Amphibia-Reptilia

Mild segregation in the breeding preferences of an invasive anuran (*Discoglossus pictus*) and its main native competitor (*Epidalea calamita*) in ephemeral ponds

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Abstract. The choice of breeding sites by pond-breeding anurans has notable consequences for the fitness of larvae. Hence, beyond pond typology and phenology, adults can also discriminate according to several other features, for instance to favour allotopy with potential competitors. However, the lack of shared evolutionary history might impede proper ecological differentiation with alien species during the first stages of invasions. Here, we studied several possible sources of ecological segregation between the invasive *Discoglossus pictus* and the native *Epidalea calamita* in ephemeral ponds, where the native toad hardly had competition before the arrival of the invasive frog. During spring of 2016, we periodically surveyed 69 ephemeral ponds in three areas with different invasion histories to detect the presence/absence of eggs and tadpoles of these species. Invasive *D. pictus* started breeding earlier than *E. calamita*, but differences were not significant. Similarly, there were not clear differences among areas with different invasion histories. However, we found for both species a mutual tendency to directly avoid larval syntopy at the end of the reproductive season. We also found interspecific differences in the features that both species use for pond choice, preferring the native species shallower and less vegetated ephemeral ponds. Globally however, co-occurrence was high, pointing at other processes as key to the coexistence between both species in these habitats.

Keywords: competitor avoidance, ephemeral ponds, interspecific competition, invasive species, niche overlap, oviposition site, tadpoles, temporary ponds.

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Supplementary material

Table S1. Exact locations and main features of the ponds studied (Depth: maximum depth; Vegetation variables: 1 = very low, 2 = low, 3 = present, 4 = abundant; Sunshine: proportion of the pond under direct sunlight).

Code	Latitude	Longitude	Surface (m^2)	Depth (m)	Vegetation inside (1–4)	Vegetation outside (1–4)	Sunshine (0 to 1)
GI.OLI.01	42° 0'15.80"N	2°51'24.36"E	23.6	0.5	1	2	0.9
GI.ABA.02	42° 0'8.78"N	2°50'45.53"E	56.5	1	2	4	0.5
GI.ARG.01	41°59'55.17"N	2°50'38.65"E	2.4	0.1	3	3	0.95
GI.ARG.02	41°59'55.10"N	2°50'38.75"E	4.7	0.5	3	4	0.8
GI.ARG.03	41°59'54.98"N	2°50'39.42"E	2	0.2	3	1	1
GI.ARG.04	41°59'55.63"N	2°50'38.65"E	31.4	1	4	3	0.9
GI.ARG.05	41°59'55.78"N	2°50'38.96"E	15.7	0.2	2	1	1
GI.ARG.06	41°59'55.71"N	2°50'40.02"E	33	1	4	4	0.1
GI.ARG.07	41°59'56.39"N	2°50'39.76"E	3.1	0.7	2	3	0.6
GI.ARG.10	41°59'41.59"N	2°50'44.02"E	7.1	0.3	2	3	0.2
RI.ESP.02	41°49'44.18"N	2°43'5.96"E	55	1	1	2	0.8
RI.MUR.01	41°49'18.12"N	2°41'18.24"E	125.7	0.2	4	4	1
RI.MUR.02	41°49'18.57"N	2°41'16.30"E	11.8	0.2	1	3	1
RI.MUR.03	41°49'18.98"N	2°41'17.29"E	35.3	1	4	3	0.8
RI.MUR.04	41°49'21.87"N	2°41'17.03"E	15.7	0.2	1	2	1
RI.MUR.05	41°49'18.29"N	2°41'17.61"E	16.5	0.2	1	1	1
RI.BAT.01	41°49'25.94"N	2°40'58.33"E	18.8	0.1	3	3	0.9
RI.BAT.02	41°49'28.78"N	2°40'59.91"E	3.9	0.2	4	2	0.8
RI.CAÇ.01	41°49'27.23"N	2°40'51.29"E	55	1.5	4	4	0.6
RI.CAÇ.02	41°49'28.29"N	2°40'45.69"E	38.5	0.5	4	3	0.8
RI.GRE.01	41°47'47.10"N	2°41'45.70"E	12.6	0.1	1	1	1
RI.GRE.02	41°47'46.94"N	2°41'45.71"E	0.2	0.1	2	1	1
RI.GRE.03	41°47'47.34"N	2°41'45.15"E	11.8	0.2	1	2	1
RI.GRE.04	41°47'50.95"N	2°41'42.21"E	12.6	0.3	1	1	1
RI.GRE.05	41°48'18.11"N	2°41'11.26"E	18.1	0.3	2	2	0.6
RI.GRE.06	41°48'12.08"N	2°41'13.59"E	4.7	0.1	1	2	0.85
RI.GRE.07	41°48'11.87"N	2°41'13.80"E	11.8	0.2	2	2	1
RI.GRE.08	41°47'46.66"N	2°41'46.37"E	12.6	0.2	1	1	0.9
RI.PON.01	41°47'54.89"N	2°42'21.67"E	15.7	0.1	3	2	0.6
HO.POL.02	41°43'55.72"N	2°37'7.36"E	2.4	0.2	1	1	1
HO.POL.04	41°44'3.76"N	2°36'38.50"E	687.2	0.3	1	1	1
HO.POL.05	41°43'57.75"N	2°36'32.23"E	9.4	0.2	1	2	0.8

HO.POL.06	41°43'54.72"N	2°36'28.98"E	15.7	0.3	1	3	0.9
HO.POL.07	41°43'54.44"N	2°36'27.90"E	12.6	0.3	1	2	0.9
HO.DUC.01	41°44'7.59"N	2°36'5.05"E	15	0.2	1	2	1
HO.DUC.02	41°44'8.44"N	2°36'4.27"E	12.6	0.4	1	2	1
HO.DUC.04	41°44'7.58"N	2°36'3.41"E	125.7	0.8	4	2	1
HO.DUC.06	41°44'6.89"N	2°36'4.01"E	6.9	0.2	1	1	1
HO.ARI.01	41°43'19.07"N	2°33'52.17"E	3.1	0.2	1	3	1
HO.ARI.02	41°43'19.19"N	2°33'53.15"E	11.8	0.3	4	4	1
HO.ARI.03	41°43'19.03"N	2°33'53.22"E	7.1	0.2	4	4	1
HO.ARI.04	41°43'19.28"N	2°33'53.52"E	7.1	0.2	4	4	1
HO.ARI.05	41°43'19.38"N	2°33'52.14"E	6.3	0.4	4	4	1
HO.MOT.01	41°43'6.53"N	2°33'10.66"E	22	0.3	1	2	1
HO.MOT.02	41°43'6.39"N	2°33'10.33"E	4.7	0.2	1	1	1
HO.MOT.03	41°43'6.46"N	2°33'9.02"E	7.1	0.2	1	1	1
HO.MOT.04	41°43'6.85"N	2°33'2.50"E	1.6	0.2	1	1	1
HO.MOT.05	41°43'6.42"N	2°33'2.98"E	12.6	0.3	1	1	1
HO.MOT.06	41°43'6.63"N	2°33'1.11"E	4.7	0.1	1	1	1
HO.MOT.07	41°43'7.28"N	2°32'58.04"E	2.4	0.2	1	2	1
HO.MOT.08	41°43'7.70"N	2°33'2.28"E	6.9	0.2	1	3	0.1
HO.MOT.09	41°43'8.00"N	2°33'2.05"E	11.8	0.3	1	2	0.1
HO.MOT.10	41°43'8.15"N	2°33'1.89"E	13.7	0.2	1	3	0.3
HO.MOT.11	41°43'8.43"N	2°33'1.37"E	16.5	0.2	1	3	0.2
HO.MEA.01	41°42'49.27"N	2°32'31.71"E	2.4	0.3	2	2	0.8
HO.MEA.02	41°42'49.55"N	2°32'31.17"E	3.1	0.3	1	2	0.3

Table S2. Survey matrix, *i.e.* summary of the presence-absence data for each species, pond and ten days period. Abbreviations: ME = Mid Expansion area, FE = Far Expansion area, EF = Expansion Front, **NA** = Pond empty of water, N = No species present, c = *Epidalea calamita* eggs during that period, C = *Epidalea calamita* tadpoles during that period, d = *Discoglossus pictus* eggs during that period, D = *Discoglossus pictus* tadpoles during that period, o = other species (*Pelodytes punctatus* or *Hyla meridionalis*) eggs during that period, O = Other species tadpoles during that period.

Area	Code	20th–30th February	1st–10th March	11th–20th March	21st–31st March	1st–10th April	11th–20th April	21st–30th April	1st–10th May	11th–20th May	21st–31st May
ME	GI.OLI.01	N	N	c	C	N	N	N	N	N	N
ME	GI.ABA.02	N	N	d	D	D	D	N	N	N	N
ME	GI.ARG.01	N	N	do	DO	DO	DO	cD	CD	CDo	CDO
ME	GI.ARG.02	N	N	d	D	D	D	D	D	N	N
ME	GI.ARG.03	N	N	N	N	d	D	N	d	D	D
ME	GI.ARG.04	N	N	d	D	D	D	D	Do	DO	DO
ME	GI.ARG.05	N	N	d	D	D	D	cD	D	D	D
ME	GI.ARG.06	N	N	d	D	D	D	D	D	D	D
ME	GI.ARG.07	N	N	N	N	d	D	N	d	Do	DO
ME	GI.ARG.10	**NA**	**NA**	d	D	N	N	N	d	D	N
FE	RI.ESP.02	**NA**	**NA**	**NA**	**NA**	**NA**	do	cDO	DO	DO	DO
FE	RI.MUR.01	N	**NA**	**NA**	**NA**	d	D	d	D	N	N
FE	RI.MUR.02	N	**NA**	**NA**	**NA**	**NA**	**NA**	N	N	N	CD
FE	RI.MUR.03	N	N	N	N	do	DO	N	N	N	N
FE	RI.MUR.04	N	**NA**	**NA**	**NA**	**NA**	**NA**	N	d	cd	CD
FE	RI.MUR.05	N	**NA**	**NA**	**NA**	N	N	c	C	N	N
FE	RI.BAT.01	N	**NA**	**NA**	**NA**	do	DO	N	N	cdo	CDO
FE	RI.BAT.02	N	**NA**	**NA**	**NA**	**NA**	CD	CD	CD	cdo	CDO
FE	RI.CAÇ.01	N	N	N	N	do	DO	DO	O	cO	CO
FE	RI.CAÇ.02	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	N	N	cd	CD
FE	RI.GRE.01	**NA**	**NA**	c	C	C	C	C	C	C	C
FE	RI.GRE.02	**NA**	**NA**	**NA**	**NA**	cd	CD	CD	CD	CD	CD
FE	RI.GRE.03	**NA**	**NA**	**NA**	**NA**	**NA**	d	D	D	D	D
FE	RI.GRE.04	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	cD	D	D	D
FE	RI.GRE.05	**NA**	**NA**	**NA**	**NA**	**NA**	c	C	Cdo	CDO	CDO
FE	RI.GRE.06	**NA**	**NA**	**NA**	**NA**	**NA**	N	c	Cd	CD	CD
FE	RI.GRE.07	**NA**	**NA**	**NA**	**NA**	**NA**	N	c	Cd	CD	CD
FE	RI.GRE.08	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	cd	CD
FE	RI.PON.01	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	c	C	**NA**	**NA**
EF	HO.POL.02	N	N	d	D	N	N	N	N	d	D

EF	HO.POL.04	N	N	N	**NA**	N	**NA**	c	C	C	C
EF	HO.POL.05	N	N	N	**NA**	d	D	**NA**	**NA**	d	D
EF	HO.POL.06	N	N	**NA**	**NA**	d	D	N	**NA**	cd	CD
EF	HO.POL.07	N	N	**NA**	**NA**	d	D	**NA**	**NA**	d	D
EF	HO.DUC.01	N	N	d	D	c	Co	CO	CO	C	C
EF	HO.DUC.02	N	N	N	**NA**	N	N	c	**NA**	do	DO
EF	HO.DUC.04	N	d	D	N	cd	CD	CD	CD	CD	CD
EF	HO.DUC.06	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	c	C	C	C
EF	HO.ARI.01	**NA**	c	d	**NA**	N	N	N	**NA**	**NA**	**NA**
EF	HO.ARI.02	d	cD	D	**NA**	cd	CD	cd	CD	**NA**	**NA**
EF	HO.ARI.03	**NA**	**NA**	**NA**	**NA**	d	cD	N	**NA**	**NA**	**NA**
EF	HO.ARI.04	**NA**	**NA**	**NA**	**NA**	cd	CD	N	**NA**	**NA**	**NA**
EF	HO.ARI.05	**NA**	**NA**	**NA**	**NA**	d	cD	N	**NA**	**NA**	**NA**
EF	HO.MOT.01	**NA**	**NA**	**NA**	**NA**	c	C	C	C	C	C
EF	HO.MOT.02	**NA**	**NA**	**NA**	**NA**	c	C	C	**NA**	**NA**	N
EF	HO.MOT.03	**NA**	**NA**	**NA**	**NA**	c	C	C	C	C	N
EF	HO.MOT.04	**NA**	**NA**	**NA**	**NA**	c	C	C	C	C	C
EF	HO.MOT.05	**NA**	**NA**	**NA**	**NA**	c	C	C	C	C	C
EF	HO.MOT.06	**NA**	**NA**	**NA**	**NA**	c	C	C	**NA**	**NA**	N
EF	HO.MOT.07	**NA**	**NA**	**NA**	**NA**	c	C	C	**NA**	**NA**	N
EF	HO.MOT.08	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	cd	CD	CD	CD
EF	HO.MOT.09	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	cd	CD	CD	CD
EF	HO.MOT.10	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	cd	CD	CD	CD
EF	HO.MOT.11	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	d	D	D	D
EF	HO.MEA.01	N	N	N	N	d	D	D	N	N	N
EF	HO.MEA.02	N	N	**NA**	**NA**	do	DO	DO	DO	DO	DO

Table S3. Tests for co-usage of ponds at some point of the reproductive season. P-values are obtained from permutation tests (see Methods) and indicate probability of significant differences between expected and observed co-occurrence in the direction that these differences are found.

Area	sp1	sp2	Observed co-occurrence	Expected co-occurrence	p
mid expansion	other anurans	*D. pictus*	3	2.7	0.700
mid expansion	other anurans	*E. calamita*	1	0.9	0.708
mid expansion	*D. pictus*	*E. calamita*	2	2.7	0.300
far expansion	other anurans	*D. pictus*	6	5.1	0.295
far expansion	other anurans	*E. calamita*	5	5.1	0.705
far expansion	*D. pictus*	*E. calamita*	13	13.5	0.578
expansion front	other anurans	*D. pictus*	3	2.0	0.279
expansion front	other anurans	*E. calamita*	2	2.3	0.545
expansion front	*D. pictus*	*E. calamita*	12	14.0	0.063

Figure S1. Model-averaged importance of terms – proportion of the 100 best models including the term – explaining the use by *Epidalea calamita* at least once of a pond for reproduction.

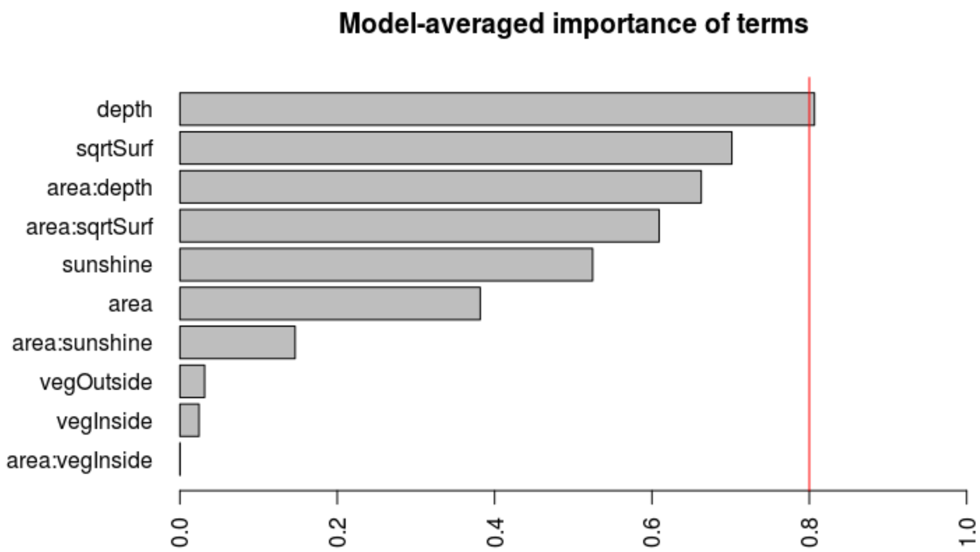

Figure S2. Predicted probabilities of use for reproduction at least once by *Epidalea calamita* according to pond maximum depth.

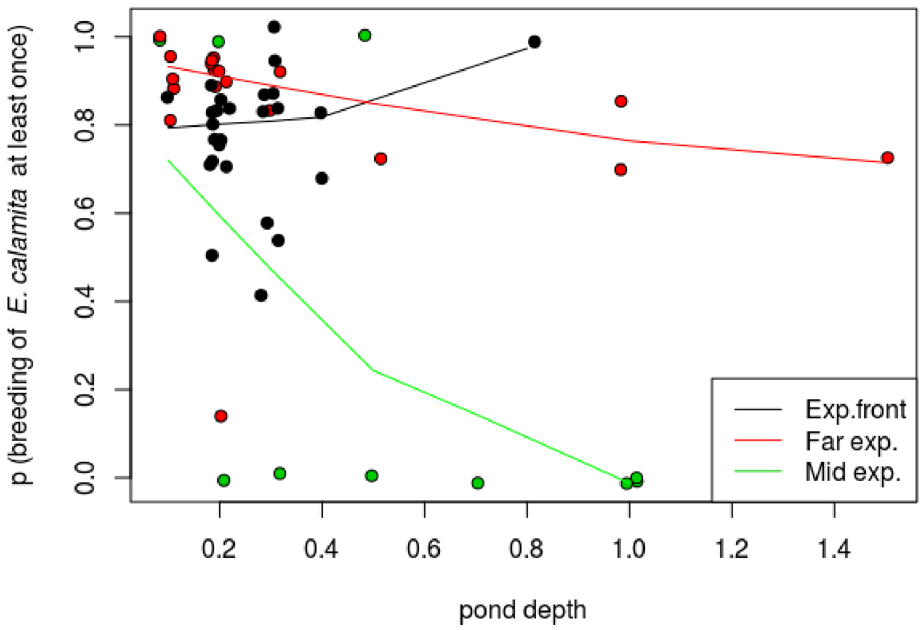

Table S4. Ten best models explaining pond choice for *Epidalea calamita*, sorted downwards starting from the best. Empty spaces indicate that the term is absent from the model, written names indicate that the term is included in the model.

model	area	vegInside	vegOutside	sqrtSurf	depth	sunshine	area:sqrtSurf	area:depth	area:sunshine	aicc	weights	Δaicc
1				sqrtSurf	depth		area:sqrtSurf	area:depth		59.29	0.23	0.00
2				sqrtSurf	depth	sunshine	area:sqrtSurf	area:depth		60.17	0.15	0.88
3	area			sqrtSurf	depth		area:sqrtSurf	area:depth		61.89	0.06	2.60
4					depth			area:depth		62.71	0.04	3.42
5					depth	sunshine		area:depth		62.85	0.04	3.56
6	area					sunshine				62.86	0.04	3.57
7	area			sqrtSurf	depth	sunshine	area:sqrtSurf	area:depth		63.46	0.03	4.17
8	area			sqrtSurf	depth	sunshine	area:sqrtSurf		area:sunshine	63.49	0.03	4.20
9	area									63.86	0.02	4.56
10	area					sunshine			area:sunshine	63.87	0.02	4.58

Table S5. Coefficients for the terms predicting the presence of *E. calamita* in a pond, including as explanatory variables in the model all terms present in more than 40% of the 100 best models.

	coefficient	Std. Error
(Intercept)	–0.93	2.26
areaRI	3.65	2.65
areaGI	–28.29	6813.96
sqrtsurf	0.59	0.54
depth	–3.39	7.03
sunshine	1.67	1.40
areaRI:sqrtsurf	–1.14	0.66
areaGI:sqrtsurf	61.05	7910.80
areaRI:depth	4.27	7.28
areaGI:depth	–503.12	59606.46

Fig S3. Predicted probabilities of use for reproduction at least once by *Epidalea calamita* according to the square root of the pond area.

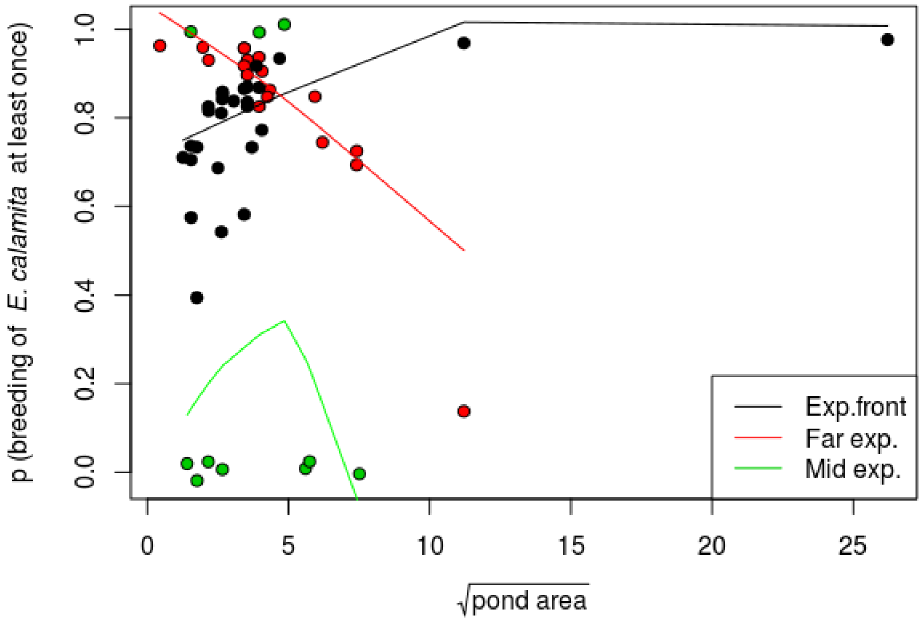

Fig S4. Predicted probabilities of use for reproduction at least once by *Epidalea calamita* according to the proportion of the pond usually under direct sunshine.

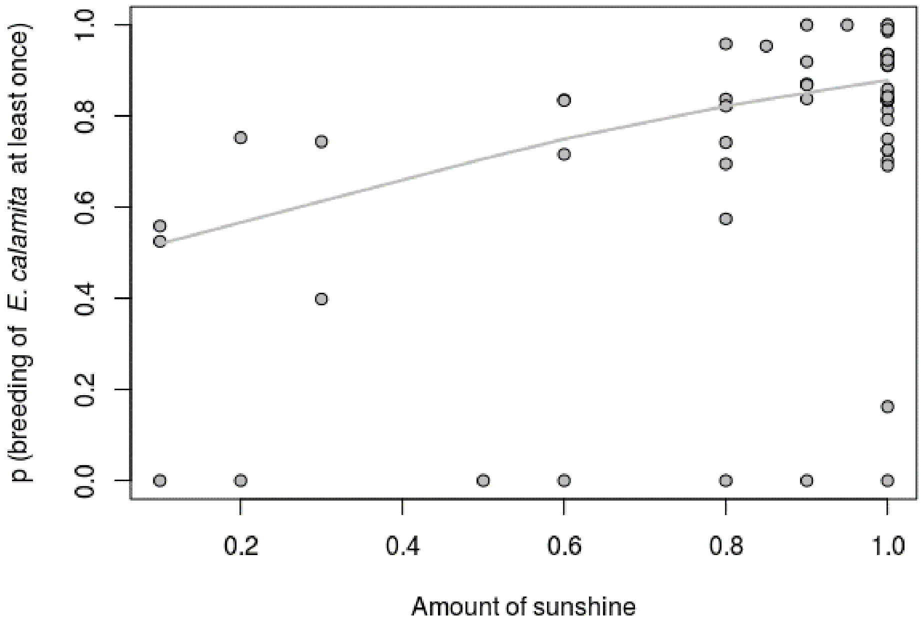

Fig S5. Model-averaged importance of terms – proportion of the 100 best models including the term – explaining the use by *Discoglossus pictus* at least once of a pond for reproduction:

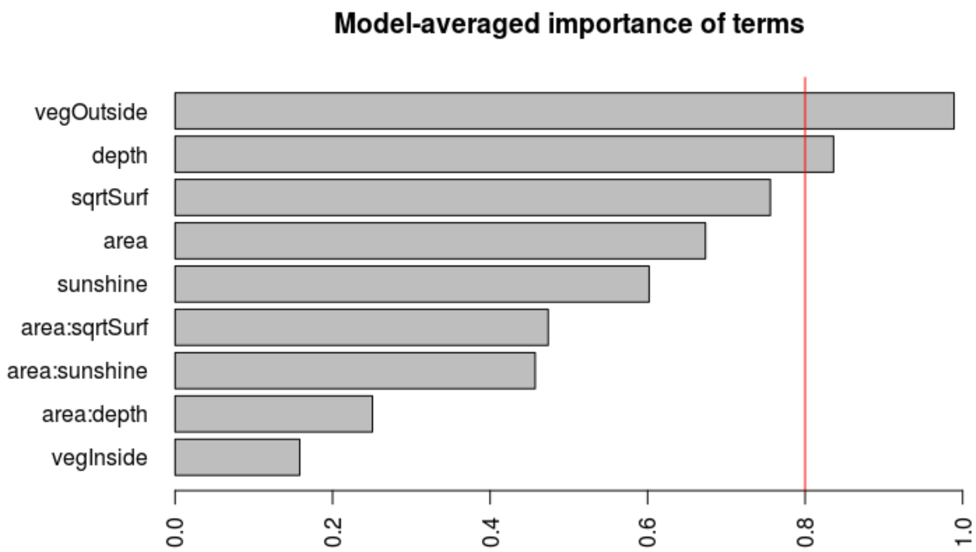

Fig S6. Predicted probabilities of use for reproduction at least once by *Discoglossus pictus* according to the square root of the pond area.

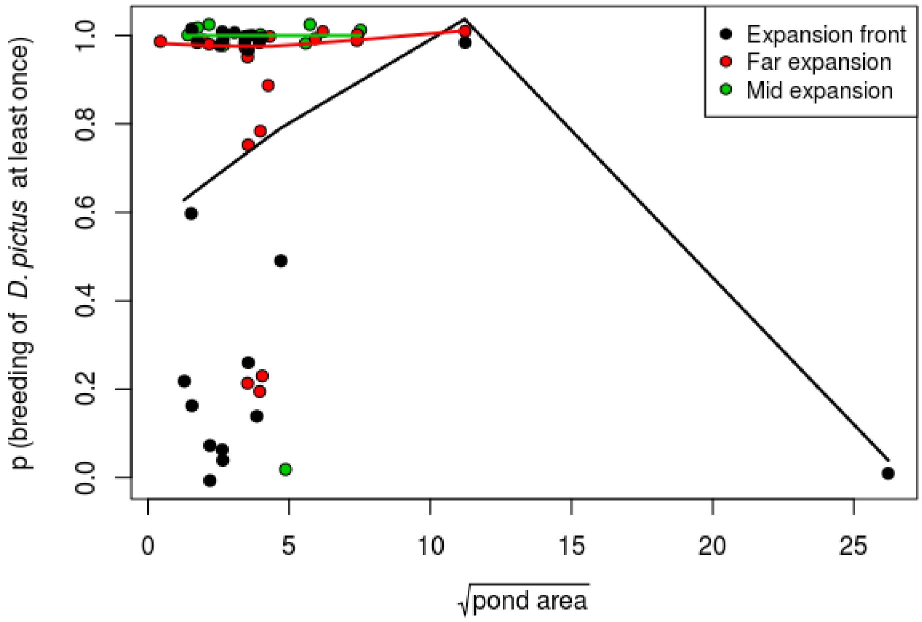

Table S6. Ten best models explaining pond choice for *Discoglossus pictus*, sorted downwards starting from the best. Empty spaces indicate that the term is absent from the model, written names indicate that the term is included in the model.

model	area	vegInside	vegOutside	sqrtSurf	depth	sunshine	area:sqrtSurf	area:depth	area:sunshine	aicc	weights	Δaicc
1	area		vegOutside	sqrtSurf	depth		area:sqrtSurf			42.88	0.20	0.00
2			vegOutside	sqrtSurf	depth	sunshine	area:sqrtSurf		area:sunshine	43.34	0.16	0.46
3	area		vegOutside	sqrtSurf	depth			area:depth		44.52	0.09	1.64
4	area		vegOutside	sqrtSurf	depth	sunshine			area:sunshine	44.85	0.08	1.97
5	area		vegOutside	sqrtSurf	depth	sunshine	area:sqrtSurf			45.51	0.05	2.63
6	area		vegOutside			sunshine			area:sunshine	45.98	0.04	3.10
7	area		vegOutside		depth	sunshine			area:sunshine	46.03	0.04	3.14
8			vegOutside	sqrtSurf	depth	sunshine		area:depth	area:sunshine	46.86	0.03	3.98
9	area		vegOutside	sqrtSurf	depth	sunshine		area:depth		47.29	0.02	4.41
10	area	vegInside	vegOutside			sunshine			area:sunshine	47.31	0.02	4.42

Table S7. Coefficients for the terms predicting the presence of *D. pictus* in a pond, including as explanatory variables all terms present in more than 40% of the 100 best models.

	coefficient	Std. Error
(Intercept)	170.01	62561.61
areaRI	-158.10	62561.61
areaGI	-137.98	73915.07
vegOutside2	2.12	1.52
vegOutside3	55.37	13714.75
vegOutside4	178.86	34783.02
sqrtSurf	-0.97	0.84
sunshine	-175.12	62561.61
depth	24.66	13.05
areaRI:sqrtSurf	-3.74	3.24
areaGI:sqrtSurf	-36.32	7193.68
areaRI:sunshine	176.21	62561.61
areaGI:sunshine	304.50	73458.00

Fig S7. Predicted probabilities of use for reproduction at least once by *Discoglossus pictus* according to the proportion of the pond usually under direct sunshine.

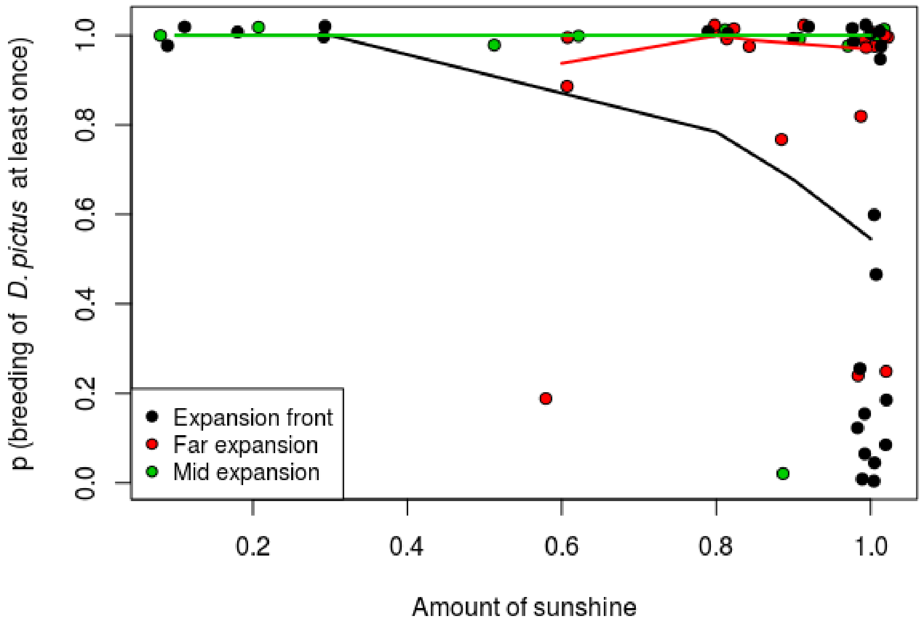

Figure S8. Flux diagram representing pond occupancy states (,) by tadpoles of two different competitors: 0 = absence of tadpoles of a species; 1 = presence of tadpoles of a species. That is, (0,0) means that the pond is empty of tadpoles, (1,1) that the both species are present as larvae, while (1,0) and (0,1) mean that only one species is present. The different types of lines in the arrows connecting the different occupancy states indicate certain types of events that lead to the presence or absence of a species, and therefore change the occupancy state. Continuous line: breeding events, add a species in the pond; Fine dashed line: drying of a pond, suppressing all tadpole species from the pond; One dash - two dots line: a whole guild of a species secures metamorphosis, suppressing the presence of tadpoles of a species from the pond. *Comment:* Transitions between (0,1) and (1,0) are improbable because they need two events to happen at once. Moreover, the fact that a whole guild of a species secures metamorphosis is really infrequent in ephemeral and temporary ponds. However, drying of ponds and breeding events happen more often. These differences in probability usually leads the system to naturally accumulate double absences (0,0) or double presences (1,1).