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JELLYFISH BLOOMS

Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review

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Abstract Studies of the trophic ecology of gelatinous zooplankton have predominantly employed gut content analyses and grazing experiments. These approaches record only what is consumed rather than what is assimilated by the jellyfish, only provide evidence of recent feeding, and unless digestion rates of different prey are known, may provide biased estimates of the relative importance of different prey to jellyfish diets. Biochemical tracers, such as stable isotopes and fatty acids, offer several advantages because they differentiate between what is assimilated and what is simply ingested, they provide an analysis of diet that is integrated over time, and may be useful for identifying contributions from sources (e.g., bacteria) that cannot be achieved using gut content approaches. Stable isotope analysis has become more rigorous through recent advances that provide: (1) signature determination of microscopic organisms such as microalgae, (2) analysis of dissolved organic carbon, and (3) improved quantification of relative source contributions. The limitation that natural tracer techniques require different dietary sources to have unique signatures can potentially be overcome using pulse-chase isotope enrichment experiments. Trophic studies of gelatinous zooplankton would benefit by integrating several approaches. For example, gut content analyses may be used to identify potential dietary sources. Stable isotopes could then be used to determine which sources are assimilated and modeling could be used to quantify the contribution of different sources to the diet. Analysis of fatty acid profiles could be used to identify contributions of bacterioplankton to the diet and, potentially, to provide an alternative means of identifying dietary sources in situations where the isotopic signatures of different potential dietary sources overlap. In this review, we outline the application, advantages, and limitations of gut content analyses and stable isotope and fatty acid tracer techniques and discuss the benefits of using an integrated approach toward studies of the trophic ecology of gelatinous zooplankton.

Keywords Gelatinous zooplankton · Trophic ecology · Diet · Gut contents

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Jellyfish Blooms: Causes, Consequences, and Recent Advances

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Introduction

Changes in the distribution and biomass of some species of jellyfish have increased concerns about their potential impacts on pelagic food webs (Brodeur et al., 2002; Lynam et al., 2006). With few exceptions (Montoya et al., 1990; Malej et al., 1993; Brodeur et al., 2002; Pitt et al., 2008), studies of the diets of gelatinous zooplankton have relied on the analysis of the prey present in the gut of the medusae or grazing experiments. Both of these approaches, however, have limitations for inferring diet and are but two of a suite of tools available for investigating trophic links. All approaches, however, have their own unique set of advantages and limitations that must be considered. The objectives of this paper are to review some of the limitations of gut content analyses and grazing experiments, outline the use of stable isotope and fatty acid tracers, describe the advantages and limitations of tracer techniques and their specific application to pelagic systems, and discuss the benefits of using multiple approaches to elucidate trophic relationships of gelatinous zooplankton.

Limitations of gut content analyses and grazing experiments

Gut content analyses record the types of prey ingested by a consumer, but they cannot easily discriminate between organisms that are assimilated by the predator and those that are ingested incidentally and either egested or pass through the gut undigested (Fry, 2006; but see Purcell et al., 1991). As most zooplankton are digested within 2–4 h (e.g., Purcell, 1997; Båmstedt & Martinussen, 2000), gut content analyses only provide evidence of recent feeding and extensive spatial and temporal sampling is required to provide a robust analysis. Variation in digestion rates of different prey types also biases estimates of their contributions to the diets of the consumers (Gee, 1989). Digestion rates of different prey have been measured for gelatinous zooplankton, but rates at which individual species of prey are digested vary among individuals, with size of predator and prey, among days, and with feeding intensity and temperature (Heeger & Möller, 1987; Purcell, 1992; Båmstedt & Martinussen, 2000), potentially confounding spatial and temporal comparisons of diet. Gut content analyses of medusae also predominantly focus on mesozooplankton and ichthyoplankton, presumably because they are more visible and retained in the gut for longer than microzooplankton. Studies of the contributions of microplankton are rarer and have been approached using grazing experiments (Stoecker et al., 1987; Sullivan & Gifford, 2004). Grazing experiments have also been used to estimate clearance rates of mesozooplankton (e.g. Fancett & Jenkins, 1988; Hansson et al., 2005). Medusae and zooplankton used in grazing experiments, however, may not behave in captivity as they would in the wild and results need to be interpreted cautiously (Toonen & Chia, 1993). For example, refugia generated by oceanographic features such as stratification may be unavailable to zooplankton in aquaria, which could artificially increase their likelihood of capture. Similarly, confinement may disrupt the flow of water around medusae and reduce their feeding efficiency.

Biochemical tracers, such as stable isotopes and fatty acids, have been used extensively in studies of trophic ecology since the 1970s. For a particular chemical to act as a tracer, its structure must be unaltered or altered in a predictable way as it passes from the dietary source to the consumer. The major advantages biochemical tracers offer over gut content analyses are that they differentiate between what is assimilated and what is simply ingested by the consumer, they provide an analysis of diet integrated over time and may be useful in identifying contributions from sources (e.g., bacteria and detritus) that are not easily determined using gut content approaches. Although tracer techniques have been used extensively to elucidate food webs in terrestrial and estuarine systems, they have been applied less frequently to pelagic systems and relatively rarely to studies involving gelatinous zooplankton (Montoya et al., 1990; Malej et al., 1993; Brodeur et al., 2002; Towanda & Thuesen, 2006; Pitt et al., 2008).

General principles of isotopic analyses

In recent years, stable isotope techniques have gained wide recognition as a tool to identify and trace energy and nutrient sources in coastal ecosystems (Fry, 2006). Stable isotope analysis of aquatic food webs involves elements that are important in the nutrition of animals and have different naturally occurring

isotopes. Their use relies on potential sources, such as different plants or types of prey, having different ratios of the common, light isotope to the heavy, rare isotope. The most commonly used element is carbon ($^{13}C/^{12}C$), which provides the basis for the majority of energetic requirements for pelagic organisms. Nitrogen ($^{15}N/^{14}N$) is also used routinely in aquatic food web studies, and is involved in protein synthesis (West et al., 2006).

Natural variability in the relative abundance of the common and rare isotopes is typically very small, and a special notation is, therefore, used to highlight the differences. Stable isotope ratios are normally reported as parts per thousand deviation from a known international standard, expressed using the delta notation:

$$\delta X = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right] \times 10^3 \quad [‰]$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ is the ratio of the atom occurrence of the rare to common isotope. The standards are Vienna PDB (equivalent to the original PeeDee Belemnite limestone standard) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

The application of stable isotopes to determine energy and nutrient pathways depends on two assumptions:

(1) that stable isotope ratios of potential sources (plants or prey) differ, and
(2) that the ratios are unaltered or altered in a predictable fashion during transfer to higher trophic levels.

In oceanic environments, phytoplankton are the dominant autotrophs that support pelagic food webs. Laboratory studies indicate that isotopic signatures of individual marine phytoplankton species can range widely (e.g., $\delta^{13}C$ −5.5 to −29.7‰; Falkowski, 1991), and that factors such as growth rate and cell size have a strong influence on the isotopic fractionation that occurs during C fixation, and therefore, on their $\delta^{13}C$ (Burkhardt et al., 1999). Thus, individual phytoplankton species can have distinct isotopic signatures that could be used to quantify their contribution to food webs supporting jellyfish. Difficulties in separating individual species in sufficient quantities for analysis from mixed samples collected in the field, however, has generally precluded measurement of species-specific signatures. New methods, such as fluorescence, to separate taxa are now enabling taxon-specific signatures to be obtained (Pel et al., 2003), which will help to elucidate the contribution of different phytoplankton taxa to jellyfish food webs. Pelagic food webs in coastal and estuarine systems may be supported by a greater diversity of autotrophs, including macrophytes such as seagrasses and mangroves. Macrophytes often have distinctive carbon isotope ratios that encompass a wide range of $\delta^{13}C$ units (e.g., Melville & Connolly, 2005; Benstead et al., 2006). These differences result from either different photosynthetic pathways (C3 versus C4 photosynthesis) or whether carbon is obtained from the air or water (Michener & Schell, 1994). The distinctive $\delta^{13}C$ for primary producers in coastal habitats may make it easier to distinguish among potential sources.

Isotopic ratios change slightly from one trophic level to the next in a process known as fractionation. The second assumption, of predictable change, is once again most likely to be met in benthic aquatic systems, where the majority of studies measuring fractionation rates have been done (McCutchan et al., 2003). Average carbon isotope fractionation per trophic level for aquatic animals is 0.4 ± 0.17‰ (McCutchan et al., 2003). Nitrogen isotope ratios generally display a much greater stepwise enrichment between producers and each higher trophic level, but average estimates for aquatic animals have varied from 2.3 ± 0.28‰ (McCutchan et al., 2003) to 3.4 ± 1.1‰ (Minagawa & Wada, 1984). The relatively larger isotopic fractionation of nitrogen has proven useful in assigning relative trophic levels to organisms for which stomach content analysis is difficult.

Although average fractionation rates are frequently applied in isotope studies, the actual range of fractionation rates varies widely among species; standard errors for average fractionation rates calculated across a range of species are ~30% of the mean (Minagawa & Wada, 1984; McCutchan et al., 2003). The degree of fractionation may also vary with food quality (van der Zanden & Rasmussen, 2001). Average fractionation rates should, therefore, be applied cautiously, and carefully controlled experiments to measure fractionation between jellyfish and their prey should be considered as part of isotope studies into jellyfish nutrition. For jellyfish species having symbiotic zooxanthellae, the tight cycling of nutrients between the host and zooxanthellae makes

fractionation particularly difficult to predict, and additional care is needed in the interpretation of isotope results for these species.

Considerations for sample preparation

Dealing with salt in jellyfish samples

Samples are dried prior to being analyzed in the mass spectrometer. Dried jellyfish samples, however, contain large amounts of salt. Since salt does not contain the elements usually used for isotopic analyses, it will not affect the ratio of the heavy to light isotopes of interest (i.e., the isotopic signature) in the sample. The amount of salt will, however, influence estimates of absolute amounts of a heavy isotope in the tissues, as a large proportion of the mass of a sample will be composed of salt rather than organic material. This may have implications for measuring assimilation rates in enrichment studies or for comparing assimilation rates between jellyfish and other taxa with much lower salt contents. Salt cannot be easily removed prior to isotopic analysis since rinsing samples with freshwater will lyse cells, resulting in the loss of dissolved organic matter and potentially change the isotopic signature. Knowledge of the relationship between dry weight and ash-free dry weight of the medusae, however, may enable estimates of absolute quantities of a heavy isotope in a sample to be calculated.

Variation in isotopic signatures among tissues

Isotopic signatures of different tissues within individual organisms may vary (e.g., Lorrain et al., 2002), and this can influence the interpretation of trophic relationships. Variation among tissues can result from different turnover times of elements (Tieszen et al., 1983). For example, carbon in the exoskeletons of mysids and krill is replaced much more rapidly than in muscle tissue (Gorokhova & Hansson, 1999; Schmidt et al., 2003). Tissues that turn over elements rapidly (e.g., gonads and exoskeleton), therefore, may provide information on recent feeding, whereas tissues with longer turnover times (e.g., muscles) may provide information about feeding over longer periods. Differences in isotopic signatures may also occur due to variations in the lipid contents of different tissues (Lorrain et al., 2002). Lipids are more depleted in ^{13}C than proteins and carbohydrates, and tissues that contain greater proportions of lipids (e.g., gonads and digestive glands) generally have lower $\delta^{13}C$ values (De Niro & Epstein, 1977; Lorrain et al., 2002). Some researchers, therefore, advocate the removal of lipids prior to isotopic analysis (e.g., Bodin et al., 2007).

Since the type of tissues selected for analysis can have a strong influence on the interpretation of trophic relationships, it is important to investigate potential variation in isotopic signatures among tissue types before deciding which type of tissue is most appropriate to use. Like most animals, the lipid content of different tissues of jellyfish vary (e.g., Lucas, 1994; Carli et al., 1991), which may influence their $\delta^{13}C$. Variation in isotopic signatures among tissues or areas of the body, however, has been examined for only two jellyfish species. Pitt et al., (2008) found no difference in the isotopic signatures of ectodermal tissue of the umbrella and mesoglea of *Catostylus mosaicus* (Quoy & Gaimard 1824), but Towanda & Thuesen (2006) observed that the mesoglea of *Phacellophora camtschatica* Brandt was greatly enriched in $\delta^{13}C$ (-10.1 ± 0.9‰) compared to the whole body (-25.7 ± 1.2‰), gonad (-27.6 ± 0.7‰), and oral arm tissue (-24.4 ± 1.1‰). Pilot studies, therefore, are needed to identify any variation in isotopic signatures among tissues and enable more informed decisions about which types of tissues should be analyzed.

Removal of inorganic material from potential sources

Gut content analyses indicate that jellyfish ingest large numbers of zooplankton that have inorganic chitinous or calcareous exoskeletons (e.g., mollusc veligers and copepods; Purcell, 2003; Browne & Kingsford, 2005). Inorganic compounds will not normally be assimilated by the jellyfish and isotopic signatures should, therefore, be obtained only for the organic component of potential prey. If the potential prey are large, the exoskeleton can be removed physically, but the simplest way of removing the exoskeletons of small zooplankton is to acidify the sample and redry it prior to analysis. Acidification has no effect on the $\delta^{13}C$ of the soft tissues (Bunn et al., 1995; Bosley & Wainright, 1999; Ng et al., 2007), and although some studies

indicate that acidification has a negligible influence on δ^{15}N (Bosley & Wainright, 1999; Ng et al., 2007), another study suggests that acidification depletes δ^{15}N (Bunn et al., 1995). The effects of acidification on δ^{15}N should, therefore, be investigated for individual taxa and, if necessary, δ^{15}N should be analyzed using separate, non-acidified samples.

Quantitative analysis of contributions of different sources

Recent advances in the analysis of stable isotope data have made isotope studies of food webs more quantitative, more rigorous, and more informative. Early studies compared consumer isotopic ratios with potential source ratios visually, but data are now routinely analyzed using mixing models to quantify the contributions from different sources (Fry, 2006). Mixing equations give unique solutions where the number of potential sources is no more than one greater than the number of elements being used (e.g., two sources for a single element analysis, three sources where, for example, carbon and nitrogen are used). Such equations have been refined so that not only mean values but also the variation around mean values can be used, giving confidence limits around estimates of source contributions (Phillips & Gregg, 2001).

Since jellyfish ingest a diverse suite of taxa, most food web studies involving jellyfish will involve too many potential sources for simple mixing equations to be useful. In these situations, the IsoSource procedure can be applied (Phillips & Gregg, 2003). The IsoSource model calculates all feasible combinations of sources that could explain the consumer isotope value, thereby placing bounds on the dietary contributions of each source. Model output is reported as the distribution of feasible solutions for each source. As an example, consider an IsoSource model that includes a consumer ($\delta^{13}C = -22.0$) and three dietary sources, A) $\delta^{13}C = -26.5$, B) $\delta^{13}C = -24.0$, and C) $\delta^{13}C = -22.0$. The model indicates that the consumer derives no less than 70% and up to 90% of its carbon from Source C and less than 30% from either of the sources A or B (Fig. 1). This methodology, however, sometimes cannot properly delineate source contributions, and a further refinement has been made that better defines potential

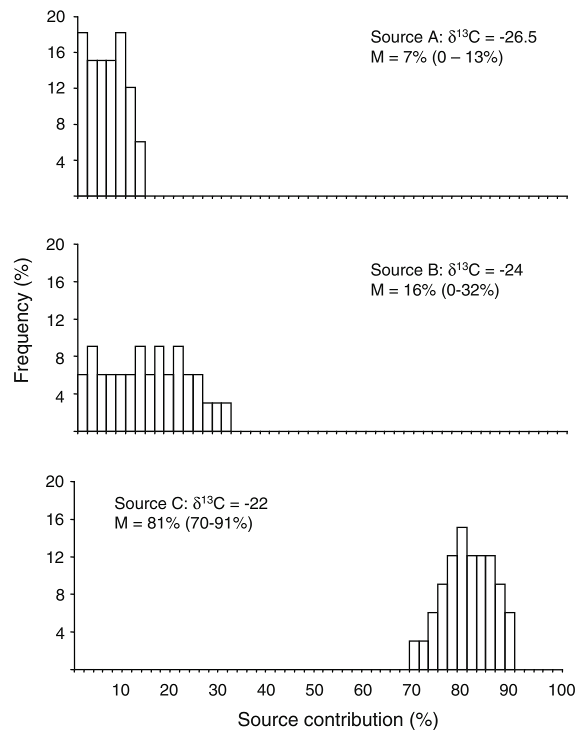

Fig. 1 Simulation of the distribution of feasible contributions of three sources to the diet of a consumer. M = median (ranges are 1 and 99 percentile values)

contributions by pooling contributions from various groups of sources selected by the researcher (Phillips et al., 2005). Quantitative analysis of isotope data is an active area of research in itself (Fry, 2006), and future developments will no doubt benefit isotope studies of jellyfish nutrition.

Limitations of isotopic techniques and potential solutions

Multiple sources with the same signature

Sometimes, multiple sources share the same isotopic signature, preventing the contribution of each source to the diet of the consumer being resolved. In such cases, analysis of an additional element may distinguish among the different sources. For example, analysis of sulfur isotopes has proven useful for distinguishing between sources that share the same carbon and nitrogen signatures (Connolly et al., 2004). Differences in sulfur ratios between different plant and algae types are, on average, much larger

than for carbon or nitrogen. Sulfur is especially useful for separating dietary contributions from benthic plants or prey (e.g., seagrass or the animals that live in seagrass) from biota in the water column. Thus, sulfur isotopes may be useful for studies of jellyfish inhabiting estuarine or shallow coastal waters, where emergent demersal zooplankton can form an important part of the diet (Flynn & Gibbons, 2007; Pitt et al., 2008).

Another means of distinguishing between food sources has been developed for situations where analysis of natural isotopic ratios cannot separate the contributions of different food sources. One or more putative sources are spiked artificially with enriched isotopes (e.g., ^{13}C or ^{15}N). Any contribution of the artificially enriched source to the diet of consumers is detected as a shift in the isotopic ratios of consumers. The first marine application was the artificial separation of the normally similar nitrogen isotope ratios of seagrass and its epiphytic algae (Winning et al., 1999). Pulse-chase experiments involving the manipulative enrichment of source signatures through the addition of enriched isotopes have since been done in small plots in seagrass (e.g., Mutchler et al., 2004) and on mudflats (e.g., Middelburg et al., 2000), and at a larger scale, in the upper reaches of estuaries (e.g., Gribsholt et al., 2005). There are, however, several difficulties that will need to be overcome for pulse-chase experiments to be applied to jellyfish. For example, since jellyfish prey on a diverse suite of taxa, only a few taxa within the assemblage of zooplankton prey could be labeled at any one time to determine if they are assimilated by the medusae (e.g., two taxa could be tested simultaneously if one taxon was labeled with ^{15}N and the other with ^{13}C). For pelagic species, pulse-chase experiments would also have to be undertaken in mesocosms. Such experiments would, therefore, suffer from the artefacts that are associated with all mesocosm experiments. At present, however, mesocosm studies offer the only option for applying this methodology to pelagic jellyfish.

Variability in isotopic signatures: problems and advantages of shifting baselines

Isotopic signatures of the autotrophs that sustain the food web can vary as a result of changes in the source of nutrients entering a system. This is commonly referred to as a "shifting baseline" and often occurs in coastal environments where terrestrial or anthropogenic nutrients, which have distinct isotopic signatures, enter waterways. For example, nitrogen derived from sewage is typically more enriched in ^{15}N than natural sources of nitrogen (Heaton, 1986). Autotrophs located close to sewage discharges, therefore, may be more enriched in ^{15}N than those located elsewhere. Temporal variation in baseline signatures may also occur following heavy rain when terrestrial sources are flushed into coastal waterways or following the commissioning or upgrading of sewage treatment plants (Costanzo et al., 2005). Changes in the isotopic signature of the autotrophs are propagated to higher trophic levels and this can result in substantial temporal and spatial variability in isotopic signatures of consumers and their sources.

The rate at which the isotopic signature of an organism shifts to reflect that of an elemental source varies depending on its turnover time. Organisms turn over isotopes in their tissues during growth and general metabolic maintenance (Fry & Arnold, 1982; Hesslein et al., 1993). Most studies indicate that, for aquatic animals, the majority of carbon and nitrogen turnover results from growth rather than metabolic maintenance (Fry & Arnold, 1982; Hesslein et al., 1993; MacAvoy et al., 2001), although results vary (e.g., Tarboush et al., 2006). Factors that affect growth rates and, to a lesser extent, metabolic rates, such as age (Sakano et al., 2005), temperature (Frazer et al., 1997), and diet (Schmidt et al., 2003), can affect rates at which isotopes are turned over in the tissues. Consequently, rates of turnover may vary within individual organisms, among conspecifics of different ages or life history stages, and among taxa. Variation in turnover times among different components of the food web, or different types of tissues, following a shift in the isotopic baseline can decouple the isotopic relationship between a consumer and its source; this limits the ability of isotopes to reliably identify trophic links (Schmidt et al., 2003). Turnover times of isotopes in gelatinous zooplankton need to be investigated to determine how rapidly they will respond to changes in the baseline signature. Given that medusae grow very rapidly (e.g., Palomares & Pauly, 2008), growth, rather than metabolic maintenance, may be expected to have a greater influence on turnover rates.

Once sources and their consumers have equilibrated to the new isotopic baseline, the shift in the

baseline can actually be useful for identifying trophic links. The difference in the isotopic signature between a consumer and its source will generally remain constant, regardless of whether their absolute values change following a shift in the baseline. Consequently, if the isotopic signature of the source and consumer vary in a consistent way through time or among places, i.e., if the signature of the consumer tracks that of its source, it can strengthen conclusions about trophic links (McCutchan & Lewis, 2002; Melville & Connolly, 2003). While two sources may have the same signature at one time or place, they may vary at another. The source can, therefore, be identified as being one of the consumer tracks. A two-dimensional correlation test has recently been developed to measure the strength of the links between sources and a consumer using two elements (e.g., carbon and nitrogen) at the same time (Melville & Connolly, 2003).

Sampling at multiple times and places is essential to determine whether baselines are consistent and to identify whether the different components of the food web have equilibrated to any observed shift in the isotopic baseline. If differences in isotopic signatures between sources and consumers are consistent, even if their actual signatures change, conclusions regarding trophic relationships are more robust. In areas where the isotopic signature of the elemental source changes frequently, variation in turnover times of different components of the food web may reduce the reliability of isotopic approaches.

Missing sources

Interpretation of dietary relationships using isotopic data depends on the relative values of the isotopic signatures of the consumer and its sources. Reliable interpretations of models of isotopic data can only be made if all dietary sources are included in the study. There are two possible outcomes for a model that is missing a source. First, the model may not be resolved. This occurs if, after allowing for appropriate fractionation, the consumer is more enriched or depleted than all sources included in the model. Alternatively, the model may be resolved, but may overemphasise the importance of a source that either makes a minor contribution to the diet or may have no dietary importance. For example, consider if, in the earlier example, a fourth, more enriched source,

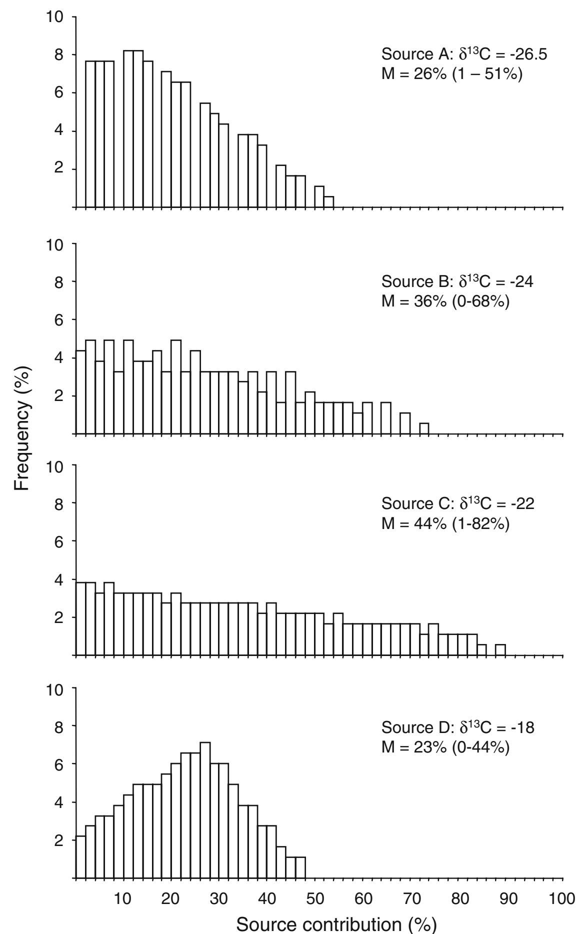

Fig. 2 Simulation of distribution of feasible contributions of four sources to the diet of a consumer. Details as in Fig. 1

D, ($\delta^{13}C = -18.0$) was found (e.g., based on gut content analysis) to have been missing from the original model. When revised, the model subsequently indicated that the contribution of Source C to the diet could range anywhere between 0 and 92%, instead of the initial 70–90% (Fig. 2). Models that are missing dietary sources are likely, therefore, to overemphasize the importance of sources that may make only a small contribution to the diet. Unless the model cannot be resolved, the deficiencies of models that fail to include a major source may not be recognised, and therefore, incorrect conclusions may be drawn regarding the dietary importance of different sources. Sources used in models, therefore, need to be carefully considered and justified. Analysis of the contents of the guts may provide an indication of possible dietary sources to include.

Obtaining sufficient material for analysis and "averaging" effects

The greatest technical difficulty in applying natural tracer techniques to planktonic food webs is obtaining sufficient quantities of each taxon for the analysis. For most zooplankton, approximately 5 μg of dried material is required for the analysis of carbon (i.e., after acid washing to remove indigestible carbonates) and 50 μg for the analysis of nitrogen. For larger species of plankton, only tens of individuals may need to be isolated (K. Schmidt, pers. comm.), but for smaller size classes, hundreds of individuals of each species may be required, which is logistically difficult. Many studies have, therefore, either obtained an average signature for bulk samples of zooplankton (e.g., Malej et al., 1993), or fractionated the zooplankton by size class (e.g., obtained by sieving, Rolff, 2000), or used differences in densities to separate zooplankton into coarse taxonomic groups (e.g., copepods and mollusc veligers, Pitt et al., 2008). Some medusae, however, feed selectively on particular types of zooplankton (Purcell, 1997). If the medusae prey on only a subset of species included in the fraction, then the average signature obtained for the fraction may not accurately reflect the actual dietary source. Mixing models are sensitive to small deviations in isotopic signatures and variations of <1‰ may determine whether a model can be resolved. For example, Pitt et al. (2008) examined the contribution of copepods, mollusc veligers, and small shrimp to the diet of the non-zooxanthellate scyphozoan, *Catostylus mosaicus*. Zooplankton was sampled during the day and night to account for possible variation in isotopic signatures associated with emergence of some taxa from the benthos at night. The daytime samples were assumed to comprise species that occurred permanently in the water column, whereas the night samples comprised species that occurred permanently in the water column and those that emerged into the water column from the benthos at night. The copepods sampled at night were $1.5 - 2.8$‰ more enriched in ^{13}C than the day samples, suggesting that emergent copepods were much more enriched than the diurnal groups (Fig. 3A). The IsoSource model, however, was unable to be resolved using the daytime and night time signatures, because after allowing for fractionation, the medusae were more enriched than all possible sources. Only when a separate signature was subsequently obtained for emergent crustaceans (sampled using emergence traps) could the model be resolved and the likely substantial contribution of emergent species (shrimp and copepods) to the diet be identified (Figs. 3B, 4).

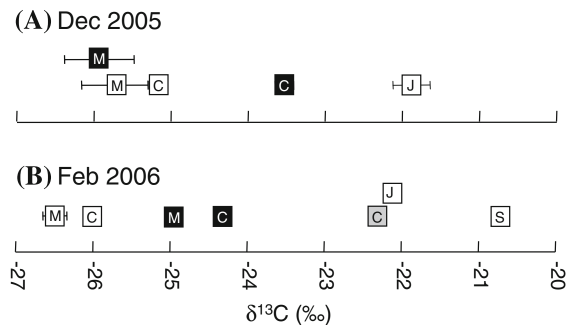

Fig. 3 (**A**) Mean (± SE) δ^{13}C values of *Catostylus mosaicus* and its potential diurnal (white) and nocturnal (black) prey in 2005, and (**B**) diurnal, nocturnal, and emergent (grey) prey in 2006. J = *C. mosaicus*, C = copepods, M = mollusc veligers, and S = mysid shrimp. Note that error bars are obscured by symbols in some cases

Recent developments have made it easier to obtain isotopic ratios of inconspicuous sources. The difficulty of isolating enough microalgae for isotope analysis has been overcome in two ways. Hamilton et al. (2005) showed that in many cases, water or sediment samples can be centrifuged in a silica gel to separate microalgae from sediment and detrital matter. Where low algal densities or high detritus loads prevent the use of this method, an alternative is to use compound specific isotopic analysis (Oakes et al., 2005). This approach involves extracting and then obtaining an isotopic signature of a compound, such as phytol, that occurs only in the source of interest. If the isotopic ratio of the compound accurately reflects the isotopic signature of the bulk algal sample, it can be used as a proxy for the algae (Oakes et al., 2005). Although these methods are time-consuming and expensive, they provide useful means for solving otherwise intractable problems. Another related benefit for isotope studies of pelagic systems is the technical advances, making routine isotope analysis of dissolved organic carbon possible (Bouillon et al., 2006).

General principles of the lipid markers approach

Lipids are key components of cell membranes and exhibit great diversity in structure. Fatty acids (FAs)

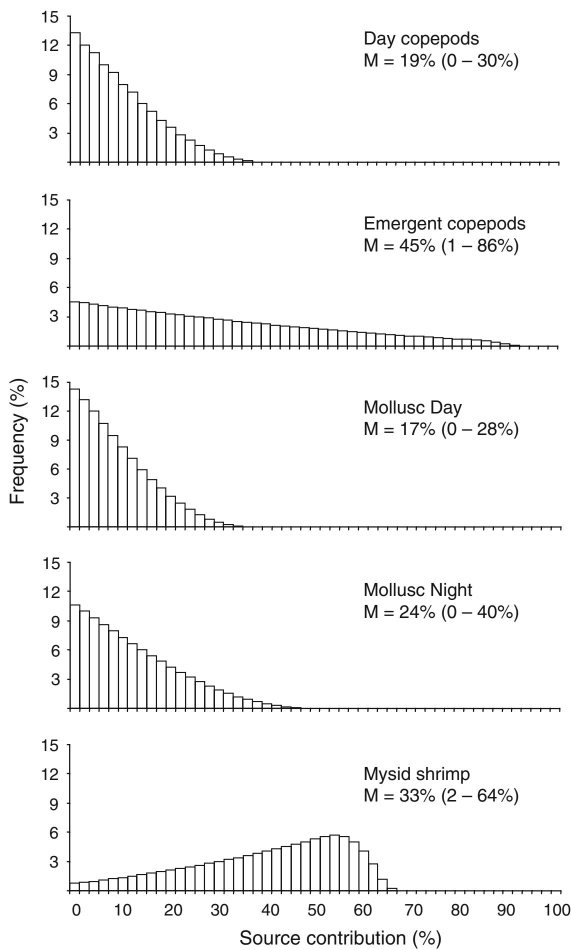

Fig. 4 Distribution of feasible contributions of diurnal, nocturnal, and emergent sources to the diet of *Catostylus mosaicus*. Details as in Fig. 1

are a particular class of lipids that have a variety of cellular functions. They have a very high energy value and are an important fuel in pelagic ecosystems (Phleger et al., 1998; Falk-Petersen et al., 2002). Their diverse structures enable them to be used as biomarkers of specific organisms (Sargent et al., 1987), because some FAs (and also some sterols, fatty alcohols, and hydrocarbons) occur only in certain taxa, thereby allowing these groups to be distinguished (e.g., Graeve et al., 2002; Meziane et al., 2007). In addition to being a valuable taxonomic tool, the specific fatty acid composition of different animal and plant groups is being increasingly used to map the transfer of the organic matter through aquatic food webs and to understand trophic relationships (Falk-Petersen et al., 2002; Copeman & Parrish, 2003).

The FA trophic marker concept is based on the observation that primary producers are characterized by the presence of certain FAs in their tissues that may be transferred conservatively to, and hence be recognized in, primary consumers (Dalsgaard et al., 2003). In practice, useful FA markers are those that, when transferred throughout the food web, provide knowledge not only about prey–predator relationships but also about the base of the food web. For example, some FAs are only synthesized de novo by plants. These include some polyunsaturated ω3- and ω6-FAs (3 and 6 are the positions of the double bond from the terminal methyl group). Producers other than plants (e.g., fungi and bacteria; Dalsgaard et al., 2003) and sometimes consumers, including jellyfish (Nichols et al., 2003), also have FAs that could be used to trace their transfer through food webs. For example, Nichols et al. (2003) discovered the presence of two rare FAs (24:6ω3 and 24:5ω6) in the tissues of *Aurelia* sp. If these FAs are unique to *Aurelia* sp., then their presence in higher-order consumers will provide evidence of predation on this jellyfish.

The composition of lipids in general has been examined in several cnidarian species. Most attention has focused on the trophic relationship between corals and their symbionts (zooxanthellae) (e.g., Harland et al., 1992; Papina et al., 2003), but more recently, profiles of jellyfish have been investigated (Fukuda & Naganuma, 2001; De Souza et al., 2007). For example, De Souza et al. (2007) used the presence of two diacylglycerols in the tissues of the medusa *Phyllorhiza punctata* von Ledenfeld 1884 as evidence of their translocation from their endosymbiotic zooxanthellae. Also, Fukuda & Naganuma (2001) used temporal variation in the FA compositions of *Aurelia aurita* (Linnaeus) to suggest that its diet may shift between the diatom-based food chain and the detritus-based food chain at different times of the year. FAs and others lipids, such as fatty alcohols, have also been used to elucidate the diet of ctenophores in the Arctic and Antarctic (Ju et al., 2004; Graeve et al., 2008). All three studies analyzed both fatty acid and fatty alcohol compositions and demonstrated that krill and copepods were the major food source of ctenophores in these waters.

Analysis of fatty acid data

If FAs are unique to particular types of organisms, then the simple presence of these FAs in the tissues of a consumer is sufficient to verify their consumption. In a semi-quantitative way, the prominence of one source over another in the diet of a pelagic consumer can be assessed by comparing the relative abundances of different types of FAs. For example, the ratio of 20:5ω3:22:6ω3 can indicate whether diatoms or dinoflagellates dominate the diet (a high value is indicative of more diatoms and a low value is more typical of a diet dominated by dinoflagellates; Budge & Parrish, 1998).

Individual FAs are useful for tracing the transfer of particular sources in a food web, but additional information can be provided by using multivariate analytical techniques (e.g., ordinations and analyses of similarities) to compare the entire FA profiles of consumers. The advantage of using the entire profile is that it fully utilizes the information that is generated in one species due the changing relative contributions of the numerous FAs present in the tissues. For example, Ju et al., (2004) did a principal components analysis (PCA) on the FA and fatty alcohol profiles of the antarctic ctenophore, *Callianira antarctica* and their potential food sources (Fig. 5). PCA enables identification of the variables that contribute the most to the variance. Principle component 1 (PC 1) accounted for 23% of the variation and PC 2 is accounted for 21%; the FA and alcohol profiles of the ctenophores more closely resembled copepods than krill, indicating that copepods make a major contribution and krill a minor contribution to the ctenophore's diet. Multivariate methods are particularly useful when a study includes very large numbers of samples (such as occurs when sampling over multiple spatial and temporal scales), which can make it difficult to readily distinguish the fate of specific markers in the ecosystem (Howell et al., 2003; Meziane et al., 2006). Such analyses can also be used to identify species that occur in similar trophic guilds (Howell et al., 2003).

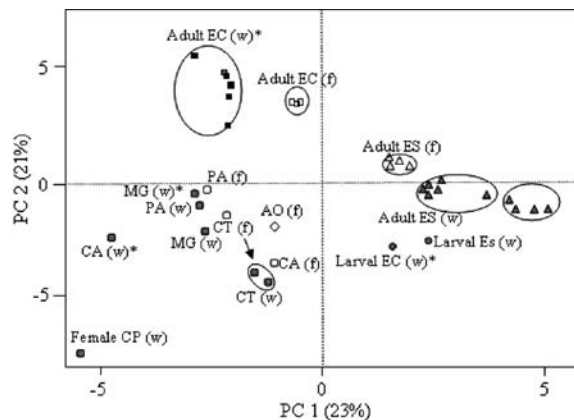

Fig. 5 Two-dimensional PCA plot of the first two principal components based on the combined fatty acid and fatty alcohol profiles for ctenophores and their potential prey in Antarctica. CT = *Callianira antarctica*, ES = *Euphausia superba*, EC = *Euphausia crystallorophias*, PA = *Paraeuchaeta antarctica*, CA = *Calanoides acutus*, AO = *Antarctomysis ohlini*, MG = *Metridia gerachei*, and CP = *Calanus propinquus*. *W* = winter, and *f* = autumn. Redrawn from Ju et al., (2004) with permission

Limitations of fatty acid analysis and possible solutions

Like stable isotopes, multiple sources can sometimes share the same FA markers, limiting their utility for tracing material from different producers through food webs (Dalsgaard et al., 2003). Thus, although some FAs are produced in large amounts by some sources, their presence, even in small amounts, in other producers can confound the assignment of food sources to the consumers. One way to overcome this limitation is to have a better understanding of temporal and spatial variations in the FA composition of organic matter at the base of the food web and of the consumer. For example, the absence of a source and its associated FA markers in a consumer at some places or times, but their presence at others, can help elucidate which sources contribute to a consumer's diet (Howell et al., 2003; Meziane et al., 2006).

Multivariate analyses of the entire FA profiles may also help to overcome this problem as they take full advantage of the information that is generated in one species due to the changing relative contributions of the numerous FAs present in the tissues. Finally, if several potential sources have similar FA profiles, the actual or dominant source can be identified using experimental approaches. For example, consumers can be fed a diet with a known FA profile (i.e., one source) and the changes in the FA profile of the consumer can be compared to that of its food source (e.g., Hall et al., 2006). This may allow verification of

whether the observed profile of the consumer resulted from the assimilation of one or multiple sources.

Analysis of stable nitrogen isotopes provides information on the number of trophic steps in a food web, but the FA approach is unable to determine whether FAs are transferred directly to consumers or via an intermediary. This is particularly true in benthic systems, where macrofauna can feed on meiofauna, which, in turn, feed on microoganisms living in the sediments (Moens et al., 1999). The FA profile of the macrofauna, therefore, will be similar to the microorganisms, even though they do not feed directly upon them. In this case, knowledge on feeding behavior of the consumers is needed to ascertain a direct trophic link as some organisms are not anatomically equipped to feed on some sources (Meziane et al., 2002). Alternatively, FA analyses may be used in association with analysis of stable nitrogen isotopes to estimate the number of trophic steps. A final limitation of FA markers is that some of these compounds can be metabolized in the tissues of the primary consumer, and therefore, may not readily traced to higher trophic levels. Indeed, polyunsaturated fatty acids (PUFAs) in some invertebrates may elongate and be used to construct other PUFAs that are also potential markers (Ito & Simpson, 1996). Although such examples are uncommon, rigorous application of the FA technique should include not only verification of the presence of specific FA markers in the potential source but their subsequent conservative transfer from the source to the primary and higher order consumers. Whether specific FA are transferred conservatively or, indeed, change structure as they are transferred across trophic levels can be determined using controlled experiments. For example, in a three-step food chain where decaying mangrove leaves were fed to shore crabs (the primary consumers), which, in turn were fed to swimming crabs (the secondary consumers), the FA 18:3ω3 was conservatively transferred from the source to both primary and secondary consumers, whereas 18:2ω6 was not transferred between the primary and secondary consumer (Hall et al., 2006). Indeed, when the secondary consumer was starved, 18:2ω6 actually accumulated in its tissues, indicating that physiological stress during starvation may have caused the synthesis of this FA, which potentially renders it inappropriate to use as a maker of the source (i.e., mangrove leafs) in the diet of higher-order consumers.

Benefits of using multiple approaches to elucidate trophic relationships in gelatinous zooplankton

All approaches used to study trophic interactions have their own suite of advantages and limitations. To date, most studies of the trophic ecology of gelatinous zooplankton have used gut content analyses or grazing experiments. Integrating multiple approaches, however, is likely to provide more useful and accurate information. For example, analysis of gut contents can provide information about which dietary sources may be useful to include in stable isotope analyses. The stable isotopes will, in turn, provide information about whether the sources are actually assimilated by the jellyfish and modeling can be used to estimate the contributions of the different sources to the diet. Due to the need to collect sufficient quantities of material for analysis, however, stable isotopes are at present difficult to use for examining the potential contributions of microplankton or bacteria to the diets of jellyfish. In such cases, analysis of FA profiles may be helpful as the presence of markers characteristic of bacteria or microplankton in the jellyfish's tissue will provide evidence of their contribution to the diet. FAs may also be useful for identifying sources that may share similar stable isotope signatures, and therefore, are not readily distinguished using isotopic approaches.

A thorough understanding of the advantages and limitations of each approach is required for reliable interpretation of data. In all cases, rigorous temporal and spatial sampling will be required to provide robust analyses. Integrating multiple approaches will provide a more comprehensive and rigorous understanding of the trophic ecology of gelatinous zooplankton.

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