Mar Biol (2011) 158:2141–2146
DOI 10.1007/s00227-011-1714-7

METHOD

Preservation methods alter stable isotope values in gelatinous zooplankton: implications for interpreting trophic ecology

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Received: 16 December 2010 / Accepted: 28 April 2011 / Published online: 15 May 2011

Abstract Jellyfish are increasingly topical within studies of marine food webs. Stable isotope analysis represents a valuable technique to unravel the complex trophic role of these long-overlooked species. In other taxa, sample preservation has been shown to alter the isotopic values of species under consideration, potentially leading to misinterpretation of trophic ecology. To identify potential preservation effects in jellyfish, we collected *Aurelia aurita* from Strangford Lough (54°22′44.73″N, 5°32′53.44″W) during May 2009 and processed them using three different methods prior to isotopic analysis (unpreserved, frozen and preserved in ethanol). A distinct preservation effect was found on δ^{15}N values: furthermore, preservation also influenced the positive allometric relationship between individual size and δ^{15}N values. Conversely, δ^{13}C values remained consistent between the three preservation methods, conflicting with previous findings for other invertebrate, fish and mammalian species. These findings have implications for incorporation of jellyfish into marine food webs and remote sampling regimes where preservation of samples is unavoidable.

Communicated by U. Sommer.

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Introduction

Gelatinous zooplankton or jellyfish (here considered as Phylum Cnidaria, Class Schyphozoa) have been viewed as peripheral and transient components within marine ecosystems, constituting little more than a carbon sink or a trophic dead end (Hansson and Norrman 1995; Arai 2005). This perception now appears outdated and international efforts are underway to redress this long-standing gap in our knowledge (Mills 2001; Purcell and Arai 2001). However, until recently, many questions surrounding the trophodynamics of jellyfish seemed somewhat intractable given the spatial and temporal variability of aggregations (Doyle et al. 2007a; Houghton et al. 2007) and the broad scale over which they can occur (Houghton et al. 2006; Doyle et al. 2008). Addressing such bottlenecks is paramount and the recent application of biochemical techniques shows great promise for future studies (see Malej et al. 1993 and Pitt et al. 2009 for review).

Advances in stable isotope techniques over the last 20 years have greatly improved ecological research in marine and estuarine systems (Peterson and Fry 1987). The use of nitrogen and carbon stable isotope ratios as food web tracers in marine ecosystems (Carabel et al. 2006; Michener and Kaufman 2007) has made it possible to characterise trophic pathways, aiding the understanding of energy transfer in these systems (e.g. Wada et al. 1987; Kaehler et al. 2000) and allowing us to assess food web structure more accurately (e.g. Peterson and Fry 1987; Hobson and Welch 1992; Davenport and Bax 2002).

Biologists seeking to unravel the trophic interactions of jellyfish face many taxa-specific experimental challenges, with three specific issues rising to the fore: (1) gut content analysis can be highly problematic as gelatinous prey (e.g. ctenophores) are difficult to quantify and identify owing to

fast deterioration in the oral arms and gut cavity (Båmstedt and Martinussen 2000; Ishii and Tanaka 2001; Pitt et al. 2009); (2) once out of the water, gelatinous samples rapidly lose physical integrity; (3) sampling at remote sites or on research cruises of extended duration can limit access to suitable processing/analytical equipment and jellyfish collected in the field are typically preserved by freezing or storage in ethanol (EtOH) (Hobson et al. 1997). Preservation methods employed prior to stable isotope analysis (SIA) have been shown to alter isotopic composition in a range of marine taxa ranging from algae (Kaehler and Pakhomov 2001; Carabel et al. 2009) through to invertebrates (Bosley and Wainwright 1999; Kaehler and Pakhomov 2001; Carabel et al. 2009) and higher vertebrates (Bosley and Wainwright 1999; Kaehler and Pakhomov 2001). Mateo et al. (2008) additionally highlighted pre-analytical biases at the class level with the greatest effects exhibited by Maxillopoda, Gastropoda and Polychaeta. Our knowledge of such effects does not currently extend to gelatinous zooplankton, presenting a potential problem for future studies.

Even though the application of SIA to gelatinous zooplankton research is in its infancy, it has the potential to provide a clearer picture of whether each species fits within established marine food webs or represents a trophic conduit to a separate gelatinous food web. Typically, $\delta^{13}C$ is used to investigate energetic pathways through food webs and identify foraging locations used by the individual (DeNiro and Epstein 1978; Wallace et al. 2009). The trophic level at which an organism feeds is reflected by its $\delta^{15}N$ value (DeNiro and Epstein 1981; Post 2002). Any mechanism altering the $\delta^{13}C$ or $\delta^{15}N$ value owing to preservation could lead to incorrect estimation of trophic position and thus overall patterns of consumption, causing problems for further integration into food web and bioenergetic models. SIA provides a useful tool for examining ontogenetic dietary shifts in a range of consumers (e.g. Olson 1996; Harrod et al. 2005; Knoff et al. 2008); however, such patterns have not been examined in jellyfish. If preservation effects exist, and are not constant across a gradient of consumer body size, this may affect our capacity to correctly interpret information regarding trophic ecology from isotope data based on preserved samples. With research on jellyfish increasing, it is essential to have an a priori knowledge of how differing preservation methodologies may bias isotopic values. In light of these considerations, the aims of this study were to determine: (1) whether there is an effect of preservation on jellyfish stable isotope values, (2) whether SIA could detect any change in isotopic value with increasing body mass (allometry) and (3) if an allometric trend was present, is it affected by preservation method? In addressing these questions, we sought to establish an effective pre-analytical processing protocol for jellyfish.

Materials and methods

The scyphozoan jellyfish *Aurelia aurita* (L.) was selected for the study as it is ubiquitous at temperate latitudes (Russell 1970; Lucas 2001) and has been previously considered in numerous studies of marine food webs (e.g. Lynam et al. 2005; Malej et al. 2007; Purcell et al. 2010). Individuals were collected from a pontoon located at the southern extreme of Strangford Lough (a large coastal embayment flowing into the Irish Sea) near the Queen's University Marine Laboratory (54°22′44.73″N, 5°32′53.44″W; Co. Down, Northern Ireland) during May 2009. A dip net (mesh size 1 mm) was used to collect smaller jellyfish from the side of a small boat, with a landing net (5 mm mesh size) used for larger individuals. Both nets were chosen as they cause minimal damage to jellyfish tissue (Fleming pers. obs.). To make a direct comparison of the isotopic value of jellyfish from the same species and same size class, individuals were collected at the same site on the same day but were subsequently randomly assigned to one of three different preservation methods (unpreserved, frozen, EtOH preserved). Originally, we aimed to split each jellyfish into three sections, and apply each treatment to each individual, allowing comparisons including individual responses to preservation. However, due to the high water content within the bell of jellyfish (~95%; Doyle et al. 2007b), small individuals did not provide adequate sample mass for replicate samples to be analysed. Our decision was also influenced by a wish to mimic procedures on research cruises as closely as possible, where individuals are typically preserved whole.

In the laboratory, jellyfish were rinsed in filtered seawater, and individual bell diameter (±1 cm) and wet mass (±1 g) recorded. To ensure that preservation treatments were balanced across all size ranges, individuals were sorted by bell diameter in 1 cm increments from 7 to 21 cm. Size-matched samples were then randomly selected and processed following the three different experimental treatments. Unpreserved samples were transferred immediately to drying trays and placed into an oven to attain dry mass (see below for details). Other specimens were either transferred individually into labelled zip-lock bags and frozen at −20°C (frozen treatment) or preserved in 75% EtOH and stored at room temperature (EtOH treatment) for 6 months before oven drying using the method described by Doyle et al. (2007b). Frozen and blotted dry EtOH samples were placed into pre-weighed aluminium containers, weighed and placed into a drying oven at a temperature of 60°C and dried to constant mass. Following drying, all samples were ground into a fine powder using an agate pestle and mortar. The samples were analysed for C and N content and $\delta^{13}C$ and $\delta^{15}N$ isotope ratios using a Thermo Scientific Elemental Analyser Isotope Ratio Mass Spectrometer model: Delta

V Advantage. Isotope analysis was carried out at the ^{14}CHRONO centre, School of Geography, Archaeology and Palaeoecology, Queen's University, Belfast. Sampling precision for δ^{13}C and δ^{15}N was estimated from the use of internal standards and was typically ±0.1‰.

Statistical analyses

The effects of preservation were investigated by performing GLM, regression and ANCOVAs on the stable isotope data using SPSS version 17.0. Wet mass and δ^{15}N values were $\log_{10}$-transformed to improve normality, stabilise variances and to linearise relationships.

Results

A. aurita isotopic values for δ^{13}C and δ^{15}N varied considerably between individuals, and following the different experimental treatments: unpreserved (δ^{13}C = −19.7 to −20.7‰, mean = −20.1‰ SD ± 0.4‰; δ^{15}N = 6.1 to 9.4‰, mean = 8.1‰ SD ± 0.9‰), frozen (δ^{13}C = −19.6 to −20.6‰, mean = −20.1‰ SD ± 0.3‰; δ^{15}N = 8.9 to 12.6‰, mean = 10.1‰ SD ± 1.2‰) and EtOH (δ^{13}C = −18.8 to −20.3 ‰, mean = −19.6‰ SD ± 0.4‰; δ^{15}N = 9.9 to 12.1‰, mean = 10.7‰ SD ± 0.6‰).

GLM was used to examine variation in mean δ^{13}C and δ^{15}N values between control (fresh) and preservation methods (Fig. 1). There were no significant differences in mean δ^{13}C values between control samples and those subjected to the two different preservation methods ($F_{2,40} = 2.1$, $p \geq 0.05$). However, mean δ^{15}N values did differ significantly between the three groups ($F_{2,40} = 27.8$, $p \leq 0.001$). Sheffe's post hoc test revealed isotope mean differences (iMD) between control and preserved samples in both freezing (iMD = −2.1‰, $p \leq 0.001$) and alcohol (iMD = −2.4‰, $p \leq 0.001$) treatments.

Using linear regression of $\log_{10}$-transformed data, we examined (1) the presence of a size-isotope relationship in *A. aurita* and (2) if this existed, whether it was affected following preservation by freezing or storage in EtOH. Non-preserved *A. aurita* showed a significant linear mass-δ^{15}N relationship ($R^2 = 0.43$, $df = 1.13$, $p \leq 0.05$; Fig. 2). Comparison of mass-δ^{15}N relationships between non-preserved and preserved samples through ANCOVA of $\log_{10}$-transformed data showed that slopes were not homogeneous ($F_{2,40} = 3.8$ $p = 0.03$). However, this reflected the lack of a relationship between bell-mass and δ^{15}N in EtOH preserved samples ($R^2 = 0.14$, $df = 13$, $p \geq 0.05$; Fig. 2). When these data were removed from the ANCOVA, it was apparent that the slope of the mass-δ^{15}N relationship was similar for both unpreserved and frozen samples ($F_{1,27} = 0.49$, $p = 0.49$). However, the intercepts differed between the two treatments ($F_{1,28} = 46.2$, $p < 0.0001$), reflecting an enrichment effect of freezing on *A. aurita* δ^{15}N values (Fig. 2).

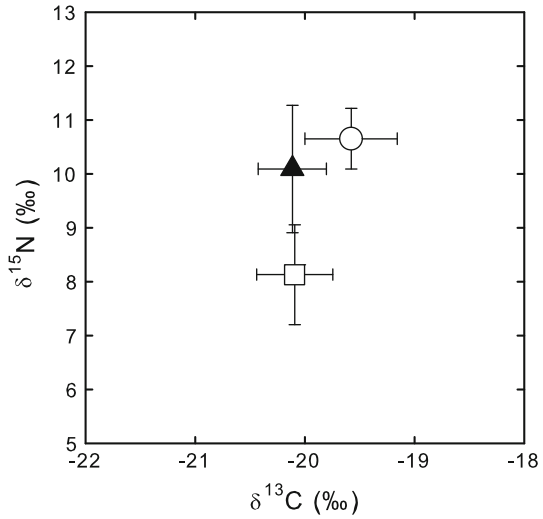

Fig. 1 Variation in mean (±SD) *A. aurita* δ^{15}N and δ^{13}C values reflect the effects of preservation (frozen-*filled triangle* ($n = 14$, size range = 7–21 cm) and EtOH *open circle* ($n = 14$, size range = 7–19 cm) relative to unpreserved *open square* ($n = 15$, size range = 7–20 cm) samples

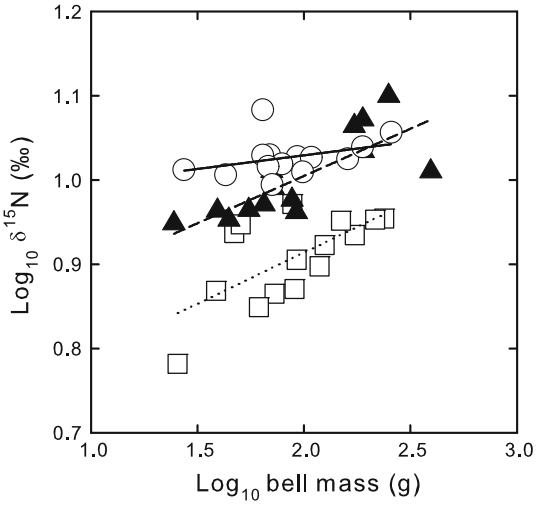

Fig. 2 An allometric effect of size on δ^{15}N was apparent in unpreserved *A. aurita* (see *open square* symbols). This revealed that preservation affected the relationship between size and δ^{15}N. ANCOVA revealed a significant preservation effect between preserved and non-preserved samples. Both frozen (*filled triangle*) and ethanol (*open circle*) preserved samples had elevated $\log_{10}$ δ^{15}N values when compared with unpreserved *A. aurita* samples (*open square*)

Discussion

Stable isotope analysis is a fundamental tool in food web studies yet comes with a number of well-documented caveats (Gannes et al. 1997; Vander Zanden and Rasmussen

2001; Mateo et al. 2008). For example, numerous studies have indicated how inconsistencies in $\delta^{13}C$ and $\delta^{15}N$ values for the same species can arise through differences in pre-analytical protocols (Feuchtmayr and Grey 2003; Sarakinos et al. 2002; Syväranta et al. 2008). In this context, the need to standardise, or at least consider the efficacy of protocols, is important for the success of future studies (Mateo et al. 2008; Carabel et al. 2009). Ideally, when only large medusae are to be considered, separating the bell into three equal components and subjecting each to a different preservation might be appropriate, but for small species such as *A. aurita* this is not always possible. Where preservation is required, freezing is often the preferred method as it typically does not affect isotopic values. Evidence for this approach can be gathered from studies of vertebrate (fish), invertebrate (cephalopod, anemone and gastropod species) and algal (kelp and fucoids) species, which demonstrated isotopic values were not affected by freezing (Bosley and Wainwright 1999; Kaehler and Pakhomov 2001; Carabel et al. 2009). Quite unexpectedly, our data suggested this was not the case for gelatinous zooplankton, as freezing enriched $\delta^{15}N$ values in *A. aurita* by ~2‰, but did not affect $\delta^{13}C$ values compared with unpreserved samples. Previous studies (see Kaehler and Pakhomov 2001) have associated shifts in $\delta^{13}C$ values following preservation, e.g., in EtOH with differential hydrolysis of ^{13}C-depleted lipids (DeNiro and Epstein 1977; Kiljunen et al. 2006) leading to altered carbon isotope ratios following preservation. However, jellyfish are lipid-poor (~1.2% by dry mass, Doyle et al. 2007b), likely resulting in the lack of a preservation effect on $\delta^{13}C$ values.

The effects of preservation prior to SIA also have implications when considering $\delta^{15}N$ values (e.g. Feuchtmayr and Grey 2003; Carabel et al. 2009; Syväranta et al. 2011). Although these shifts appear less pronounced than they are for $\delta^{13}C$ (Bosley and Wainwright 1999; Kaehler and Pakhomov 2001), they are important to consider as $\delta^{15}N$ can elucidate the trophic relationships or identify the ecological niche occupied by an organism (consumer) within a food web (DeNiro and Epstein 1978, 1981; Post 2002; Wallace et al. 2009). Ontogenetic trophic shifts have been observed in a wide range of marine mammals and fish (e.g. Olson 1996; Harrod et al. 2005; Knoff et al. 2008), but the existence of such dietary changes within gelatinous species is not well documented.

EtOH preserved *A. aurita* were ^{15}N enriched by ~2.5 ‰ relative to freshly prepared samples. Moreover, there was a distinct allometric component to the preservation effects with an apparent ontogenetic shift of ca. one trophic level between small and large *A. aurita*. Preservation by freezing and EtOH each led to elevated $\delta^{15}N$ values along the same size gradient, though interestingly EtOH dampened the allometric signature of the individuals tested and decreased the variability around the mean isotopic values. It is possible EtOH elevated the $\delta^{15}N$ values in our samples through disruption of fluid membranes allowing leakage of cellular components (Goldstein and Chin 1981). If mean trophic fractionation between consumer and prey is interpreted as 3.4‰ for $\delta^{15}N$ (Post 2002), then the effect of EtOH preservation could lead to the overestimation of trophic position, with obvious effects for those aiming to build food webs.

When sampling regimes may be protracted or geographically disparate, attention should also be given to the duration of tissue preservation. *A. aurita* frozen for 180 days showed elevated $\delta^{15}N$ values, reflecting similar data from freshwater zooplankton by Feuchtmayr and Grey (2003) (in both studies, samples were stored at −20°C). In contrast, Barrow et al. (2008) reported no effect on marine turtle tissue after 30 days and depletion in $\delta^{15}N$ values after 60 days (samples stored at −10°C). These conflicting results highlight preservation effects can be specific to different faunal groups and that assumptions based on previous studies regarding duration or method might prove problematic. For example, Carabel et al. (2009) showed that freezing had no effect on isotopic values of another cnidarian species (*Anemonia sulcata*). Pinpointing the processes that drive these inconsistencies is not simple. Feuchtmayr and Grey (2003) and Barrow et al. (2008) suggested that potential sources of error can arise at the cellular level through the mechanical or chemical processes associated with preservation. For frozen samples, these factors can cause the breakdown of cells and loss of ^{12}C and/or ^{13}C components via leaching during thawing or filtration. It is also feasible that denaturing of proteins or lipid protein complexes as a consequence of freezing may lead to variation in perceived $\delta^{15}N$ values (e.g. Lovelock 1957; Jiang et al. 1987; Wroblowski et al. 1996). This idea is supported by evidence of protein denaturation in a range of fish (Mackie 1993; Paredi et al. 2010), cephalopod (Paredi et al. 2006; Reyes et al. 2009) and bivalve species (Makri 2010; Syväranta et al. 2011). What is clear, however, is that although freezing is often considered the preferred method of sample preservation for isotope studies, it is not a panacea and the assumptions of no preservation effect of freezing should not be taken for granted.

With concern about potential increases in scyphomedusae biomass and geographical coverage through climatic forcing and overfishing (e.g. Lynam et al. 2004; Doyle et al. 2008; Kirby et al. 2009), the need to assess the role of jellyfish within marine systems is paramount. Refining and standardising SIA methodology is an important step to achieving this goal. The scale of isotopic changes reported here following preservation suggest that future studies examining the isotope ecology of jellyfish and other gelatinous zooplankton should seek to process samples without preservation where possible. If preservation is unavoidable

then researchers should conduct calibration experiments prior to ecological studies so that correction factors can be derived.

Acknowledgments This study was supported by a Department of Education and Learning (DEL) PhD studentship awarded to NECF by the Northern Ireland Assembly. We are grateful to Phillip Johnston from Queen's University, Belfast Marine Laboratory (QUML) for boat support and sample collection, and Michelle Thompson from the ^{14}CHRONO centre, School of Geography, Archaeology and Palaeoecology, Queen's University, Belfast for SIA processing. Thanks also go to Donal Griffin and Tony Lilley for help with sample processing.

References

Arai MN (2005) Predation on pelagic coelenterates: a review. J Mar Biol Assoc UK 85:523–536

Båmstedt U, Martinussen MB (2000) Estimating digestion rate and the problem of individual variability, exemplified by a scyphozoan jellyfish. J Exp Mar Biol Ecol 251:1–15

Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation method on stable carbon and nitrogen isotope values. Physiol Biochem Zoo 81:688–693

Bosley KL, Wainwright SC (1999) Effects of preservation and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two marine species of marine animals. Can J Fish Aquat Sci 56:2181–2185

Carabel S, Godínez-Domínguez E, Verísimo P, Fernández L, Freire J (2006) An assessment of sample processing methods for stable isotope analyses of marine food webs. J Exp Mar Biol Ecol 336:254–261

Carabel S, Verísimo P, Freire J (2009) Effects of preservatives on stable isotope analyses of four marine species. Estuar Coast Shelf S 82:348–350

Davenport SR, Bax NJ (2002) A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. Can J Fish Aquat Sci 59:514–530

DeNiro M, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J (2007a) The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia 579:29–39

Doyle TK, Houghton JDR, McDevitt R, Davenport J, Hays GC (2007b) The energy density of jellyfish: estimates from bomb-calorimetry and proximate-composition. J Exp Mar Biol Ecol 343:239–252

Doyle TK, De Haas H, Cotton D, Dorshell B, Cummins V, Houghton JDR, Davenport J, Hays GC (2008) Widespread occurrence of the jellyfish *Pelagia noctiluca* in Irish coastal and shelf waters. J Plankton Res 30:963–968

Feuchtmayr H, Grey J (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Commun Mass Spectrom 17:2605–2610

Gannes LZ, O'Brien DM, Martínez Del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276

Goldstein DB, Chin JH (1981) Interaction of ethanol with biological membranes. Fed Proc 40(7):2073–2076

Hansson LJ, Norrman B (1995) Release of dissolved organic carbon (DOC) by the scyphozoan jellyfish *Aurelia aurita* and its potential influence on the production of planktonic bacteria. Mar Biol 121:527–532

Harrod C, Grey J, McCarthy TK, Morrissey M (2005) Stable isotope analyses provide new insights into ecological plasticity in a mixohaline population of European eel. Oecologia 144:673–683

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. Mar Ecol Prog Ser 84:9–18

Hobson KA, Lisle Gibbs H, Gloutney ML (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75:1720–1723

Houghton JDR, Doyle TK, Wilson MW, Davenport J, Hays GC (2006) Jellyfish aggregations and leatherback turtle foraging patterns in a temperate coastal environment. Ecology 87(8):1967–1972

Houghton JDR, Doyle TK, Davenport J, Lilley MKS, Wilson RP, Hays GC (2007) Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusa (Cnidaria: Scyphozoa). Hydrobiologia 587:1–13

Ishii H, Tanaka F (2001) Food and feeding of *Aurelia aurita* in Tokyo Bay with an analysis of stomach contents and a measurement of digestion times. Hydrobiologia 451:311–320

Jiang ST, Hwang BS, Tsao C (1987) Protein denaturation and changes in nucleotides of fish muscle during frozen storage. J Agr Food Chem 35:22–27

Kaehler S, Pakhomov EA (2001) Effects of storage and preservation on the δ^{13}C and δ^{15}N signatures of selected marine organisms. Mar Ecol Prog Ser 219:299–304

Kaehler S, Pakhomov EA, McQuaid CD (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by δ^{13}C and δ^{15}N analysis. Mar Ecol Prog Ser 208:13–20

Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI (2006) A revised model for lipid-normalising d13C values from aquatic organisms, with implications for isotope mixing models. J App Ecol 43:1213–1222

Kirby RR, Beaugrand G, Lindley JA (2009) Synergistic effects of cimate and fishing in a marine ecosystem. Ecosystems 12:548–561

Knoff A, Hohn A, Macko S (2008) Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Mar Mammal Sci 24(1):128–137

Lovelock JE (1957) The denaturation of Lipid-protein complexes as a cause of damage by freezing. Proc R Soc B Biol Sci 147:427–433

Lucas CH (2001) Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. Hydrobiologia 451:229–246

Lynam CP, Hay SJ, Brierley AS (2004) Interannual variability in abundance of North Sea jellyfish and links to the North Atlantic oscillation. Limnol Oceanogr 49(3):637–643

Lynam CP, Heath MR, Hay SJ, Brierley AS (2005) Evidence for impacts by jellyfish on North Sea herring recruitment. Mar Ecol Prog Ser 298:157–167

Mackie IM (1993) The effects of freezing on flesh proteins. Food Rev Int 9(4):575–610

Makri M (2010) The biochemical, textural and sensory properties of king scallop (*Pecten maxinus*) meats frozen at different characteristic freezing times. Afr J Biotechnol 9(28):4374–4385

Malej A, Faganeli J, Pezdic J (1993) Stable isotope and biochemical fractionation in the marine pelagic food chain: the jellyfish *Pelagia noctiluca* and net zooplankton. Mar Biol 116:565–570

Malej A, Turk V, Lučić D, Benović A (2007) Direct and indirect trophic interactions of *Aurelia* sp. (Scyphozoa) in a stratified marine environment (Mljet Lakes, Adriatic Sea). Mar Biol 151:827–841

Mateo MA, Serrano O, Serrano L, Michener RH (2008) Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157:105–115

Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine food webs: an update. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental science, 2nd edn. Wiley-Blackwell, London, pp 238–282

Mills CE (2001) Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? Hydrobiologia 451:55–68

Olson MH (1996) Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. Ecology 77(1):179–190

Paredi ME, Roldan HA, Crupkin M (2006) Changes in myofibrillar proteins and lipids of squid (*Illex argentinus*) during frozen storage. J Food Biochem 30:604–621

Paredi ME, Pagano MR, Crupkin M (2010) Biochemical and physicochemical properties of actomysin and myofibrils from frozen stored flounder (*Paralichthys patagonicus*) fillets. J Food Biochem 34(5):983–997

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Pitt KA, Connolly CM, Meziane T (2009) Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review. Hydrobiologia 616(1):119–132

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83(3):703–718

Purcell JE, Arai MN (2001) Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451:27–44

Purcell JE, Fuentes V, Atienza D, Tilves U, Astorga D, Kawahara M, Hays GC (2010) Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645(1):135–152

Reyes G, Finol H, Bello R (2009) Effect of freezing and cooking on the ultrastructure of arms of octopus (*Octopus vulgaris*). Acta Microscopia 18(2):76–80

Russell FS (1970) The medusae of the British Isles II. Cambridge University Press, Cambridge

Sarakinos HC, Johnson ML, Vander Zanden MJ (2002) A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Can J Zool 80(2):381–387

Syväranta J, Vesala S, Rask M, Ruuhijärvi J, Jones R (2008) Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia 600:121–130

Syväranta J, Martino A, Kopp D, Céréghino R, Santoul F (2011) Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (*Corbicula fluminea*). Hydrobiologia 658:383–388

Vander Zanden MJ, Rasmussen JB (2001) Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Wada E, Terazaki M, Kabaya Y, Nemoto T (1987) ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep Sea Res 34:829–841

Wallace BP, Avens L, Braun-McNeill J, McClellan CM (2009) The diet composition of immature loggerheads: insights on trophic niche, growth rates, and fisheries interactions. J Exp Mar Biol Ecol 373(1):50–57

Wroblowski B, Diaz JF, Heremans K, Engelborghs Y (1996) Molecular mechanisms of pressure induced conformational changes in BPTI. Proteins 25:446–455