and Scroggie, 2008; Mitchell and Bergmann, 2016). Most amphibians are active at night and frequently shelter in underground refuges during the day (Sutherland, 2006). Such refuges can be critical to avoid extreme temperatures and dehydration notably under hot or arid climate (Seebacher and Alford, 2002). Under temperate (constraining) climates, thermal conditions can be limiting and diurnal thermoregulation can be important to achieve preferred temperature. In this context, diurnal

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refuge use may primarily be driven by thermoregulatory purposes.

The use of artificial refuges (AR) can provide an effective technique for detecting secretive taxa (Hoare et al., 2009) and have been efficiently used to detect and monitor reptile populations (Milne, Bull and Hutchinson, 2003; Michael, Lunt and Robinson, 2004; Graitson and Naulleau, 2005; Lettink and Cree, 2007; Hoare et al., 2009). Although, some amphibian species use AR (Michael, Lunt and Robinson, 2004; Graitson and Naulleau, 2005), only few studies have specifically used this method (e.g., Wakelin et al., 2003; Hampton, 2007; Latham and Knowles, 2008).

The aim of our study was to experimentally examine the use of diurnal refuges by the midwife toad, *Alytes obstetricans*. We compared two refuge types (wood *versus* rubber boards) and two substrates (sawdust *versus* natural substrate). Because of vulnerability to water loss, we posit that the hydric properties of the substrate beneath refuges will likely influence refuge selection. We tested the following predictions:

1) Because of their hydric requirements, Midwife toads should prefer sawdust substrate that should maintain the highest humidity level.

2) Because dark rubber boards should provide warmer conditions, they should be more attractive when environmental temperatures are low.

The common midwife toad, *Alytes obstetricans*, is a terrestrial species from the Alytidae family, ranging across Western Europe. Midwife toads often shelter under refuges such as rocks and slabs and are frequently encountered in the vicinity of anthropogenic habitats, as stone walls, wood pile or screes. Reproduction is partially independent of the aquatic environment. Oviposition occurs on land and males actively contribute to oviposition in a lumbar amplexus. This species shows male-only parental care with males carrying eggs during early development (2 to 3 weeks). Aquatic bodies such as ponds are required for tadpole deposition.

We studied surface activity in an anthropic habitat (a sawmill), located in Southern France within the Chizé forest (46.146315N; −0.395969W). The site (1 hectare) offers numerous ground shelters including woodpiles, logs, accumulated sawdust and is largely occupied by midwife toads. A small pond close to the site is used for tadpole deposition.

We examined the influence of refuge type by comparing two types of materials: wood *versus* rubber board (conveyor belt). The two refuges were of similar size: 40×80 cm. We also tested the influence of the substrate (sawdust *versus* bare soil) placed beneath artificial refuges using a 2×2 factorial design.

We positioned 17 groups of artificial refuges on the study sites. Each group was composed of four artificial refuges with four modalities:

1) Wood board and sawdust substrate.
2) Wood board and natural substrate.
3) Rubber board and sawdust substrate.
4) Rubber board and natural substrate.

Within each group the four artificial refuges were placed side by side on a linear transect favouring the access to the different modalities (total $n = 68$ refuges). Refuges were placed in May, two weeks before conducting the survey.

From June to August 2016, we visited the study site on 11 occasions. Each refuge was gently lifted in order to determine the presence of midwife toads. When detected, we counted the number of individuals under the refuge and assessed age class based on body size (adult > 4 cm, juvenile < 4 cm, only three individuals were not attributed to an age-class). When possible, we also assessed the sex and reproductive status of some individuals (i.e., male transporting eggs). Temperatures data were collected from www.meteociel.fr website, recorded in Niort, the closest station from our study site (∼19 km straight line). Temperature was recorded every hour. Mean temperature was calculated by averaging the last record before the survey and the first record after the survey.

We used Generalized Linear Mixed-Effects Models to examine the determinants of presence and abundance under artificial refuges. The probability of presence was modelled using a binomial distribution. The abundance (i.e., the number of toads when present) was modelled using a Poisson distribution. The group number (1 to 17) was treated as a random factor and the four different refuges in each group as a random effect nested in the group. The same procedures were used when considering either all individuals or caring males only. Analyses were carried out using R 3.5.0 software (R Development Core Team, 2005), lme4 package (Bates et al., 2015) and MuMIn package (Barton, 2019). The figures were designed using ggplot2 package (Wickham, 2016).

Over the 11 visits, a total of 110 positive refuge controls were obtained (14.7%). Toads were detected in 11 of the 17 groups of artificial refuges. The abundance ranged from 1 to 7 (mean $\pm$ SD = 2.2 $\pm$ 1.7). The total number of contacts was 238 individuals. The majority of individuals were adults ($n = 223$) and we observed relatively few juveniles ($n = 12$). Among adults, 108 individuals (48%) that were

Table 1. Statistical models used to test for the influence of refuge type and substrate on toad presence and abundance. AR: artificial refuge. Est.: estimate. Juv.: juveniles. R^2m: marginal R square. R^2c: Conditional R square. SE: standard error, Temp.: temperature. Group/AR stands for random intercept varying among group and AR nested within group.

Response variable	GLMER family	Random-effect	Explanatory variables	Est.	SE	Z	r^2m	r^2c	P
All toads presence (juv. included)	Binomial	Group/AR	(Intercept)	−6.553	1.387	−4.725			<0.001
			Material	−3.553	1.482	−2.398	1.500e−4	0.620	<0.05
			Substrate	−4.388	0.928	−4.729	0.206	0.633	<0.001
			Temp.	−0.007	0.038	−0.175	0.006	0.630	0.861
			Material:Substrate	1.246	1.131	1.102	0.222	0.640	0.271
			Material:Temp.	0.124	0.056	2.240	0.013	0.638	<0.05
			Model				0.233	0.656	
Caring males presence	Binomial	Group/AR	(Intercept)	−12.272	3.304	−3.714			<0.001
			Material	0.366	2.507	0.146	1.285e−10	5.475e−6	0.884
			Substrate	5.808	1.878	3.093	6.374e−7	5.223e−6	<0.001
			Temp.	−0.066	0.057	−1.164	8.174e−9	5.582e−6	0.244
			Material:Substrate	−1.575	2.312	−0.681	6.172e−7	5.256e−6	0.496
			Material:Temp.	0.028	0.076	0.374	1.009e−8	5.622e−6	0.708
			Model				6.458e−7	5.413e−6	
All toads abundance (juv. included)	Poisson	Group/AR	(Intercept)	−0.440	0.682	−0.646			0.518
			Material	0.185	0.806	0.230	9.739e−5	0.251	0.818
			Substrate	0.720	0.655	1.098	0.024	0.260	0.272
			Temp.	0.014	0.016	0.908	0.024	0.262	0.364
			Material:Substrate	−0.493	0.759	−0.650	0.031	0.267	0.516
			Material:Temp.	0.016	0.023	0.694	0.026	0.261	0.488
			Model				0.050	0.274	
Caring males abundance	Poisson	Group/AR	(Intercept)	−0.021	1.068	−0.019			0.985
			Material	−0.582	1.305	−0.446	0.122	0.576	0.656
			Substrate	0.641	1.035	0.619	0.027	0.619	0.536
			Temp.	−0.000	0.021	−0.008	0.006	0.592	0.994
			Material:Substrate	−0.410	1.175	−0.349	0.044	0.628	0.727
			Material:Temp.	0.032	0.035	0.892	0.029	0.596	0.372
			Model				0.073	0.194	

found carrying eggs were confidently sexed as males.

The presence of midwife toads was strongly associated to substrate type with more frequent contacts with sawdust substrate (table 1, fig. 1a) and to AR type with more frequent contacts under rubber boards (table 1, fig. 1b). We found an interaction between the AR type and the temperature, showing that rubber boards were more attractive at low temperatures (table 1, fig. 1c). In contrast, the use of wood board refuges was independent of thermal conditions (table 1, fig. 1c). Restricting our analyses to adult individuals yielded similar results. Finally, when focusing on reproductive males (carrying eggs), we found an influence of substrate solely (table 1). When focusing on abundance data,

we found no influence of artificial refuge type or substrate (table 1). However, the abundance of toads observed with no substrate was low compared to sawdust ($n = 13$ versus 225) and the proportion of caring males tended to be lower (30.8% versus 44.9%).

We found that sawdust substrate had a major influence on refuge selection. This effect likely reflects the hygrometric properties of this substrate. Indeed, sawdust accumulates large amount of water within wood fibers (Gruda, 2008). This hygroscopic property should provide high humidity and reduce the water vapour deficit. Such benefits are particularly important for amphibians that can face high hydric and thermal needs (Köhler et al., 2011). In addition, sawdust allows burrowing or digging behaviour
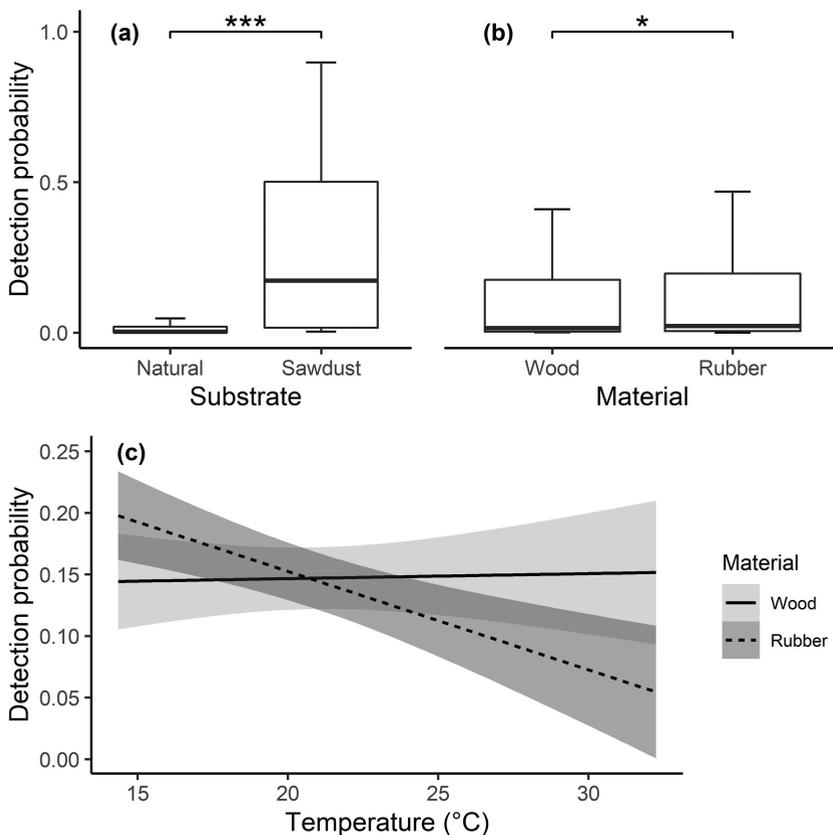
Figure 1. Effect of (a) substrate and (b) refuge type on detection probability. Detection probability was determined from extraction of predicted values of toad presence (binomial glmer, see text for details). The boxes represent the interval between the 25% and 75% quartiles and the whiskers represent the range. The middle horizontal line in the box plot represents the median. ***: $P < 0.001$; *: $P < 0.05$. (c) Influence of temperature and artificial refuge type on detection probability. Detection probability was determined from extraction of predicted values of toad presence (binomial glmer, see text for details). The lines represent the fitted linear regression and the grey areas the 95% confidence intervals.

and small-scale adjustments within this micro-habitat. Accordingly, individuals were often observed within circular spaces made by arranging the topsoil. That should provide specific micro-climatic conditions. Such advantages are presumably missing with the natural substrate (i.e., bare soil) with more important air exchanges and dehydration risks. High humidity can notably benefit to caring-males to enhance eggs development (Janzen, Ast and Paukstis, 1995) and avoid desiccation (Taigen, Pough and Stewart, 1984). The relatively lower proportion of caring males using bare soil suggests differential substrate selection. In support of these results, previous studies have also demonstrated

the role of substrate humidity on refuge use in amphibians (Grant et al., 1992).

We found an interaction between the AR type and the ambient temperature on toads' observations. Specifically, rubber boards were more attractive when environmental temperatures were lower, while toads' presence under wood boards was similar across temperature. Dark rubber covers warm up more and more rapidly than wood boards when exposed to the sun (OL, pers. obs.) and likely provide thermal benefit when environmental temperatures were low. This further indicates that midwife toads thermoregulate during the day in the refuge they choose at night. On the other hand,

when environmental temperature are high, rubber covers can potentially achieve high temperature that may determine toads' presence, while wood refuges appear as attractive, no matter the temperature (Grant et al., 1992). Wood refuges are therefore expected to provide more stable microenvironments when thermal conditions are critical. While reproduction often influence thermoregulation effort and accuracy (Klug and Bonsall, 2014), we did not find an influence of AR type on refuge selection by caring-males. Future studies including more controls across the entire reproductive season will help to test for this hypothesis.

Several studies have demonstrated that AR are effective for amphibians and reptiles detection (Ryan et al., 2002; Scheffers et al., 2009; Michael et al., 2018). Our study provides clear evidence that substrate type and AR type in interaction with temperature can have a major influence on Midwife toads' diurnal refuge selection. Future studies are required to test the influence of refuge type over an entire season and test for a possible differential attractiveness according to ambient conditions for caring-males.

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