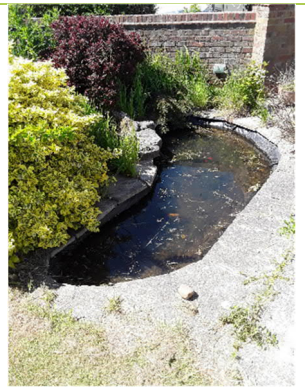
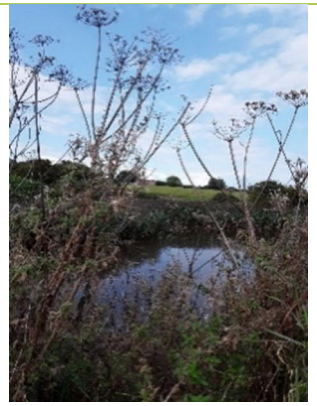
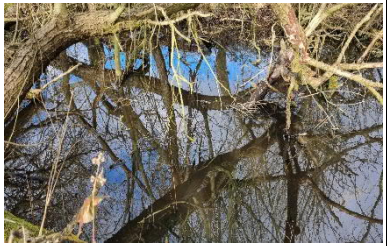
Factor	Pond 273			Pond 274		
	Value	Score	Photo	Value	Score	Photo
Description	Small pond at the end of a field, overshaded by trees.			Small ornamental garden pond with water lillies.		
Location (SI 1)	Zone A	1		Zone A	1	
Surface Area (SI 2)	50-100m2	0.1		<50m2	0.05	
Pond Drying (SI 3)	Sometimes	0.5		Never	0.9	
Water Quality (SI 4)	Moderate	0.67		Moderate	0.67	
Shade (SI 5)	66-70%	0.8		0-60%	1	
Waterfowl (SI 6)	Absent	1		Absent	1	
Fish (SI 7)	Absent	1		Possible	0.67	
Pond Density per km^2(SI 8)	>12	1		>12	1	
Terrestrial Habitat (SI 9)	Moderate	0.67		Moderate	0.67	
Macrophyte Cover (SI 10)	21-25%	0.55		<1%	0.3	
HSI Score and Suitability	0.63 (Average)			0.58 (Below Average)		

Factor	Pond 275			Pond 276		
	Value	Score	Photo	Value	Score	Photo
Description	Small depression within arable wheat field large enough to contain GCN.			Very small 5x2 m lined garden pond with stone tiles overhanging, and no aquatic vegetation		
Location (SI 1)	Zone A	1		Zone A	1	
Surface Area (SI 2)	250m2	0.5		<50m2	0.05	
Pond Drying (SI 3)	Annually	0.1		Never	0.9	
Water Quality (SI 4)	Good	1		Moderate	0.67	
Shade (SI 5)	0-60%	1		0-60%	1	
Waterfowl (SI 6)	Minor	0.67		Absent	1	
Fish (SI 7)	Absent	1		Absent	1	
Pond Density per km^2(SI 8)	10	0.95		6	0.84	
Terrestrial Habitat (SI 9)	Poor	0.33		Poor	0.33	
Macrophyte Cover (SI 10)	<1%	0.3		<1%	0.3	
HSI Score and Suitability	0.56 (Below Average)			0.55 (Below Average)		

Factor	Pond 277			Pond 278		
	Value	Score	Photo	Value	Score	Photo
Description	Small school pond which has concrete banks and base.			Five x 40 m, 1 m deep max. recently dug, occasional yellow flag and bulrush. No other aquatic vegetation present.		
Location (SI 1)	Zone A	1	No Photo	Zone A	1	
Surface Area (SI 2)	<50m2	0.05		200m2	0.4	
Pond Drying (SI 3)	Never	0.9		Rarely	0.9	
Water Quality (SI 4)	Poor	0.33		Moderate	0.67	
Shade (SI 5)	81-85%	0.5		0-60%	1	
Waterfowl (SI 6)	Absent	1		Absent	1	
Fish (SI 7)	Possible	0.67		Absent	1	
Pond Density per km^2(SI 8)	>12	1		4	0.72	
Terrestrial Habitat (SI 9)	Moderate	0.67		Moderate	0.67	
Macrophyte Cover (SI 10)	1-5%	0.35		1-5%	0.35	
HSI Score and Suitability	0.51 (Below Average)			0.73 (Good)		

Factor	Pond 279			Pond 280		
	Value	Score	Photo	Value	Score	Photo
Description	Recently dug oblong pond, 4 m x 25 m, max depth 1 m. Occasional bulrush, otherwise not well vegetated.			Recently dug oblong pond, 3 m x 20 m, max depth 1 cm. Occasional bulrush, otherwise not well vegetated.		
Location (SI 1)	Zone A	1		Zone A	1	
Surface Area (SI 2)	150m2	0.3		100m2	0.2	
Pond Drying (SI 3)	Rarely	0.9		Never	0.9	
Water Quality (SI 4)	Moderate	0.67		Moderate	0.67	
Shade (SI 5)	0-60%	1		0-60%	1	
Waterfowl (SI 6)	Absent	1		Absent	1	
Fish (SI 7)	Absent	1		Absent	1	
Pond Density per km^2(SI 8)	4	0.72		4	0.72	
Terrestrial Habitat (SI 9)	Moderate	0.67		Moderate	0.67	
Macrophyte Cover (SI 10)	1-5%	0.35		1-5%	0.35	
HSI Score and Suitability	0.71 (Good)			0.68 (Average)		

Factor	Pond 281			Pond 283		
	Value	Score	Photo	Value	Score	Photo
Description	Large waterbody between arable/cow pasture. Surrounded by scrub and tall ruderal vegetation and common reed.			Fresh water lagoon, run-off feeding back to plant on quarry.		
Location (SI 1)	Zone A	1		Zone A	1	
Surface Area (SI 2)	500m2	1		200m2	0.4	
Pond Drying (SI 3)	Never	0.9		Never	0.9	
Water Quality (SI 4)	Good	1		Moderate	0.67	
Shade (SI 5)	0-60%	1		0-60%	1	
Waterfowl (SI 6)	Major	0.01		Absent	1	
Fish (SI 7)	Possible	0.67		Absent	1	
Pond Density per km^2(SI 8)	3	0.65		7	0.85	
Terrestrial Habitat (SI 9)	Moderate	0.67		Poor	0.33	
Macrophyte Cover (SI 10)	21-25%	0.55		<1%	0.3	
HSI Score and Suitability	0.51 (Below Average)			0.68 (Average)		

Factor	Pond 284		
	Value	Score	Photo
Description	Irregular shaped pond in woodland, choked with leaf litter and overshaded. No aquatic vegetation present.		
Location (SI 1)	Zone A	1	
Surface Area (SI 2)	800m2	0.99	
Pond Drying (SI 3)	Never	0.90	
Water Quality (SI 4)	Poor	0.33	
Shade (SI 5)	96-100%	0.20	
Waterfowl (SI 6)	Absent	1	
Fish (SI 7)	Absent	1	
Pond Density per km^2(SI 8)	>12	1	
Terrestrial Habitat (SI 9)	Moderate	0.67	
Macrophyte Cover (SI 10)	<1%	0.30	
HSI Score and Suitability	0.54 (Below Average)		

Annex 5 Supplier Environmental DNA Analysis reports

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	10-May-2021
Order Number:	GCN21-1348
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
[redacted]					
EL2 P085	S21-011991	Negative	0	No	No
EL2 P0247	S21-011992	Negative	0	No	No
EL2 P087	S21-011993	Negative	0	No	No
[redacted]					
EL2 P052	S21-011995	Negative	0	No	No
[redacted]					
EL2 P092	S21-012007	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	18-May-2021
Order Number:	GCN21-1348
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 [redacted] P269	S21-011996	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	Clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	10-May-2021
Order Number:	GCN21-1355
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P229-B	S21-012039	Negative	0	No	No
EL2 P229	S21-012040	Positive	12	n/a	n/a
EL2 P253	S21-012041	Negative	0	No	No
EL2 P230	S21-012042	Positive	11	n/a	n/a
EL2 P225	S21-012043	Positive	11	n/a	n/a
EL2 P228	S21-012036	Negative	0	No	No
EL2 P235	S21-012037	Negative	0	No	No
EL2 P254	S21-012153	Negative	0	No	No

The results indicate that eDNA for great crested newts was detected in three of the samples and in the remaining samples eDNA was not detected (as detailed in the table above). Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	Clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	18-May-2021
Order Number:	GCN21-1355
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P197	S21-012154	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	Clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	01-Jun-2021
Order Number:	GCN21-1355
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 [redacted] P259	S21-012038	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	10-May-2021
Order Number:	GCN21-1361
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P246	S21-012084	Negative	0	No	No
EL2 P198-B	S21-012086	Negative	0	No	No
EL2 P172	S21-012087	Negative	0	No	No
EL2 P173	S21-012088	Negative	0	No	No
EL2 P171	S21-012089	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	18-May-2021
Order Number:	GCN21-1361
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P218	S21-012085	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	01-Jun-2021
Order Number:	GCN21-1383
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P129	S21-012212	Negative	0	No	No
EL2 [redacted] P271	S21-012215	Negative	0	No	No
EL2 P129a	S21-012216	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Clare Mcilwraith
Email:	clare.mcilwraith@aecom.com
Tel:	[redacted]
Report date:	13-Jul-2021
Order Number:	GCN21-1412
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
EL2 P277	S21-012422	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

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SureScreen Scientifics

Folio No: E10698
Report No: 1
Purchase Order: 60641917
Client: AECOM INFRASTRUCTURE & ENVIRONMENT
Contact: Clare Mcilwraith

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 01/06/2021
Date Reported: 09/06/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4090 P274	Eastern Link 2 [redacted]	SE7516430347	Pass	Pass	Pass	Negative	0
4091	EL2 P 110	483543, 437409	Pass	Pass	Pass	Negative	0
4096	Eastern Link P093	484437, 437699	Pass	Pass	Pass	Negative	0
4097	Eastern Link 2 P108	483843, 437563	Pass	Pass	Pass	Negative	0
4098	Eastern Link P109	SE837373	Pass	Pass	Pass	Negative	0
4099 P273	Eastern Link 2 [redacted]	SE7535330460	Pass	Pass	Pass	Negative	0
4101	Eastern Link 098	SE843376	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for

population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

SureScreen Scientifics

Folio No: E10851
Report No: 1
Purchase Order: 60641917-3.15/1472867
Client: AECOM INFRASTRUCTURE & ENVIRONMENT
Contact: Clare Mcilwraith, Jo Atkinson

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

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RESULTS

Date sample received at Laboratory: 09/06/2021
Date Reported: 16/06/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4102	[redacted] Pond 275	507179, 457295	Pass	Pass	Pass	Negative	0
5675	Pond 45	TA02529 54731	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Gabriela Danickova **Approved by:** Gabriela Danickova

METHODOLOGY

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