Box 6.1 Molecular techniques to establish trophic links

A promising new strategy for assessing feeding in small invertebrates is the use of molecular methods to detect prey-specific nucleic acid molecules as biomarkers of trophic interactions (Sheppard and Harwood 2005). Various different assays have been developed, but the general strategy of these methods is to purify DNA from stomach contents followed by detection and possible quantification using Polymerase Chain Reaction (PCR) amplification-based methods targeting gene fragments associated with prey organisms. Increasingly, this approach is being utilized to tease apart food webs, establish tropic links, and to estimate *in situ* feeding rates. Molecular approaches provide a means by which stomach content analyses can be conducted directly on field-caught animals without the potential of bias from incubation-based methodologies (Nejstgaard *et al.* 2003, 2007). A distinct advantage of a DNA-based molecular approach compared to gut fluorescence and direct microscopic observation is the ability to detect non-pigmented and macerated prey. Two general approaches have been used. The first is to use end-point PCR to qualitatively identify prey species in the guts of predators, while the second is to use real-time quantitative PCR (qPCR) to quantify the amount of DNA of a prey species in the stomach of a predator.

In the first approach PCR amplification primers with different specificities for prey have been used for amplification of genetic markers including species-specific (Bucklin *et al.* 1998; Nejstgaard *et al.* 2003; Vestheim *et al.* 2005), group-specific (Jarman *et al.* 2006), and universal (Blankenship and Yayanos 2005). Species-specific primers only amplify gene fragments associated with a prey species of interest and require a priori knowledge of the marker gene sequence of the prey species to design primers. In contrast, universal primers for a particular genetic marker amplify DNA of all of the different prey species. These amplification products may be separated through the use of clone libraries or other DNA profiling techniques (Troedsson *et al.* 2008a) and sequenced, enabling prey species to be identified (Blankenship and Yayanos 2005). Because predator DNA is always abundant compared to prey DNA, PCR amplification using universal primers is typically biased towards amplification of predator DNA and rarer prey sequences from the stomachs may fail to amplify. Different approaches have been utilized to attempt to overcome this problem. Blankenship and Yayanos (2005) attempted to minimize it by dissecting the stomachs to reduce the amount of predator DNA in their DNA purifications. They were able to further reduce the amount of predator DNA amplified by digesting with a restriction enzyme that cut only predator DNA within the target PCR amplicon prior to amplification with universal primers (Blankenship and Yayanos 2005).

qPCR offers a method for quantifying the amount of prey DNA present in the stomachs of predators and provides a basis for determining predator feeding rates. This approach allows quantification of the starting amount of DNA template and is based on the detection of a fluorescent reporter molecule that increases exponentially as PCR amplicons accumulate with each cycle of amplification. Fluorescence is measured at each cycle and an amplification plot is generated from the fluorescence data for standards and samples. Samples with higher amounts of target DNA exhibit increases in fluorescence after fewer number of amplification cycles than samples with less target DNA.

During the past decade qPCR has begun to be applied widely in ecological studies including the quantification of algal species in marine planktonic and sediment environments and for investigations of protist parasites and pathogens of marine metazoans (Frischer *et al.* 2006; Handy *et al.* 2006; Lyons *et al.* 2006). PCR-based assays are

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