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Molecular trophic markers in marine food webs and their potential use for coral ecology☆

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ABSTRACT

Notable advances in ecological genomics have been driven by high-throughput sequencing technology and taxonomically broad sequence repositories that allow us to accurately assess species interactions with great taxonomic resolution. The use of DNA as a marker for ingested food is particularly relevant to address predator–prey interactions and disentangle complex marine food webs. DNA-based methods benefit from reductionist molecular approaches to address ecosystem scale processes, such as community structure and energy flow across trophic levels, among others. Here we review how molecular trophic markers have been used to better understand trophic interactions in the marine environment and their advantages and limitations. We focus on animal groups where research has been focused, such as marine mammals, seabirds, fishes, pelagic invertebrates and benthic invertebrates, and use case studies to illustrate how DNA-based methods unraveled food-web interactions. The potential of molecular trophic markers for disentangling the complex trophic ecology of corals is also discussed.

1. Introduction

Food webs represent predator–prey interactions in ecological communities and provide information on nutrient recycling and energy pathways among trophic levels (Allgeier et al., 2015; Thompson et al., 2015). These networks, which are based on complex interactions among individuals, illustrate an important case study of eco-systems biology, a relatively recent discipline that focuses on the intersection between reductionist individual-level approaches and holistic viewpoints of ecosystem function and its underlying mechanisms (Raes and Bork, 2008). Individual links between resources and consumers ultimately characterize the transfer of nutrients and energy among species and trophic levels, thus providing information on large-scale ecosystem processes such as community structure and biogeochemical cycles.

Researchers have been trying to characterize food webs as an initial step in understanding ecosystems. Important parameters, such as species richness and number of species interactions or links, have been determined for various food webs (e.g., Jeppesen et al., 2000; Vinagre et al., 2015). However, because of the large number of species and their interactions, large cryptic biodiversity, high level of species aggregation, and limited spatio-temporal extent of the investigations, information on marine food webs is still limited as compared to freshwater and terrestrial networks (Dunne et al., 2004; Link, 2002). As our understanding of food webs is inherently limited by the methods available to study the links between predators and preys, our knowledge of such complex networks has been driven by the development of innovative methods and methodological approaches (Kelly and Scheibling, 2012; Pompanon et al., 2012). Multidimensional assessments of individual interactions in space and time and across trophic levels are, therefore, critical to provide an overarching and integrative approach addressing the multilevel complexity of marine food webs.

The continuous progress of ecological genomics during the past decades has enabled transdisciplinary studies where individual genotype–phenotype interactions are explored at the population, community and ecosystem levels (e.g., Ellegren, 2014; Fitzpatrick and Keller, 2015). Notable advances in high-throughput sequencing together with increased access to data repositories and bioinformatic tools to manage and explore large data sets open great perspectives for an accurate and efficient assessment of large-scale species-level interactions. The use of high-throughput approaches, such as DNA-based methods, has been particularly useful to address individual predator–prey interactions in natural settings (King et al., 2008; Sheppard and Harwood, 2005). Prey DNA sequences present in predator guts and/or scats can be used to unravel links between resources and consumers that are not possible to detect using traditional techniques (e.g., visual observations of gut contents and fecal material, stable isotopes, and fatty acid biomarkers).

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This ultimately allows recreating complex food webs composed of previously unobserved interactions (e.g., Cleary et al., 2012; Olsen et al., 2014).

Several reviews already summarized the development of molecular trophic interactions (Sheppard and Harwood, 2005; Symondson, 2002) and methodological best practices (King et al., 2008; O'Rorke et al., 2012a), as well as the use of next generation sequencing (NGS) for diet assessments (Pompanon et al., 2012) and its application for conservation purposes (Clare, 2014). In view of such literature reviews, it is not our goal to provide a thorough and overarching assessment of the development and use of molecular trophic markers. The purpose of this review is to focus on how DNA-based methods have been used to investigate trophic interactions in the marine environment, which have never been reviewed. Moreover, we focus on how molecular trophic markers can be used to better understand the trophic ecology of corals and their role in coral reef food webs. Coral reefs are one of the most diverse and important coastal ecosystems with complex food web pathways that have been poorly investigated as compared to other marine environments (Fry et al., 1982; Glynn, 2004; Valentine and Heck, 2005). Although global climate change is impacting these fragile ecosystems at an unprecedented rate, the role of corals in coral reef food webs and how environmental change impacts their trophic ecology is still dramatically limited and may notably benefit from the use of molecular trophic markers.

2. Molecular trophic markers in marine ecosystems

Molecular techniques have been used in the past decades to detect prey DNA and identify prey species using gut contents or fecal samples from predators (Symondson, 2002; Symondson and Harwood, 2014). The increasing use of this technique has been mostly driven by the problems associated with traditional visual identification of prey species in gut contents and scats. This is a particular issue for soft-bodied prey that are rapidly digested and for prey species without taxonomically relevant morphological structures (Sheppard and Harwood, 2005). For instance, although algae are often difficult to identify to species level using morphology (Leliaert et al., 2014), molecular trophic markers are able identify ingested algae species by marine predators (Leal et al., 2014a; Nejstgaard et al., 2003). Moreover, some marine animals feed on soft tissues or prey remains that cannot be visually identified but have DNA that can be traced (Albaina et al., 2012; Olsen et al., 2014). DNA-based methods allow the identification of most species with high level of taxonomic resolution with very low amount of sample biomass and have no bias associated with observer, prey size or its hardness. Nevertheless, there are biases in molecular techniques, especially when it comes to PCR and DNA barcoding. For instance, false positive results may occur with simple PCR assays (O'Rorke et al., 2013) or DNA barcoding (Meyer and Paulay, 2005). Other common issues are PCR biases if multiple primer sets are used (Sint et al., 2012), extremely high sensitivity to cross-contamination and other PCR errors (Pompanon et al., 2012; Traugott et al., 2013).

Different molecular methods, from a simple endpoint PCR (hereafter referred as PCR) to high-throughput NGS, can be used to produce both qualitative and quantitative data on trophic interactions. PCRs using prey-specific primers or multiplex PCRs have been used to provide qualitative information, i.e., presence/absence, on prey species ingested by predators (King et al., 2008; Sint et al., 2012). PCRs have also been combined with denaturing gradient gel electrophoresis (PCR-DGGE) to simultaneously detect diverse assemblages of organisms based on the presence of unique DNA signatures. This is an efficient method for evaluating sample diversity in large sample sizes, and has been used to examine complex dietary profiles of marine invertebrates and discriminate among diet constituents in gut and fecal material (Maloy et al., 2009; Martin et al., 2006). Finally, denaturing high-performance liquid chromatography (DHPLC) is a chromatographic method that separates a mix of amplicons based on DNA sequence differences, which has also been used to identify trophic interactions without prior knowledge of prey diversity in the predator sample (Olsen et al., 2012, 2014). After optimization, PCR–DHPLC is able to decrease the generic PCR bias of dominant templates, thus enhancing the less abundant DNA sequences. Other sequencing approaches, such as cloning or NGS, have been used to provide a qualitative characterization of diets (Maloy et al., 2013; O'Rorke et al., 2014). These sequencing approaches often use universal primers that target highly conserved DNA regions and theoretically allow the amplification of all prokaryotic or eukaryotic diversity. Universal primers can be used to gain insights into the feeding ecology of organisms without any prior knowledge of dietary composition. However, universal primers are also subject to bias. They favor DNA with exact complimentary sequences and preferentially amplify DNA of higher quality, which is particularly problematic because prey DNA is usually more degraded than predator DNA (Blankenship and Yayanos, 2005).

In contrast to qualitative approaches, quantitative studies using DNA-based methods are still scarce. Quantitative approaches use mostly quantitative PCR (qPCR) to assess the amount of prey DNA in the predator's gut and compare it to a standard curve composed of prey DNA extracted from a known amount of prey biomass or individuals. Most quantitative studies have been performed in laboratory settings to quantify prey ingestion and digestion of marine invertebrates preying either on phytoplankton or zooplankton (Durbin et al., 2011; Frischer et al., 2014; Leal et al., 2015; Nejstgaard et al., 2008). The application of such quantitative approaches in field-collected samples is still poorly explored, mostly because it is difficult to establish the link between the amount of prey DNA and prey biomass/numbers that may change with species and ontogenetic stage (Jungbluth et al., 2013). Other issues that affect quantitative studies using DNA-based methods are associated with the effect of digestion processes on the amount of prey DNA and the variable resistance of prey fragments to pre- and post-digestive mechanisms. All these limitations for accurate quantitative diet assessment still need to be thoroughly addressed in order to use DNA-based information for quantitative food web models that rely on metrics such as prey biomass or energy contribution (Cury et al., 2008).

In order to circumvent the limitation of absolute quantification of prey DNA, several studies have followed a semi-quantitative approach using frequency data (Deagle et al., 2013; Pompanon et al., 2012). This has been a common approach to analyze data derived from NGS. Sequences are often grouped in operational taxonomic units (OTUs) and counted, thus providing quantitative assessments through relative abundances (e.g., O'Rorke et al., 2012b, 2014). However, no direct correlation between sequence numbers and prey contribution to predator's diet is often observed due to amplification bias and other methodological issues (for a thorough review on this topic please read Pompanon et al., 2012). Nevertheless, the research community has acknowledged this problem that impedes the use of genomic methods to obtain valid quantitative diet assessments. Consequently, recent studies have been improving estimates of prey species biomass using correction factors that relate sequence frequencies in a gut or fecal sample to the frequency of what has been initially ingested (Deagle et al., 2010; Thomas et al., 2014, 2015). These quantitative issues and methodological approaches to tackle them are similar to recent advances in gut microbiome analysis (Benson et al., 2010; Ghanbari et al., 2015), which is a topic highly relevant for DNA sequence analysis of gut contents and fecal material as every vertebrate and most invertebrate have bacteria, protozoa and fungi in their guts that aid digestion (Douglas, 1994, 2014).

Although most research on the use of molecular trophic markers in the marine environment is still addressing the methodological issues previously described, DNA-based methods have been able to unravel new trophic links in marine food webs and still hold a great potential to investigate may other trophic ecology features of marine organisms either in natural or laboratory settings (Calado and Leal, 2015). However, it is important to highlight that the increasing use of NGS approaches, is inherently limited by the availability of reference libraries. Although