

Table S1. Crayfish survey data and sample collection dates at each site as part of the *in-situ* validation study.

Site	Survey 1	No. captured 1	Survey 2	No. captured 2	No. recaptured 2	Survey 3	No. captured 3	No. recaptured 3	Survey 4	No. captured 4	No. recaptured 4
1	26/06/2018	3	28/06/2018	5	0						
2	26/06/2018	9	28/06/2018	10	0						
3	05/07/2018	33	11/07/2018	62	2	31/07/2018	68	0	01/08/2018	110	5
4	03/07/2018	0	21/07/2018	0	0	01/08/2018	0	0			
5	04/07/2018	40	06/07/2018	45	17						
6	09/07/2018	84	12/07/2018	75	11						

Table S2. eDNA sampling collection dates and volume filtered at each site as part of the *in-situ* validation study.

Site	Date	Time	Filtered volume	Water temp °C
1	22/06/2018	09:50	500ml	12.4
2	22/06/2018	11:05	1000ml	12.5
3	22/06/2018	15:50	500ml	14.1
4	22/06/2018	18:06	150ml	14.8
5	29/06/2018	11:40	1000ml	19
6	29/06/2018	15:00	250ml	16.7

Table S3. Table showing mismatches between the species-specific primers (WC2302) and the respective COI targeting sequence in *A. pallipes* and various other species closely related or likely to co-occur. Base pair mis-matches are highlighted in grey and matches in bases are highlighted in white.

Forward Primer		GCTGGGATAGTAGGGACTTCTTT			
Probe (reverse compliment)				GAATTAGGGCAGCCGGGCAG	
Reverse Primer (reverse compliment)					GTAGTGGTTACCGCCCATG
		1	23 43	62 91	109
AB443445.1	A.pallipes	GCTGGGATAGTAGGGACTTCTTT	GAATTAGGGCAGCCGGGCAG	GTAGTGGTTACCGCCCATG	
EU921148.1	P.leniusculus	GCTGGTATAGTGGGAACCTTCTCT	GAATTAGGTCAACCTGGAAG	GTTGTAGTCACGGCACATG	
GU727619.1	A.astacus	GCTGGGATAGTAGGAACCTCTTT	GAACTCGGTCAACCTGGGAG	GTAGTAGTAACTGCTCATG	
KP205431.1	F.limosus	GCTGGCATAGTAGGAACCTCATT	GAGTTGGGTGAGCCGGGAAG	GTGGTAGTTACAGCTCATG	
JQ421465.1	A.leptodactylus	GCTGGAATAGTGGGAACCTCTTT	GAACTAGGTCAACCAGGGAG	GTCGTAGTAACTGCTCATG	
KC499604.1	P.clarkii	GCTGGTATAGTCGGAACCTCATT	GAGTGCAGTCAACCAGGAAG	GTGGTAGTTACAGCTCATG	
LC228303.1	P.fallax	GCTGGTATAGTAGGGACTTCATT	GAGTTAGGTCAACCTGGTAG	GTAGTAGTTACAGCTCATG	
KY745779.1	C.quadricarinatus	TCCGGTATAGTAGGCACTTCCCT	GAACTTGGTCAACCAGGAAG	GTAATCGTCACAGCCCAAG	
MF744674.1	F.virilis	GCTGGGATAGTAGGGACTTCATT	GAGTTAGGTGAGCCAGGAAG	GTGGTAGTTACAGCTCATG	
MH235946.1	P.acutus	GCTGGGATAGTAGGGACTTCATT	GAGTTAGGTGAGCCAGGAAG	GTAGTAGTTACAGCTCATG	

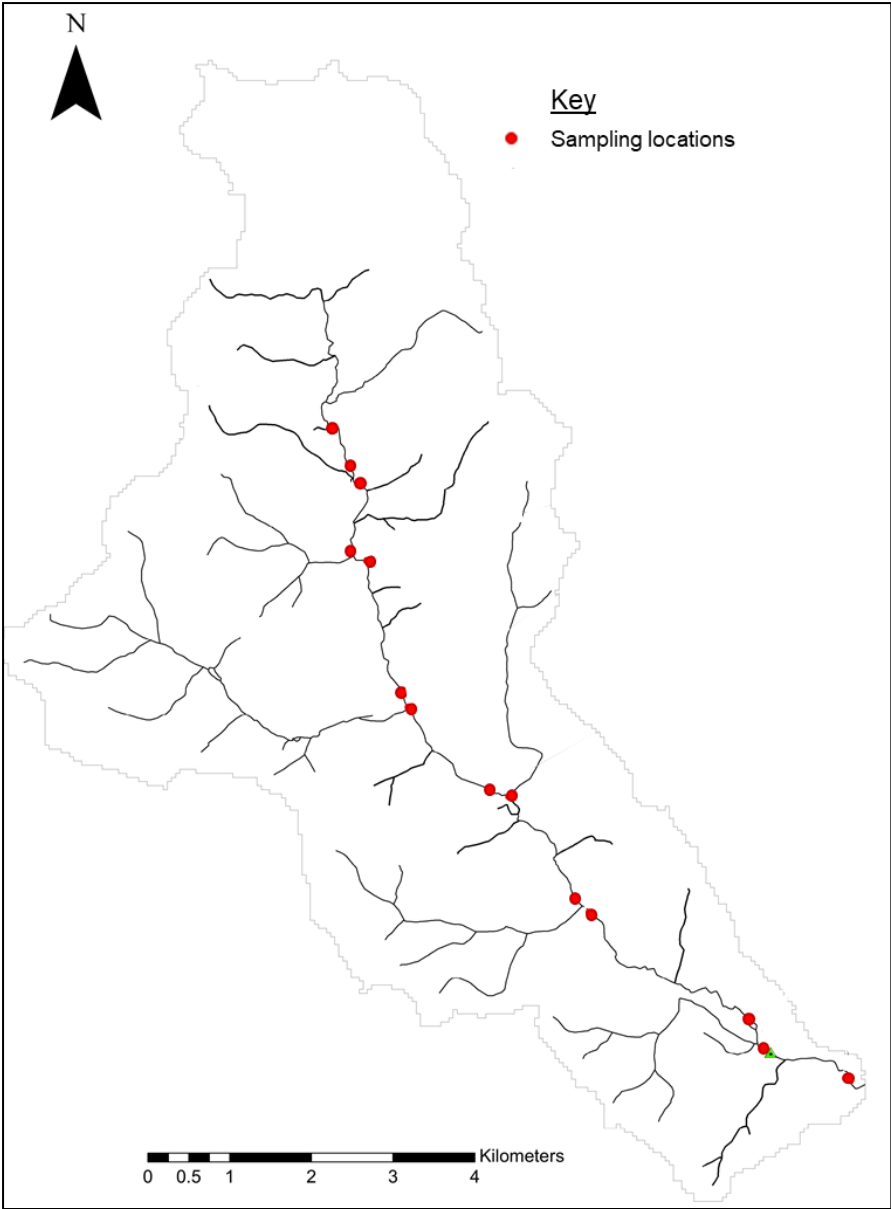


Figure S1. Location of sample collection sites within the Derbyshire river catchment.