Introduction: Special Issue on Molecular Detection of Trophic Interactions

Unpicking the tangled bank

William O.C. Symondson[1], *James D. Harwood*[2]

[1]Cardiff School of Biosciences, Sir Martin Evans Building, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK

[2]Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546, USA

It is now seven years since a conference was held at the University of Innsbruck, Austria, that for the first time was dedicated exclusively to the emerging field of the Molecular Detection of Trophic Interactions. Since then the technology, and as a consequence the ecological questions that can now be addressed, has moved on considerably and the field continues to grow exponentially. In this Special Issue we have published 27 papers prepared by attendees at the second such meeting held in May 2013, at the University of Kentucky, organised locally by James Harwood. These papers cover a diverse array of ecological studies and diagnostic techniques, and include predation, parasitism and herbivory, by and/or on vertebrates and invertebrates, in both terrestrial and aquatic systems. Together they provide, in a single issue of the journal, an excellent opportunity to assess the current state of play in this rapidly developing field.

Probably the biggest change since the last meeting has been the application of Next Generation Sequencing (NGS) to trophic analyses. What a predator eats depends upon what else is available, and for the first time it is possible to rapidly detect the whole spectrum of prey or plant remains that a predator or herbivore may have eaten. Nine of the 27 papers use this technology, which has dropped enormously in price, bringing it within reach of cash-strapped ecologists. This has partly been brought about by the introduction of bench-top sequencers, such as the Ion Torrent machines, but these in turn are being replaced by even smaller and cheaper equipment as new developments are made at an accelerating pace. In most recent studies, DNA from predator gut or faecal samples has been tagged, by individual or sub-group/treatment, permitting the spectra of prey consumed to be separately analysed across a population or between populations. Seven of these nine papers analyse the diets of bats (Burgar *et al.* 2014; Clare *et al.* 2014b,b; Emrich *et al*. 2014; Krüger *et al.* 2014a,b; Sedlock *et al.* 2014), one the diets of shrews and skinks (Brown, Burger *et al*. 2014a), and one prey consumption by seals (Thomas *et al.* 2014), reflecting the dominant (but not exclusive) use of NGS to date for analysis of vertebrate (versus invertebrate) predator diets. In all cases, these were predators that could have chosen, in the field, to eat from among tens to hundreds of different prey items; no other approach could have analysed such samples as efficiently.

One of the problems with NGS, particularly in tropical rainforest and other biodiversity hot spots, is that many prey species cannot be identified from their DNA sequences. Barcoding of species in such locations is very patchy, requiring molecular operational taxonomic units (MOTUs) detected within faecal samples to be classified (as far as possible) by family or even order. Where diets are compared between different predator species, it is possible to analyse niche breadth and niche overlap entirely by MOTUs, even when few or none of the sequences can be identified to species (e.g. Brown *et al*. 2014a; Burgar *et al*. 2014; Sedlock *et al*. 2014). Thus, even though it is not known precisely what they were eating, it is possible, for example, to detect resource partitioning (e.g. Burgar *et al*. 2014; Emrich *et al.* 2014; Krüger *et al*. 2014a; Sedlock *et al*. 2014) and direct competition (Brown *et al.* 2014). Nevertheless, this gap in our knowledge should provide a further incentive for extensive barcoding of biota. Where possible, and where the range of items in the diet is limited, those using

NGS may in the meantime need to barcode biota within the habitats under study if they are to improve the resolution of their analyses.

The range of ecological questions being addressed by the papers using NGS is broad but like almost all molecular diagnostic work on diets there are biases that have to be considered. The hope originally was that numbers of sequences generated by NGS would provide a good estimate of biomass consumed. Unfortunately, this is not the case. Homology at the primer site, rates of digestion of different tissues, density of mtDNA copies per cell, competitive PCR biases and other factors all affect numbers of sequences amplified, even when the same predator species and the same primers are used throughout. Here, Thomas *et al*. (2014) found that lipid content of different fish fed to seals can also affect results and could be incorporated into models adjusting for bias. Such adjustments may, as in this case, be specific to a particular study system. With NGS, therefore, many authors now simply record numbers of predators testing positive for a target prey or plant species, providing a pragmatic and useful surrogate for truly quantitative information.

Next Generation Sequencing is certainly not a universal panacea. For example, analysis of the role of predators in crops or even natural ecosystems, where relatively few potentially influential prey species are present, can be done cost-effectively using species-specific primers (Raso *et al*. 2014; Schmidt *et al*. 2014; Šerić Jelaska *et al*. 2014). Some studies are concerned with analysing the range of predators attacking a single target species (e.g. Lundgren & Fergen 2014). The great advantage of species-specific primers is that large numbers of predators can be individually and rapidly screened, providing the levels of replication often needed for analysis of invertebrate food webs.

The question of biases is greatest where different predator species are compared and/or where results are obtained using different primers. The wider use of qPCR can increase sensitivity and help quantify prey DNA in samples (Eitzinger *et al*. 2014; Leal *et al.* 2014a; Lundgren & Fergen 2014; Redd *et al.* 2014), and is excellent for comparing relative predation rates of particular predator-prey pairings over time or between treatments, but is still subject to these biases. Thus, the whole question of biases remains problematic, but in this Special Issue there is a spectrum of studies where biases are reduced or managed in a range of effective ways. Invertebrate food webs are the primary challenge, where there is great interest in how interactions between and within several trophic levels can lead to changes in populations. The opinion piece by Greenstone *et al*. (2014) highlights the need for adjustments to be made based on feeding trials and digestion rates, allowing the sum effects of these biases to be parameterised. Where few species are involved, this is relatively simple (e.g. Welch *et al*. 2014). However, such feeding trials are not always practical in complex food webs, where multiple predators are feeding on multiple prey (and each other). One option is to do feeding trials on representative species within predator groups (defined by taxonomy, hunting mode or feeding mode), but not all species (Schmidt *et al*. 2014). Alternatively, recognising that suppression, for example, of crop pests can depend upon the concerted action of whole communities, it is possible to avoid making comparisons between individual predator species and look instead (separating predators only by feeding mode) at how predator abundance and diversity affect overall predation on a target pest (Lundgren & Fergen 2014). The approach of Wallinger *et al.* (2014) was to design sets of primer pairs that amplified DNA fragment of similar length, and they found (in earlier trials) that this allowed detectability of plant DNA in rootworms to be similar across most plant species. Eitzinger *et al*. (2014) looked at the interesting question of whether predator size, within species, might affect digestion rates too, and found that it did not (at least in centipedes).

It was very good to see aquatic systems well represented within the Special Issue, one freshwater and six marine. Krüger *et al.* (2014a) compared the diets of two sympatric 'aquatic' bats that trawl for insects (using feet and tail membranes) across the surface of rivers. Small differences in bat morphology were thought to be related to prey choice, one species feeding more on terrestrial prey

than the other. Alonso *et al.* (2014) analysed the diets of Cory's shearwaters feeding on fish and invertebrates, comparing factors such as sex, breeding status and breeding phase. Both of these papers report comparisons of morphological analysis of samples with molecular analysis, finding many more trophic links and greater taxonomic precision with the latter. The Alonso *et al*. (2014) paper in particular shows how the two approaches can be used in a complimentary way. Thomas *et al.* (2014) performed captive feeding trials with seals, to try to calibrate quantitative variables discussed above. Redd *et al.* (2014) highlight sources of error too, in their temporal and spatial analysis of Rock Lobsters feeding on sea urchins, finding that juveniles in particular may be picking up urchin DNA from the benthos, a problem that could potentially cause interpretive challenges with other studies involving benthic food webs. Dietary work is also reported from coral reefs and deep sea vents, by cnidarians (corals and sea anemones) and amphipods, respectively. It was shown in the lab that both an anemone and a coral digested their prey far more slowly than had previously been thought (Leal *et al*. 2014b), while some corals failed to capture and consume the microalgae offered or showed evidence of choice (Leal *et al.* 2014a). Studying trophic interaction in the deep ocean is even more of a challenge as the species will often only survive *in situ*. Olsen *et al*. (2014) found, using denaturing high performance liquid chromatography of 18S rDNA, that amphipods feeding at hydrothermal vents and cold seeps were far more omnivorous than previously suspected. The range of these aquatic studies illustrates well the breadth of species, habitats and trophic associations that can be studied using molecular diagnostics.

One group of predators that have rarely been studied before using molecular diagnostic are the reptiles. Brown *et al*. (2014a), as mentioned above, examined the diet of the rare Telfair's Skink on a Mauritian island, competing for invertebrate prey with the invasive Asian Musk Shrew. They also analysed the diet of the locally rare Smooth Snake in the UK, using a panel of species-specific primers, demonstrating that these snakes may be confined to small pockets of habitat where their main prey, other reptiles, are abundant (Brown *et al*. 2014b). One little-recognised advantage of analysing faecal DNA is that the same samples can be used to detect not only diet (and where necessary the species and genotype of the animal producing the faeces) but also gut parasites. Thus Brown & Symondson (2014) were able to examine a range of factors affecting prevalence of nematodes in the Slow Worm, a legless lizard.

From its infancy, molecular diagnostics has been used to better understand trophic interactions (both predation and parasitism) between invertebrates, in both natural and agricultural systems, and such papers are well represented here. A novel application of the analysis of trophic pathways is to use them to explain the movements of heavy metals through the environment. Šerić Jelaska *et al*. (2014) were able to analyse the bioaccumulation of such metals in carabid beetles, by linking the heavy metal burdens of the beetles to those of their main prey (in this case earthworms and slugs in forest ecosystems). Equally interesting is work on pioneer communities on land exposed as glaciers retreat. Few prey species are available to the spiders and carabids inhabiting such environments and Raso *et al*. (2014) showed clearly (using a combination of molecular diagnostics and stable isotope analysis) the intensity of competition between predator species, demonstrated by high rates of intraguild predation.

Much has been published to date on the application of molecular diagnostics to the effective detection of parasitoids within their hosts (often at an early stage of development), mostly in agricultural systems. Here we have three papers that take this approach in new directions, both technically and ecologically. Derocles *et al*. (2014) analyzed parasitoids attacking aphids in agricultural fields vs. field margins, and showed that there was strong compartmentalization, with few parasitoids attacking aphids in both environments. This suggests that, with parasitoids at least, field margins may be a poor source of useful natural enemies, contrary to much evidence already published. Two papers report the ability of molecular diagnostics to identify host and parasitoid

from empty pentatomid eggs (Gariepy *et al.* 2014) and empty aphid mummies (Varennes *et al*. 2014). Gariepy *et al*. (2014) were interested in determining the range of native parasitoids attacking an alien pentatomid bug, and were only limited in this endeavor by lack of a comprehensive parasitoid barcode database. Varennes *et al.* (2014), using general primers followed by single-stranded conformation polymorphism (SSCP), experimented in the laboratory with detection of parasitoid and hyperparasitoid DNA from empty aphid hosts. Such work is truly forensic and further extends the reach of molecular diagnostics.

Useful work continues to be conducted on biocontrol of crop pests by invertebrate generalist predators (Lundgren & Fergen 2014; Schmidt *et al*. 2014), although far fewer papers are represented here than were reported at the last meeting in Austria. The opinion piece by Greenstone *et al*. (2014) is aimed primarily at ways in which the accuracy of such work can be improved, using calibratory feeding trials and modelling to estimate a rank order of predators in terms of their effectiveness as biocontrol agents at a study site. Such efforts allow us to approach, if not finally answer, the question of precisely how many prey individuals are being consumed. Conventional food webs (such as host-parasitoid webs) require such numerical estimates in order to evaluate web dynamics. However, much can be done based simply on number of predators testing positive, as shown in the study of Lundgren and Fergen (2014), in which it was shown that both numbers and diversity of natural enemies had significant effects on a focal prey, corn root worm. Similarly, Schmidt *et al.* (2014) examined the wide range of predators attacking squash bugs, major pests of cucurbits particularly in organic farming systems. They were able to compare the value of different natural enemies as biocontrol agents of this pest under various organic management practices.

To conclude, we have hardly begun to exploit the full potential of molecular diagnostics as a tool for improving our understanding of trophic interactions. There seems to be no limit to the interactions that can be studied in this way, or to the habitats in which trophic interactions can be explored. A major strength of this field had been the willingness of participants to embrace new technologies which have taken us from early precipitin tests, using antibodies, through to NGS. This process will continue, as will the use of data obtained by such means to test ecological theory. Current work embraces everything from applied work, on ecosystem services, through analysis of behaviour to conservation applications. It is safe to predict that all these areas, and more, will expand rapidly over the next few years.

Acknowledgements

The 2nd International Symposium on the Molecular Detection of Trophic Interactions was generously supported, in part, by funds from the College of Agriculture and Department of Entomology at the University of Kentucky. We would like to thank Tim Vines for inviting us to compile this Special Issue for *Molecular Ecology*.

References

Alonso H, Granadeiro JP, Waap S, Xavier J, Symondson WOC, Ramos JA, Catry P (2014) An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. *Molecular Ecology,* **23**, 3719-3733.

Brown DS, Symondson WOC (2014) Sex and age-biased nematode prevalence in reptiles. *Molecular Ecology*, **23**, 3890-3899.

Brown DS, Burger R, Cole N, Vencatasamy D, Clare EL, Montazam A, Symondson WOC (2014a) Dietary competition between the alien Asian Musk Shrew (*Suncus murinus*) and a re-introduced population of Telfair's Skink (*Leiolopisma telfairii*). *Molecular Ecology*, **23**, 3695-3705.

Brown DS, Ebenezer KL, Symondson WOC (2014b) Molecular analysis of the diets of snakes: changes in prey exploitation during development of the rare smooth snake *Coronella austriaca*. *Molecular Ecology*, **23**, 3734-3743.

Burgar JM, Murray DC, Craig MD *et al.* (2014) Who's for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed. *Molecular Ecology*, **23**, 3605-3617.

Clare EL, Symondson WOC, Broders H *et al*. (2014a) The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology*, *23*, 3618-3632.

Clare EL, Symondson WOC, Fenton MB (2014b) An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology*, **23**, 3633-3647.

Derocles SAP, Le Ralec A, Besson MM, Maret M, Walton A, Evans DM, Plantegenest M (2014) Molecular analysis reveals high compartmentalization in aphid–primary parasitoid networks and low parasitoid sharing between crop and noncrop habitats. *Molecular Ecology*, **23**, 3900-3911.

Emrich MA, Clare EL, Symondson WOC, Koenig SE, Fenton MB (2014) Resource partitioning by insectivorous bats in Jamaica. *Molecular Ecology*, **23**, 3648-3656.

Eitzinger B, Unger EM, Traugott M, Scheu S (2014) Effects of prey quality and predator body size on prey DNA detection success in a centipede predator. *Molecular Ecology*, 23, 3767-3776.

Gariepy TD, Haye T, Zhang J (2014) A molecular diagnostic tool for the preliminary assessment of host–parasitoid associations in biological control programmes for a new invasive pest. *Molecular Ecology*, **23**, 3912-3924.

Greenstone MH, Payton ME, Weber DC, Simmons AM (2014) The detectability half-life in arthropod predator–prey research: what it is, why we need it, how to measure it, and how to use it. Molecular Ecology, **23**, 3799-3813.

Krüger F, Clare EL, Greif S, Siemers BM, Symondson WOC, Sommer RS (2014a) An integrative approach to detect subtle trophic niche differentiation in the sympatric trawling bat species *Myotis dasycneme* and *Myotis daubentonii. Molecular Ecology*, **23**, 3657-3671.

Krüger F, Clare EL, Symondson WOC, Keišs O, Pētersons G (2014b) Diet of the insectivorous bat *Pipistrellus nathusii* during autumn migration and summer residence. *Molecular Ecology*, **23**, 3672-3683.

Leal MC, Ferrier-Pagès C, Calado R, Thompson ME, Frischer ME, Nejstgaard JC (2014a) Coral feeding on microalgae assessed with molecular trophic markers. *Molecular Ecology*, **23**, 3870-3876.

Leal MC, Nejstgaard JC, Calado R, Thompson ME, Frischer ME (2014b) Molecular assessment of heterotrophy and prey digestion in zooxanthellate cnidarians. *Molecular Ecology*, **23**, 3838-3848.

Lundgren JG, Fergen JK (2014) Predator community structure and trophic linkage strength to a focal prey. *Molecular Ecology*, **23**, 3790-3798.

Olsen BR, Troedsson C, Hadziavdic K, Pedersen RB, Rapp HT (2014) A molecular gut content study of *Themisto abyssorum* (Amphipoda) from Arctic hydrothermal vent and cold seep systems. *Molecular Ecology*, **23**, 3877-3889.

Raso L, Sint D, Mayer R, Plangg S, Recheis T, Brunner S, Kaufmann R, Traugott M (2014) Intraguild predation in pioneer predator communities of alpine glacier forelands. *Molecular Ecology*, **23**, 3744-3754.

Redd KS, Ling SD, Frusher SD, Jarman S, Johnson CR (2014) Using molecular prey detection to quantify rock lobster predation on barrens-forming sea urchins. *Molecular Ecology*, **23**, 3848-3869.

Schmidt JM, Barney SK, Williams MA, Bessin RT, Coolong TW, Harwood JD (2014) Predator–prey trophic relationships in response to organic management practices. *Molecular Ecology*, 23, 3777-3789

Sedlock JL, Krüger F, Clare EL (2014) Island bat diets: does it matter more who you are or where you live? *Molecular Ecology*, **23**, 3694-3694.
Šerić Jelaska L, Jurasović J, Brown DS, Vaughan IP, Symondson WOC (2014) Molecular field analysis of trophic relationships in soil-dwelling invertebrates to identify mercury, lead and cadmium transmission through forest ecosystems. *Molecular Ecology*, **23**, 3755-3766.
Thomas AC, Jarman SN, Haman KH, Trites AW, Deagle BE (2014) Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. *Molecular Ecology*, **23**, 3706-3718.
Varennes Y-D, Boyer S, Wratten SD (2014) Un-nesting DNA Russian dolls – the potential for constructing food webs using residual DNA in empty aphid mummies. *Molecular Ecology*, **23**, 3925-3933.
Wallinger C, Staudacher K, Schallhart N, Mitterrutzner E, Steiner E-M, Juen A, Traugott M (2014) How generalist herbivores exploit belowground plant diversity in temperate grasslands. *Molecular Ecology*, **23**, 3826-3837.
Welch KD, Schofield MR, Chapman EG, Harwood JD (2014) Comparing rates of springtail predation by web-building spiders using Bayesian inference. *Molecular Ecology*, **23**, 3814-3825.