

Institution: University of Derby		
Unit of Assessment: 7		
Title of case study: The application and improved functionality of environmental DNA (eDNA) for assessing species distribution in freshwater environments		
Period when the underpinning research was undertaken: 2015–2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Environmental Sustainability Research Centre (ESRC) researchers:		
Michael Sweet	Associate Professor	September 2013–present
Mark Bulling	Senior Lecturer	August 2010–present
Andrew Ramsey	Head of Discipline	June 2013–present
Alfred Burian	Researcher	August 2017–July 2020
Period when the claimed impact occurred: 2015–2020		
Is this case study continued from a case study submitted in 2014? No		
1. Summary of the impact (indicative maximum 100 words) <p>Environmental DNA (eDNA) barcoding is set to increase the cost-efficiency for detection and monitoring of species in aquatic habitats. The research underpinning this case study has so far entailed the development and accurate validation of eDNA assays for 5 invertebrate and 2 vertebrate freshwater species with either endangered/protected or invasive status. The impact generated by the application of the eDNA assays has occurred straightaway: one of the eDNA assays developed, the white-clawed crayfish assay has already been transferred to industry and released as a commercial product. This has brought economic, capacity-building and environmental benefits extending synergistically beyond academia. Furthermore, the validation of the assays in the field has already transformed the ecological monitoring programmes of governmental (<i>Environmental Agency, Natural Resource Wales</i>), charitable (<i>Dorset Wildlife Trust, Zoological Society of London</i>) and independent scientific wildlife and habitat-protecting organisations (<i>Freshwater Biological Association</i>), which have endorsed the developed eDNA assays in their freshwater species surveys.</p>		
2. Underpinning research (indicative maximum 500 words) <p>The development of efficient and cost-effective surveying techniques is crucial for the success of monitoring programmes aimed at the conservation of threatened species and charting the spread of invasive species. One relatively new monitoring method is the use of environmental DNA (eDNA). This is nuclear or mitochondrial DNA that is released from an organism into any given environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin or hair, and carcasses. In aquatic environments, eDNA is diluted and distributed by currents and other hydrological processes and the quality or abundance of this fragmented DNA is highly influenced by environmental parameters such as temperature, salinity, and flow rates for example. Therefore, although showing great promise, a significant level of validation must be conducted when designing new assays and implementing their usage in the field.</p> <p>Since 2015, researchers in the Environmental Sustainability Research Centre (ESRC, University of Derby), Sweet, Bulling, Ramsey and Burian (please see current roles and employment dates in</p>		

above), have carried out novel research on the development, validation and improvement of eDNA assays for the detection of species of invertebrates and vertebrates, with either endangered/protected or invasive status.

The research outputs underpinning this case study are:

- Development of a new eDNA technique targeting both the native white clawed crayfish (*Austropotamobius pallipes*) and invasive crayfish species. The white clawed crayfish is classified as endangered (by the International Union for Conservation of Nature, IUCN) throughout its range in Western Europe. One of the major threats to this species is the spread of invasive, non-native, crayfish species which can out-compete the species and can carry 'crayfish plague', a lethal fungal disease to which white clawed crayfish are particularly susceptible. This is the first study to design and rigorously validate eDNA as a tool for assessing the presence/absence of white clawed crayfish, taking degradation rates and seasonality into the framework [3.1].
- Validation and assessment of an eDNA assay designed to target the freshwater pearl mussel, *Margaritifera margaritifera* and recommendations for standardisation of protocols for all eDNA assays in the future [3.2]. This species is classified by the IUCN as endangered throughout its Holarctic range and it is protected by law in all European countries.
- Development and validation of eDNA assays for the detection of the highly invasive demon shrimp, *Dikerogammarus haemobaphes* [3.3] and the Marmorkrebs or marbled crayfish, *Procambarus virginalis* [3.4].
- Validation of eDNA assays targeting two critically endangered freshwater fishes: *Valencia robertae* and *V. letourneuxi*. [3.5].
- Molecular (with double droplet PCR, ddPCR) and field validation of eDNA assays for the detection of the critically endangered stonefly *Isogenus nubecula*. This research has involved the adaptation of assays to be utilised by ddPCR to improve detection rates [3.6].

This body of research has led to:

- a) The commercialisation of one of the eDNA assays.
- b) The application of the eDNA assays by end-users to undertake impact surveys at regional (Environment Agency), national (Dorset Wildlife Trust, Natural England, Freshwater Biological Association, Natural Resources Wales), and international locations (The Hellenic Centre for Marine and Inland Waters Research, HMCR, Greece).

3. References to the research (indicative maximum of six references)

Publications in peer-reviewed journals

University of Derby staff researchers are indicated by black, underlined text:

[3.1] Troth C., Burian, A., Mauvisseau, Q., Bulling, M., Nightingale, J., Mauvisseau, C. and Sweet, M. (2019) 'Development and application of eDNA-based tools for the conservation of white-clawed crayfish', *Science of The Total Environment*, online 30 July 2020, 141394. DOI: <https://doi.org/10.1101/732941>. [Accurate validation of a new eDNA assay in the lab and field in order to launch it as commercial product.]

[3.2] Mauvisseau, Q., Burian, A., Gibson, C., Brys, R., Ramsey, A. and Sweet, M. (2019) 'Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches', *Scientific Reports* 9, 580. DOI: <https://doi.org/10.1038/s41598-018-37001-y>.

[Rigorous assessment on how to improve the efficiency of eDNA sampling strategies for the rare and protected freshwater pearl mussel *M. margaritifera*.]

[3.3] Mauvisseau, Q., Troth, C., Young, E., Burian, A. and Sweet, M. (2019) 'The development of an eDNA based detection method for the invasive shrimp *Dikerogammarus haemobaphes*', *Management of Biological Invasions* 10(3), 449-460.

DOI: <https://doi.org/10.3391/mbi.2019.10.3.03>. [A novel eDNA assay is presented to detect this shrimp species, including a comprehensive validation in silico, ex-situ and in the field.]

[3.4] Mauvisseau, Q., Tönges, S., Andriantsoa, R., Lyko, F. and Sweet, M. (2019) 'Early detection of an emerging invasive species: eDNA monitoring of a parthenogenetic crayfish in freshwater systems', *Management of Biological Invasions* 10(3), 461-472.

DOI: <https://doi.org/10.3391/mbi.2019.10.3.04>. [Design and in situ validation in Germany of a new assay for the highly invasive Marmorkrebs.]

[3.5] Mauvisseau, Q., Kalogianni, E., Zimmerman, B., Bulling, M., Brys, R. and Sweet, M. (2020) 'eDNA based monitoring: advancement in management and conservation of critically endangered killifish'. *Environmental DNA* online 26 May 2020. DOI: <https://doi.org/10.1002/edn3.92>.

[Groundwork research on the development of an eDNA assay targeting 2 critically threatened and 1 invasive fish species, including accurate validation in freshwaters from Greece.]

[3.6] Mauvisseau, Q., Davy-Bowker, J., Bulling, M., Brys, R., Neyrinck, S., Troth, C. and Sweet, M. (2019) 'Combining ddPCR and environmental DNA to improve detection capabilities of a critically endangered freshwater invertebrate', *Scientific Reports* 9, 14064.

DOI: <https://doi.org/10.1038/s41598-019-50571-9>. [Development of a new eDNA assay to detect the extremely rare stonefly *I. nubecula* and comparison with traditional ecological methods to detect this species.]

This Research has been part-funded by SureScreen Scientifics (GBP89,750), which supported two part-funded PhD studentships, plus the Zoological Society of London (GBP7,500).

4. Details of the impact (indicative maximum 750 words)

The majority of published eDNA assays cannot immediately be applied on a commercial scale for conservation and monitoring schemes. The key contribution of the ESRC eDNA team is the rigorous assessment of the validation requirements for commercially applicable eDNA assays and the utilisation of this information to **develop novel eDNA assays ready for commercial, end-user application**.

Consequently, a primary **commercial innovation impact** has been underpinned by the research. The white-clawed and signal crayfish eDNA assays developed by ESRC researchers at the University of Derby were adopted and released as a commercial service by SureScreen Scientifics on 1 October 2018 [5.1]. As the website of the company stated (access date 8 January 2020), there is a "strong partnership with Dr Michael Sweet and The University of Derby to bridge the gap between researching useful assays and delivering real solutions to help the ecological industry" [5.2]. The partnership is ongoing and is articulated through the development and validation of additional eDNA assays targeting, for example, the detection of the endangered freshwater pearl mussel *Margaritifera margaritifera*, and the invasive demon shrimp *Dikerogammarus haemophabes*. The significant and far-reaching repercussions of this collaboration were contextualised by the Science Minister of the UK in his speech delivered on the 10 July 2019. The Minister advocated the role of the University of Derby through his words "... universities are remarkably skilled at identifying where they can have the greatest impact – locally, regionally, nationally and globally – and just getting on and doing it. For example, the work being spearheaded at the University of Derby on environmental DNA, working with SureScreen" [5.3]. All this evidence augurs a long-lasting entrepreneurial relationship generated by the innovative research of the University of Derby's ESRC eDNA team and the transfer of its expertise to the company.

The research on eDNA by the ESRC team has also acted as a catalyst for additional benefits that extend well beyond academia:

Economic impact: The white clawed crayfish test has provided a new service for SureScreen Scientifics which has had a multi-faceted impact, spreading the company's risk, increasing profit and revenue, and extending the client base and the period for seasonal business. As evidenced by SureScreen Scientific's Managing Director testimonial: *"The new crayfish service has increased SureScreen Scientific's eDNA revenue by 5%..."* [5.1].

Moreover, the application of the ESRC research informed eDNA assays has made sample collection and water testing more economically efficient for wildlife and environmental organisations and charities. As the Chief Officer at the Zoological Society in London (ZSL) states [5.4]: *"....in terms of time needed, the eDNA method requires approx. 1 h for field work (to obtain and filter the water samples) as opposed to 2 h for fish sampling. Similarly, eDNA sampling requires a 2-member field team as opposed to a 4-member field team for sampling by electrofishing. This implies that all the rest of the costs for field work (transportation, accommodation, etc) is reduced to half in the case of eDNA sampling"*. The time-effectiveness of this assay has been evidenced by a comprehensive study in streams of the Wyre Forest, where the time effort was estimated to be 4 times less than obtained with a catchment-wide survey [5.5].

Added to this, ESRC researchers have had a positive **Pedagogical impact** by increasing University of Derby graduate employment prospects through their partnership with SureScreen. This has entailed promoting their undergraduate and postgraduate student talent into SureScreen's workplace. This has also benefitted the company by augmenting its capacity and expertise in eDNA. Effectively, SureScreen Scientifics has funded two PhD studentships within the ESRC eDNA team (£65,000 each), for Quentin Mauvisseau (April 2017 to June 2020) and for Chris Troth (November 2015 to December 2019). Employment opportunities have also been generated from this partnership with industry: the increase of the company's revenue due to the new crayfish service has permitted Dr. Troth to transition to a permanent staff position with SureScreen [5.1].

The ESRC eDNA research has also triggered a very clear **Environmental impact**. The environmental benefit of the barely habitat-disturbing eDNA surveys has been evidenced by the improved detection for rare, threatened and invasive species in the field. Moreover, the eDNA assays for the detection of the white clawed crayfish and the pearl mussel open a new avenue for the conservation of these species [5.1]. The National Centre for Universities and Business explicitly includes the University of Derby eDNA research in its last report (2019), where it is stated that: *"University of Derby has an international reputation in the use of eDNA as an emerging tool for monitoring and quantifying biodiversity in the natural environment"* [5.6, p. 25].

Since 2017, and as a result of the expertise developed through the underpinning research, the ESRC eDNA team has been pivotal in instrumenting their novel techniques for species-distribution surveys through charitable (**Dorset Wildlife Trust, Zoological Society of London**) and scientific independent wildlife and habitat protecting organisations (**Freshwater Biological Association**), as well as governmental institutions in UK (**Environment Agency, Natural Resource Wales**) and overseas (**The Hellenic Centre for Marine and Inland Waters Research**).

The Zoological Society of London (ZSL), a conservation charity with a strong focus on freshwater biodiversity monitoring, has also benefitted from ESRC eDNA research as their own testimony acknowledges [5.4]: *"this eDNA assay allows a potential expansion of our work to other species and other locations that we work in. This increases our ability to monitor threatened species in our projects, helps influence conservation planning and ultimately provides monitoring options in remote locations where electrofishing is not possible or desired"*.

The Environment Agency (East Midlands Fisheries Team) commissioned the ESRC eDNA team to use the novel assay developed for white-clawed crayfish, signal crayfish and crayfish plague to

inform on a weir removal project in Derbyshire, where traditional ecological survey methods had been unsuccessful in detecting the crayfish [5.7].

The ESRC eDNA research has also informed the Dorset Wildlife Trust through a report that found white-clawed crayfish in a river where detection of individuals using traditional methods had failed, and assessed why disease outbreak had occurred in the apparent absence of signal crayfish [5.8]. Natural Resources Wales entrusted the ESRC eDNA team to undertake a state of the art survey on the critically endangered stonefly using their novel combined eDNA assay and double droplet PCR (dd PCR) technique [5.9]. The significant impact of this work was vividly evidenced by an Honorary Fellow of the Freshwater Biology Association (FBA) *"This technology could transform our ability to locate populations of our rarest freshwater invertebrates – an essential first step in their conservation. I envisage eDNA assays becoming an increasingly important technology in UK freshwater invertebrate conservation. The pioneering work by the Derby team has made great progress in achieving this advance"*. [5.10]. Moreover, the FBA has been involved with this landmark research. As a FBA Honorary Fellow claims: *"This work has raised the profile of the FBA and enabled the FBA to contribute to the development of this exciting new technology"*. [5.10].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[5.1] Testimonial Letter by the Managing Director of SureScreen Scientifics Ltd. 11 February 2020.

[5.2] SureScreen Scientifics Ltd. website, html version (Accessed: 8 January 2020).

[5.3] Transcript of the speech 'Becoming an Innovation Nation: Driving up private investment into research and development' as it was delivered by the Minister of Science on the 10 July 2019. Source: Government Releases. 'The Minister speaks about the role of private investment in achieving the Industrial Strategy ambition to invest 2.4% of GDP in R&D by 2027'. Available at: <https://www.gov.uk/government/speeches/becoming-an-innovation-nation-driving-up-private-investment-into-research-and-development> (Accessed: 20 November 2019).

[5.4] Testimonial Letter by the Chief Officer from the Zoological Society of London (ZSL). 29 January 2020.

[5.5] Testimonial Letter by eDNA assay end-user. 2 February 2020.

[5.6] Page 25 of the National Centre for Universities and Business, 'State of the Relationship Report 2019'. Available at: <https://www.ncub.co.uk/images/reports/State-of-the-Relationship-Report-2019.pdf> (Accessed: 4 December 2019).

[5.7] Report commissioned by the Environment Agency, 'Assessing Signal Crayfish Distribution to Inform Fish Pass Installation in the River Ecclesbourne (Derbyshire) Using Environmental DNA (eDNA)', January 2018. Authors: Chris Troth and Michael Sweet (University of Derby).

[5.8] Report commissioned by the Dorset Wildlife Trust, 'Assessing Crayfish Distribution in the River Allen (Dorset) Using Environmental DNA', May 2018. Authors: Chris Troth and Michael Sweet (University of Derby).

[5.9] Evidence Report Number 326 commissioned by Natural Resources Wales (NRW), 'A survey of the River Dee for the Scarce Yellow Sally stonefly *Isogenus nubecula* in spring 2018', February 2019. Authors: J. Davy-Bowker, Q. Mauvisseau, M. Hammett, M. Sweet and C. Troth. Available at: <https://cdn.naturalresources.wales/media/689743/nrw-evidence-report-no-326-a-survey-of-the-river-dee-for-the-scarce-yellow-sally-stonefly-i.pdf> (Accessed: 23 February 2020).

[5.10] Testimonial Letter by an Honorary Fellow of the Freshwater Biology Association. 7 December 2019.