Application of nitrogen stable isotope analysis in size-based marine food web and macroecological research†

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Interacting human and environmental pressures influence the structure and dynamics of marine food webs. To describe and predict the effects of these pressures, theoretical advances need to be supported by a capacity to validate the underlying models and assumptions. Here, we review recent applications of nitrogen stable isotope analysis in marine food web and macroecological research, with a focus on work that has paralleled a resurgence of interest in the development and application of size-based models. Nitrogen stable isotope data have been used to estimate intra- and inter-specific variation in trophic level, predator-prey size ratios, transfer efficiency, food chain length, relationships between predator and prey species diversity and the dynamics of energy use. Many of these estimates have contributed to the development, testing and parameterisation of food web and ecosystem models, some of which have been used to establish baselines for assessing the scale of human impacts. The interpretation of results depends on assumed fractionation but, when supported by sensitivity analyses and experimental validation, nitrogen stable isotope data provide valuable insights into the structuring of marine communities and ecosystems. Copyright © 2008 John Wiley & Sons, Ltd.

Nitrogen stable isotope analysis provides a powerful tool for ecologists, giving new insight into the origins and transformations of organic matter and the migrations of animals in terrestrial and aquatic environments.1–3 This tool has been used to identify the sources of energy that support consumers,4 to describe interactions among small groups of species4 and to investigate migration.5,6 The development of automated and subsequently more cost-effective methods for measuring nitrogen stable isotope natural abundance7 has facilitated many of the recent ecological applications of stable isotope analysis. This is because inherent variability in life history and ecology among individuals, populations, communities and ecosystems means that high levels of replication in space and time are needed to gain sufficient statistical power to test competing hypotheses.

It is rarely feasible to study the ecology of all species that comprise food webs in such detail that the results can be used to describe community and ecosystem processes. Indeed, while species are often a focus of food web research at high trophic levels, low trophic levels are invariably dealt with at higher levels of aggregation (e.g. phytoplankton or plants). Given these constraints, an informative process is to assess the properties of communities and ecosystems in their own right, irrespective of the identities of component species. In marine ecology, interest in this approach was stimulated by the invention of the Coulter counter, which allowed the size distributions of living particles in the ocean to be described for the first time.8 The regularity of these distributions prompted research into the processes responsible,9 and provided a good example of what was later dubbed the macroecological approach.10

Here, we focus on recent applications of nitrogen stable isotope analysis in marine food web and macroecological research; work that has paralleled the resurgence of interest in size-based modelling of marine food webs. This is a necessarily short review to meet the requirements of this conference volume, and does not cover significant advances in using stable isotope analysis to study source materials, pathways and conventional species-based food web structures; as recently reviewed by Fry.2

Size-based analyses of food webs treat body size rather than species identity as the principal descriptor of an individual’s role in the food web. In such analyses, a small individual of a large species is treated as functionally

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equivalent to a large individual of a small species in the same body mass class. A strength of the focus on body size is that body size dictates much of the variation in the biological properties of individuals and underpins predator-prey interactions. Size-based approaches are especially relevant in aquatic ecosystems where many species grow through five or more orders of magnitude in body mass and two or more trophic levels during their life cycle and where most primary production is attributable to pelagic phytoplankton.

Increases in mean trophic level with size were implied by theoretical analyses and first examined empirically using nitrogen stable isotope analysis in size-fractionated zooplankton samples. If representative sampling of organisms in different size classes is feasible, then knowledge of relationships between trophic level, abundance, production, diversity and size can be used to describe structures and processes in food webs. Here, we discuss how size-based nitrogen stable isotope analysis has been used to quantify intra- and inter-specific variation in trophic levels, predator-prey size ratios, transfer efficiency, food chain length, relationships between predator and prey species diversity and the dynamics of energy use by populations and communities and to establish baselines for assessing the scale of human impacts on ecosystems. In addition, we consider the effects of assumptions about fractionation on the outcomes of such analysis.

Size-based analysis

A principal assumption of size-based analysis is that body size accounts for a large proportion of the variance in trophic level compared with species identity. If this assumption were not met, then categorisation by species would be necessary, greatly complicating the analysis of food web structures in ecosystems that vary widely in species composition. Many marine food chains are, however, strongly size-based. For example, analysis of the relative contributions of intra- and inter-specific differences in trophic level (as indicated by $\delta^{15}N$) to trophic structure in a North Sea food web showed that the inter-specific relationships between maximum body mass and $\delta^{15}N$ at a fixed proportion of that body mass were weak or non-significant for invertebrates and fishes. This result was confirmed with a comparative analysis that accounted for phylogenetic relationships among species. Conversely, when all animals in the communities were divided into body mass classes without accounting for species identity, trophic level rose near continuously with body mass (e.g. Fig. 1).

Additional analyses of intra- and inter-specific relationships between body mass and $\delta^{15}N$ in fishes and invertebrates have shown that increases in trophic level across the size spectrum were predominantly a consequence of intra-specific increases in trophic level with body mass rather than larger species (species with greater maximum body mass) feeding at higher trophic levels. Thus the increase in trophic level of individual species with body size makes an important contribution to the increase in the trophic level of the community with size (Fig. 2). Analyses of this type have yet to be done for a complete size spectrum but, within the size range considered, most of the animals contributing to the spectrum were included.

Figure 1. Relationships between the mean $\delta^{15}N$ ($\pm$95% CL) and maximum body mass of North Sea fish species (a) and between the mean $\delta^{15}N$ ($\pm$95% CL) of fishes of all species combined into log$_2$ body mass classes (b). The relationship between body size and trophic structure is expected to be stronger in marine food webs that are based on phytoplankton than in marine food webs based on benthic algae, macroalgae and detritus or food webs in freshwater and terrestrial environments. However, phytoplankton do account for around 90% of global marine primary production and around 35% of global primary production.

Figure 2. Relationships between the $\delta^{15}N$ of invertebrates/fishes and log$_2$ body mass class in a size-fractionated community (broken line) and between the $\delta^{15}N$ of individual species of invertebrates or fishes and log$_2$ body mass class (continuous lines). The individual species shown are those that accounted for the greatest proportion of the biomass of the whole community.
Predator-prey mass ratios and transfer efficiency

If the fractionation of $\delta^{15}N$ with trophic level is known then relationships between $\delta^{15}N$ and body mass class can be used to predict the predator-prey mass ratio (PPMR) in the community. The PPMR is the ratio of the mean body mass of predators in a food web to the mean body mass of their prey. PPMR is an important attribute of a food web because it can be used to predict the strength of predatory interactions, the length of food chains and the pathways of energy transfer. Estimates of the PPMR are necessary inputs to models of the structure and function of marine food webs. Mean PPMR ($\mu$) is a function of the slope ($b$) of the relationship between $\delta^{15}N$ and $\log_{10}$ body mass class, and is calculated as $\mu = n^{-1/\Delta}$, where $n$ is the base of the logarithm of the body mass classes and $\Delta$ the fractionation of $\delta^{15}N$.20

The use of the relationships between $\delta^{15}N$ and body mass class to calculate the PPMR requires representative sampling of the animals in each body size class in the food web. This is challenging but possible in most marine environments. Representative sampling will usually require different sampling gears to target different groups of animals. The mean $\delta^{15}N$ weighted by biomass for all groups can be calculated from the abundance and $\delta^{15}N$ data for each group, provided that the sampling gears allow abundance to be determined in a standard way for all groups (e.g. mean biomass $m^{-2}$).20

Body mass is closely related to other attributes of animals, such as metabolism, respiration, production, mortality and intrinsic rates of population increase.11 For this reason, the distributions of abundance by body mass class can be used to approximate the distributions of other attributes, provided that relationships between body mass and these attributes are known. The relationship between body mass ($M$) and individual biomass production ($P/M$) is approximated by $P/M = aM^{-b}$, where $a$ is a constant. The existence of this relationship underpins an approach where size-based nitrogen stable isotope analysis can be used to predict transfer efficiency ($\epsilon$); where $\epsilon$ is defined as the proportion of prey production that is converted into predator production ($\epsilon = P_{i+1}/P_i$, where $P_{i+1}$ is predator production and $P_i$ is prey production).20 Production in each body mass class is estimated from biomass ($B$) in the body mass class $[P = B \times (P/M)]$ and trophic level in each class from $\delta^{15}N$. The transfer efficiency (TE) can be calculated from the slope of the relationship between $\log_{10} P$ ($y$) and $\delta^{15}N$ ($x$) where $\epsilon = n^{\Delta}$.

Total species richness often decreases systematically with increasing body mass and this allows size-based nitrogen stable isotope analysis to be used to estimate relationships between numbers of predator and prey species.20 For a relationship between $\log_{10}$ normalised species richness by body mass class ($y$) and $\delta^{15}N$ by body mass class ($x$), the ratio between the number of predator and prey species will be $n^{\Delta}$.

The approaches for estimating PPMR, TE and the ratios between numbers of predator and prey species all rely on representative sampling of biomass and species richness in each size class and knowledge of the fractionation of $\delta^{15}N$ ($\Delta^{15}N$). The logistical challenge of sampling can be considerable, but it has been possible to conduct such sampling for defined size windows in the overall size spectrum and estimates of these parameters have been published for some marine food webs.21,22 These size-based methods of food web analysis make a number of gross simplifications about the size-based nature of predator-prey relationships, the significance of omnivory in marine ecosystems and the estimation of trophic level from nitrogen stable isotope analysis.15 However, they do provide a basis for assessing the structure and function of ecosystems at large spatial scales and the results have been broadly consistent with those obtained from costly and labour-intensive diet and ecosystem modelling studies.23

Food chain length

Food chain length affects rates of nutrient recycling, levels of contaminants in top predators and pathways of energy flow in food webs. Nitrogen stable isotope data have been used to estimate food chain length,24 with much of the existing emphasis on work in freshwater and terrestrial environments.25–28 In size-based food webs, PPMR and food chain length are closely linked since, in a community of given size composition, a smaller PPMR will result in longer food chains. The drivers that affect food chain length could act on the PPMR or the length of food chains directly, by removing top predators. An investigation of the links between PPMR and food chain length at 74 sites in the North Sea21 showed that the PPMR was smaller in longer food chains and in less variable environments. Also notable was the observation that the heaviest predator at each site rarely fed at the highest trophic level (Fig. 3) and the longest food chains supported predators with intermediate body size. This suggested that it would not be sufficient to sample only the largest predators when estimating food chain length. On average, trophic level rose consistently and significantly with body mass (Fig. 4), and the mean predator-prey mass ratio among sites was 424:1 if $\Delta^{15}N = 3.4^{\%}$ was assumed.21

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**Figure 3.** The relationship between the maximum trophic level of any individual and the maximum trophic level of the largest individual in North Sea food webs.21 Trophic level was estimated from $\delta^{15}N$ assuming a fractionation of 3.4% with each trophic level and that ‘base’ $\delta^{15}N$ varied according to a model linking base $\delta^{15}N$ and environmental variables.29
Size spectra

Abundance-body size relationships capture the distribution of body sizes in food webs. These are expressed in two ways. First, as cross-species relationships between log mean abundance and log mean M, often for taxonomically or functionally defined groups of species, such as plants, birds or mammals. Second, for all individuals in a food web or specified size range, from relationships between log abundance in a body mass class and log M at the lower bound or midpoint of the class. The former approach has principally been explored for terrestrial communities and the latter for aquatic communities, where abundance-body size relationships are usually referred to as size spectra.8

In circumstances where there is a linear reduction in energy availability with size, as in strongly size-based aquatic food webs, it is straightforward to estimate energy availability in different size classes of animals from knowledge of trophic level, TE and PPMR.9 The energy available ultimately constrains abundance and therefore determines the slope of the size spectrum.10 However, calculations of slope also depend on knowledge of how abundance changes with body size when energy is shared. This is predicted by the energetic equivalence hypothesis, which suggests that numerical abundance N scales as $M^{-0.75}$ in plants or animals sharing a resource (and therefore biomass abundance $B$ as $M^{0.75}$. Since the metabolic rate is known to scale as approximately $M^{0.75}$, energy use is independent of $M$ and body size does not provide any population with an energetic advantage.30 When the availability of energy ($E$) to animals changes with body size, as is expected when trophic level changes, then the scaling of $N$ or $B$ with $M$ can be predicted from the scaling of $E$ and $M$ which is given as $E = M^{0.75}$. The slope of the size spectrum (relationship between log $B$ and log $M$) can then be calculated as $M^{0.75}$.25

Based on the preceding theory, nitrogen stable isotope analysis of animals fractionated into body mass classes has been used to predict slopes of size spectra in complete food webs and subsets of those webs. For example, the observed biomass scaling of $M^{-0.2}$ in a marine food web was not significantly different from the scaling of $M^{-0.24}$ predicted from energy availability at site.22 Applications of the method to subsets of food webs have provided further evidence of the central role of energy availability in determining abundance in body size classes. In some benthic food webs, for example, the $\delta^{15}N$ value suggested that trophic level fell with body size because the smaller size classes in the food web were dominated by predatory worms and the larger size classes by deposit and suspension feeders such as bivalves.31 When the positive scaling of $E$ and $M$ was used to predict the relationship between log $B$ and log $M$, the expectation that the slopes would be more positive than $M^{0.25}$ was confirmed.31

Human impacts

Human activities can modify size spectra. Fishing, for example, increases the slopes of size spectra.32 Comparisons between slopes that describe food web structures in the absence of human impacts and slopes that describe impacted systems provide a measure of the magnitude of human impacts.

The unimpacted slope of a size spectrum can be predicted using the methods described in the preceding section because the PPMR and TE are largely unaffected by fishing.21 For the North Sea, a comparison of the slopes of contemporary (2001) size spectra and slopes predicted from measurements of PPMR and TE33 revealed reductions in the biomass of large fishes, suggesting that the biomass of fishes weighing 4–16 kg and 16–66 kg, respectively, was 97.4% and 99.2% lower than in the absence of fisheries exploitation (Fig. 5). The method is advantageous because it provides a common baseline for assessing fishing impacts in different regions and because the structure of unexploited communities cannot always be predicted from historical data. This is because fisheries exploitation usually precedes scientific investigation and non-fisheries impacts, such as climate change, modify ecosystems over time.

Figure 4. Relationship between body mass and mean trophic level at 74 North Sea sites (± SD).21 Trophic level was estimated from $\delta^{15}N$ assuming a fractionation of 3.4‰1 and that base $\delta^{15}N$ varied according to a model linking base $\delta^{15}N$ and environmental variables.29

Figure 5. Predicted slope of an unexploited size spectrum (dashed line) and the size spectrum for the exploited North Sea in 2001 (continuous line and data).33 Trophic level was calculated from body mass and $\delta^{15}N$ assuming a fractionation of 3.4‰ and that ‘base’ $\delta^{15}N$ varied according to a model linking base $\delta^{15}N$ and environmental variables.28 The height (intercept) of the size spectrum was predicted from an estimate of primary production.33
Stability of trophic structure

Time-series δ¹⁵N data are starting to provide insight into the relative stability of trophic levels of different species and body size classes in food webs, and of the food web as a whole. In addition, valuable insights into the stability of trophic structure and feeding strategies are starting to emerge from comparisons among sites subject to different levels of human impact. Thus fragmentation of coastal marine habitats appears to be associated with reductions in the niche width of predators using those habitats.

Regular annual sampling of species that dominate the total biomass of the North Sea fish community shows the range of trophic strategies employed by these species and their variation through time (Fig. 6). Indeed, for some species such as plaice Pleuronectes platessa, trophic level actually decreases with body size reflecting a shift in diet from small predatory worms to larger filter-feeding bivalve molluscs. Such strategies are only adopted by relatively few species, however, and the main pathways of energy flow in the community are strongly size-based. Further, the overall relationship between trophic level and body mass, and hence PPMR, is remarkably consistent despite variations in the abundance of component species.

Fractionation

All the preceding work is predicated on the assumption that δ¹⁵N provides a good index of trophic level and that Δδ¹⁵N is known. For this reason, the effects of assumed Δδ¹⁵N need to be understood and Δδ¹⁵N should ideally be validated in comparable food webs. A simple approach to understanding the effects of assumed Δδ¹⁵N on the results of analyses is to conduct sensitivity analysis. These show, for example, how estimated PPMR and TE might vary with Δδ¹⁵N in a marine food web (Fig. 7). The effects of the propagation of errors associated with TE or PPMR can then be assessed by looking, for example, at their effect on the calculated slope of the size spectrum (Fig. 8).

Absolute values of trophic level as required in the analyses of energy flow are also dependent on assumptions about δ¹⁵N at the base of food chains (δ¹⁵Nbase). In large areas of the marine environment that have been studied δ¹⁵Nbase is positively correlated with salinity, probably reflecting the contribution of sewage-derived nitrogen inputs to coastal waters. In a study of spatial variation in the δ¹⁵N of a bivalve mollusc that filters sea water and is a good integrator of the very variable short-term δ¹⁵N signals that characterise detritus and phytoplankton, a model of the relationship between δ¹⁵N and environmental variables was used to show that 51% and 77% of spatial variance in the δ¹⁵N of two fish species, and hence apparent trophic level, could be attributed to differences in δ¹⁵N at the base of the food chain. Since temperature and salinity are correlated with base δ¹⁵N, and since gradients in these physical variables are particularly pronounced in coastal areas, spatial comparisons of trophic level in these areas are easily biased if fine-scale information on base δ¹⁵N is not available. Conversely, in offshore regions, where temperature and salinity show little variation over large areas, variations in base δ¹⁵N and the associated bias are expected to be less (Fig. 9).

Understanding the effects of uncertainty in Δδ¹⁵N on the outputs of analyses is only one part of a process that should also include validation; to help determine what the most likely values of Δδ¹⁵N would be. In studies that aggregate across individuals under ‘natural’ conditions it is likely that some of the extreme variability in Δδ¹⁵N that is recorded for individuals in experimental studies will not be relevant and the observation that PPMR, as assessed with δ¹⁵N and diet data, seems to be relatively constant across at least ten orders of magnitude in body mass suggests that strong size-related effects on Δδ¹⁵N are unlikely. At the level of individuals and populations, variation in Δδ¹⁵N is likely to be greater and will affect the relationships among calculated trophic levels for
different species. $\Delta\delta^{15}N$ has often been assumed to be $3.4 \pm 1.1\%$ (1 standard deviation (SD)) and although this estimate was based on a very small number of individual estimates, it proved to be a surprisingly reliable mean value when further results were compiled.

In the context of the food web work previously described, we have placed recent emphasis on experimentally validating $\Delta\delta^{15}N$ for fishes under experimental regimes that approximate natural conditions of temperature, light, food type and food availability (temperatures and/or temperature variations encountered in the wild, natural photoperiods, low stocking densities, food types eaten in the wild and feeding rates representing the expected minimum and maximum for wild fish). For example, when European sea bass (Dicentrarchus labrax) were reared on constant diets of dab (Limanda limanda) muscle or whole sandeel (Ammodytes marinus) for 2 years under natural light and temperature regimes, mean values of $\Delta\delta^{15}N$ were 3.83% and 3.98% for muscle tissue. Fractionation appeared to be independent of body mass, but there was a very small time effect on muscle $\Delta\delta^{15}N$, described by a sinusoidal function with a period of 1 year and amplitude $/C2/C0.3\%$. Although the $\Delta\delta^{15}N$ for bass muscle on both diets approached 4%, the data from this study were combined with literature data for other species and these suggested that, when species-specific data were not available, a mean $\Delta\delta^{15}N$ for fish muscle of 3.2% should be applied (Fig. 10), close to the previously reported mean values for a wider range of animals.

To further investigate effects of temperature and feeding rate on $\Delta\delta^{15}N$ for bass muscle, bass were also reared on identical sandeel diets at 11 and 16°C and at three ration levels for 600 days. The $\Delta\delta^{15}N$ was affected by a temperature x ration interaction (Fig. 11), with $\Delta\delta^{15}N$ falling from 4.41% at 11°C to 3.78% at 16°C for all rations combined. The effects of ration were relatively small. Thus at all rations $\Delta\delta^{15}N$ was higher in the cooler temperature but the difference in $\Delta\delta^{15}N$ between cool and warm varied depending on the ration, with the greatest difference (−0.2% per 1°C) at medium ration and the least difference at high ration.
The experimental studies largely confirm the expectation that \( \delta^{15}N \) is a better indicator of relative rather than absolute trophic level and that differences in \( \Delta\delta^{15}N \) among species should be expected (Fig. 10). Values of \( \Delta\delta^{15}N \) around 3.4% still represent the mean expectation and may be realised when animals of many species are combined in size classes. In studies of single species the use of a mean value may be more misleading. These concerns surrounding the value of \( \Delta\delta^{15}N \) should be weighed against the recognition that there is currently no better method for looking at many aspects of food web structure and function, and that approaches based on nitrogen stable isotope analysis often require lower levels of replication than diet studies to provide realistic time- and space-integrated assessments of food web structure and function.

CONCLUSIONS

The use of nitrogen stable isotope analysis in size-based marine food web and macroecological research provides one example of the many potential applications of this methodology when assessing food web structure and function. One valuable feature of the size-based work, however, is that it has paralleled a resurgence of interest in the development and application of size-based models of marine food webs.\(^{11,41,42}\) and empirical analyses have been guided by theory, and vice versa. Thus, nitrogen stable isotope data have been used to quantify intra- and inter-specific variation in trophic level, predator-prey size ratios, transfer efficiency and food chain length, and many of these estimates have been to support the development, testing and parameterisation of models.

The precision, and thus the power to resolve competing hypotheses, has been much improved by the automation and falling relative cost of nitrogen stable isotope analysis. However, the methods of marine macroecological and food web analysis described would have wider applicability if there were more confidence in the accuracy of the outputs, especially when attempting to assess energy fluxes. An increase in accuracy is most likely to be achieved with compound-specific rather than bulk analysis,\(^{43-44}\) but at present there is an obvious requirement to balance the benefits of more accurate predictions of trophic level against the costs of low replication. If methods for compound-specific analyses can be more effectively automated and if amino acids that provide reliable information on trophic fractionation are identified, then compound-specific analyses will help to deal with many of the factors that probably influence bulk signals, such as differences in the amino acid supply from diet and protein breakdown.\(^{45}\)

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REFERENCES

44. McClelland JW, Montoya JP. *Ecology* 2002; 83: 2173.