

Power of stable isotope techniques to detect size-based feeding in marine fishes

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ABSTRACT: Stable isotope techniques are now the most frequently applied method to investigate size-based variation in fish trophic position (TP), and data suggest changes in TP with body size are common. However, of 131 collated investigations of the relationship between body size and $\delta^{15}\text{N}$ for inshore coastal fishes, approximately 60% were non-significant. The present study tests whether non-significant trends reflect a true feeding pattern, a lack of statistical power, or a function of both. Results suggest none of these studies for which correlation coefficients were available, achieved enough statistical power to rigorously assess observed slopes. The sample size required to detect statistically significant trends of different magnitude, given assumptions of dispersion and observed data on body size range sampled, was then assessed. Even under lenient assumptions of below average dispersion and uniform sampling effort across the sampled body size range, <10% of the statistical tests classifying trends as non-significant had the power to detect a slope corresponding to a 0.25 change in TP over 80% of maximum length (proportional length range: L_{max}) throughout life. Therefore non-significant trends in $\delta^{15}\text{N}$ are predominantly the result of poor power, and no conclusion can be drawn as to associated fish trophodynamics. Analysis here suggests that size-based feeding studies should aim to sample over 40% L_{max} range of the population, as the sample size required to maintain sufficient statistical power diminishes almost exponentially with increasing body size range. Researchers should strive to minimise other sources of variation as linear increases in dispersion lead to virtually linear increases in sample size necessary to maintain power. Finally, a key component of hypothesis testing for size-based feeding should be not just whether a trend is significant, but whether sampling is adequate to detect a minimum ecologically relevant threshold slope. This will separate tests that lack power from those where size-independent feeding is a true feeding mode.

KEY WORDS: Diet · Regression · Ontogenetic · Sulphur · Nitrogen · Trophodynamic · Production

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INTRODUCTION

Body size is a fundamental determinant of fish trophodynamics (Peters 1983). Food intake, foraging ability, and potential range of prey all generally increase with size (Cohen et al. 1993, Reid et al. 2007) resulting in predators being typically larger than their prey (Jennings et al. 2008a). Predators generally increase in trophic position (TP) throughout their life as a result of ontogeny first and then through subsequent growth. The need to understand these 2 processes of size-based changes in feeding are particularly important for understanding the driving forces behind community structure and fisheries management.

While size-based feeding has been documented in a number of studies based on gut content (Stergiou & Karpouzi 2002), the time- and labour-intensive nature of this technique often limits application to commercially or ecologically important species. Stable isotope analyses (SIA) provide an alternative approach to estimation of TP as well as the potential to describe the source of primary production sustaining different species or size classes. The use of SIA in size-based feeding studies has reduced the sample size compared to gut content based studies because stable isotopes can derive TP data for individuals in relation to suitable baseline values. TP has repeatedly been shown to be positively correlated to the stable isotope ratio of ^{15}N to ^{14}N , expressed as $\delta^{15}\text{N}$ (e.g. Jennings et al. 2002).

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Improvements in analytical instrumentation and reductions in per sample cost have ensured widespread adoption of the stable isotope technique (Jennings et al. 2008a).

Numerous studies have applied isotope techniques to the investigation of size-based feeding. The most comprehensive datasets to date (Davenport & Bax 2002, Jennings et al. 2002) suggest that size-based feeding is common. Both derive results from planktonic-driven continental shelf ecosystems where size-based feeding is expected to be strong. Despite this, substantial numbers of species (~40%) exhibit non-significant trends (Jennings et al. 2002). In more complex systems with a greater array of production sources and weaker size structuring (Layman et al. 2005), the potential for non-significant trends is greater.

It is, however, unclear whether non-significant data in more complex inshore systems reflect a true feeding pattern or a lack of statistical power. Defining the relative proportions of each would be informative in interpreting size-based feeding behaviour and stimulating correct experimental design. The majority of studies that test size-based feeding apply linear regression techniques. This choice, as opposed to an ANOVA with multiple size classes, is the right and powerful choice (Cottingham et al. 2005) for size-based trends.

The earliest isotope study to examine size-based feeding using SIA was Rau (1981) on Dover sole *Microstomus pacificus*. With a sample size of ≤ 6 , the study would fail to detect a significant body size effect despite an R^2 in excess of 0.70. Menard et al. (2007) also found $\delta^{15}\text{N}$ independent of body size in yellowfin tuna *Thunnus albacares*, although in this case a sample size of 244 individuals lends confidence to their conclusions. These 2 studies are illustrative of the issue of separating studies genuinely exhibiting feeding patterns from studies with limited power.

The statistical power ($1-\beta$) of a linear regression to detect a significant dependence of the response variable on a predictor is related to both sample size (n) and to the strength of the correlation (r) (Dupont & Plummer 1998). r in turn is a function of the magnitude of the slope (b), the range of predictor (Δx), the distribution of data within Δx , and the degree of dispersion (σ_ϵ) in the response. Dispersion can be quantified as σ , the standard deviation (SD) of the residuals ϵ . Expressed in terms of size-based diet shifts in fish, statistical power is a function of the sampled body size range ($\Delta x = \Delta L_{\text{max}}$), the distributions of samples within that size range, the magnitude of the trophic change (ΔTP) with body size ($b = \text{slope of the } \delta^{15}\text{N}-L_{\text{max}} \text{ regression}$) and the variability in $\delta^{15}\text{N}$ for a given body size ($\sigma_\epsilon = \text{SD of } \delta^{15}\text{N}_{\text{observed}} - \delta^{15}\text{N}_{\text{predicted}}$).

Given R^2 and n , the power of a regression is easily determined. However, in individuals with no size-based feeding, hundreds of samples may be required to

achieve acceptable levels of power ($1-\beta = 0.80$), and almost all data eventually exhibit significance given enough sampling effort regardless of how meaningful the effect. A more pertinent question is whether the statistical analyses had the power to detect some ecologically meaningful threshold effect. In this way, power analysis may be used to separate studies revealing true feeding patterns from under-sampled studies if ecologically meaningful threshold levels of b can be defined. Given observed levels of n , σ_ϵ and ΔL_{max} , it is possible to test whether any given study had the power to detect the desired b . Those that do have sufficient power but detect no significant effect, suggest $\delta^{15}\text{N}$ is independent of body size and represent a true feeding behaviour, while those that do not achieve the desired power lack the ability to differentiate trends.

The threshold effect approach described above is used here to assess how frequently non-significant trends between $\delta^{15}\text{N}$ and body size in marine fishes are attributed to true feeding behaviour or simply result from a lack of statistical power, particularly size-based trends in fish sampled at complex inshore habitats. The definition of an ecologically significant threshold effect may vary with perspective, the isotope used, and study aims. Similarly, power will vary with the level of σ_ϵ and with other factors influencing r . Specifically, this study investigates (1) the proportion of non-significant tests of size-based feeding in a data set of marine inshore coastal fishes; (2) how many of these tests achieved sufficient statistical power for detecting observed slopes based on associated r or R^2 data; and (3) given lenient assumptions of dispersion, how many tests would have been powerful enough to detect ecologically meaningful diet shifts of between 0.25 and 1.0 TPs in magnitude. In investigating the impacts of assumptions of dispersion and threshold effect on conclusions, the present study also allows the provision of guidance for testing size-based feeding behaviour.

MATERIALS AND METHODS

Ontogenetic or size-based feeding data for inshore coastal fishes described by $\delta^{15}\text{N}$ were collated from the literature using Institute for Scientific Information (ISI) literature database key word searches (combinations of "isotop*", "trophic", "size", "ontogenetic", "fish*", "diet", "feeding", "reef", "lagoon", "estuarine", and "marine") and followed up by a search through forward and backward citations. The literature survey covered January 1977 to July 2009 (see Supplement 1 at www.int-res.com/articles/suppl/m407p271_app1.pdf). Further criteria for inclusion were data obtained from nominally complex communities including brackish, vegetated, and biogenic habitats.

Information on n , b , R^2 , body size range, and slope significance was then extracted from collated literature. The sampled body size range and slope of the isotope-size regression were converted from minimum and maximum unit length (mm or cm) to proportional length range (ΔL_{\max}) to standardise among species. L_{\max} were obtained from FishBase (Froese & Pauly 2009). Regressions were then assessed for $1-\beta$ using a 1-tailed test, Eq. (1) (Pérttega Díaz & Pita Fernández 2002):

$$Z_{1-\beta} = \sqrt{n-3} \frac{1}{2} \ln\left(\frac{1+r}{1-r}\right) - Z_{1-\alpha} \quad (1)$$

where n represents sample size and α is the probability of falsely accepting the alternative hypothesis when in fact the null hypothesis is true and was set at 0.05. $1-\beta$ was set at 0.80 and equals the probability of falsely accepting the null hypothesis when in fact the alternative hypothesis is true.

To categorise data retrospectively as either (1) adequate to identify true feeding behaviour or (2) insufficient in sample size, the sample size needed to achieve $1-\beta$ of 0.80 was determined based on a threshold b and estimated σ_ϵ in combinations with the observed ΔL_{\max} , assuming a uniform distribution of data across the sampled body size range (ΔL_{\max}). Where the observed n (n_{obs}) was greater than n required to achieve sufficient power ($n_{1-\beta}$), data were categorised as adequate (true feeding behaviour). When n_{obs} was less than $n_{1-\beta}$, data were categorised as under-sampled (lacking statistical power).

$n_{1-\beta}$ was derived by fitting linear models to simulated datasets of 100 000 samples with an independent variable X created as a random sample with uniform distribution on ΔL_{\max} intervals and a dependent variable Y constructed by Eq. (2):

$$Y = bX + \epsilon \quad (2)$$

where Y represents the $\delta^{15}\text{N}$ value, X the sampled body size range as L_{\max} , and ϵ the error term, which was constructed as 100 000 samples normally distributed with mean = 0 and SD = 0 σ . All the calculations were done using the statistical software R V.2.5.0 (R Development Core Team 2007) (source code in Supplement 2 at www.int-res.com/articles/suppl/m407p271_app2.txt).

This paper determined the minimum threshold effect as a 0.25 change in TP throughout life. This equates to $b = 0.01$ assuming a nitrogen diet-tissue fractionation of 3.2‰ (Sweeting et al. 2007a). This was based on ultimate interests in impacts of exploitation on marine populations.

As this study retrospectively estimates statistical power of studies, observed σ_ϵ was not available. σ_ϵ was set at ± 0.3 , derived from Sweeting et al. (2005) for fish

on a constant diet. Although ΔL_{\max} was collected, the distribution of samples within ΔL_{\max} was unavailable. For simplicity, sampling of body size was assumed uniform throughout the range and this combined with a σ_ϵ from fish on constant diet represents the lenient scenario most readily assessing studies to have adequate power. Stricter scenarios were assessed by altering σ_ϵ .

As σ_ϵ , ΔL_{\max} , and the threshold b may vary among studies, and both b and R^2 are derived variables that may be altered by context, the effect of variation in the former parameters on $n_{1-\beta}$ was assessed. $n_{1-\beta}$ requirements were also assessed for TP changes of 0.33, 0.5, and 1.0 over 80% L_{\max} ($b = 0.0166$, 0.02, and 0.04, respectively). Variation on σ_ϵ was assessed in 0.02 increments from ± 0.1 to ± 1.5 , representing the best analytical precision and high variability of young wild fishes with high turnover feeding on an unknown diet (Sweeting et al. 2005), respectively.

RESULTS

The literature review obtained 131 tests of trends or differences in $\delta^{15}\text{N}$ with body size from 31 studies (Supplement 1) and 86 species. Linear regression was the dominant form of statistical analysis, constituting 64.89%. Pearson's correlation constituted 6.87%. 14.50% of tests utilised Spearman's rank, while all remaining analyses were of the ANOVA family (ANOVA, mixed ANOVA, t -test, and so on) and totalled only 13.74%.

There was no evidence that researchers have increased sample size through time when assessing feeding behaviour by means of linear regression. Of all tests, 60.31% were non-significant, with this proportion almost uniform among statistical techniques. Of the linear regressions and Pearson's correlations, 60.63% exhibited no significant trend between $\delta^{15}\text{N}$ and size; 36.17% exhibited significant positive trends; and 3.19% significant negative trends. Only a quarter of tests reporting non-significant results gave correlation coefficients. Utilising these, none of the tests achieved a satisfactory power of 80% to adequately test slope significance. Power instead ranged from 0.06 to 58.39%.

However, it is more informative to examine whether tests had the statistical power to detect effects of ecological significance. Using the sampled ΔL_{\max} and n_{obs} for each regression and assumptions of dispersion ($\sigma_\epsilon = 0.3$), proportions of 6, 9, 27, and 63% of the non-significant regressions achieved a statistical power of 0.8 with threshold effects of $\Delta\text{TP} = 0.25$, 0.33, 0.50, and 1.0, respectively (Fig. 1). These represent the maximum number of studies with reliable non-significant

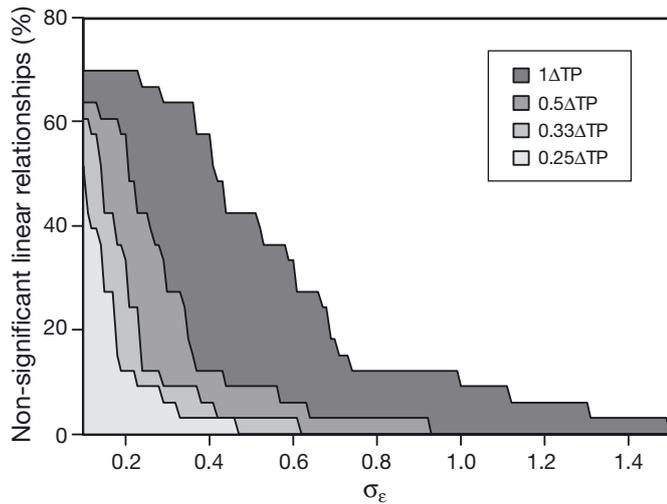


Fig. 1. Percentage of non-significant linear relationships with sample size (n) bigger than the minimum n required to achieve power $(1-\beta)$ of 0.8 ($n_{1-\beta}$) given observed proportional length range (ΔL_{\max}) and assuming uniform sampling across ΔL_{\max} , degree of dispersion $\sigma_\epsilon = 0.3$, and different threshold lifetime changes in trophic position (ΔTP)

trends, i.e. studies that can be considered having adequate power, because higher levels of σ_ϵ and infringements of the assumption of uniform sampling effort across ΔL_{\max} will only reduce power and thus the proportion of the dataset that was allocated to a true feeding behaviour. For example, for $\sigma_\epsilon > 0.46$ or 0.61 , no conclusion could be drawn regarding size-based feeding from any study using $\Delta TP = 0.25$ and 0.33 as threshold effects, respectively (Fig. 1). This suggests that in the majority of cases where non-significant trends were identified, sampling strategies were insufficient for detecting shifts in feeding behaviour of less than 1 TP, which inadequate for many ecological hypotheses.

The formulation of the above post hoc power testing equally allows provision of guidance on better sampling strategy. The statistical power to detect trends dramatically improved with increasing threshold effect and sampled size range (Fig. 2A,B) and with decreasing dispersion (Fig. 2C,D). For any given body size range, linear increases in slope (ΔTP) led to approximately exponential decreases in the sample size required to obtain sufficient power (Fig. 2B). Thus $n_{1-\beta}$ was very sensitive to ΔL_{\max} throughout but particularly when ΔL_{\max} was low. Sampling intensity required to detect the defined ecologically important threshold effect began to equalise between 40 and 60% ΔL_{\max} and asymptotically converged to a sample size of ~ 6 with increasing threshold (ΔTP). For example, when assessing $\Delta TP > 0.5$, sample size changed little $> 30\%$ ΔL_{\max} . Similarly, increases in $n_{1-\beta}$ were particularly pronounced when trying to detect effects smaller than

0.4 TP, although this was mediated by increasing ΔL_{\max} (Fig. 2B). In comparison with other parameters, $n_{1-\beta}$ increased almost linearly with increasing σ_ϵ given the range of dispersion assessed (Fig. 2C,D). However, the impact of increasing dispersion on $n_{1-\beta}$ was reduced when both the magnitude of the slope and the sampled size range increased.

DISCUSSION

The analyses presented here suggest that significant bias exists in current assessment of size-based feeding in marine fishes from inshore coastal habitats. This review indicates the dominant observation was for size-independent feeding. This conclusion is, however, flawed as few tests were capable of detecting even large shifts in feeding behaviour with only 2 papers recognising that sample size was too small and ΔL_{\max} too narrow.

Sample size, per statistical test of the SIA size-based feeding studies evaluated in the present study, did not increase over time despite reductions in per sample analysis costs. It appears instead that authors have either sampled more species, or the same species across a greater number or spatial/temporal points. This suggests analytical advances have not led to more robust sampling, only to a greater quantity of under-sampled data.

Analysis also highlights that the currently applied simple classification of feeding into significant and non-significant size-based feeding is inadequate. These conclusions are unlikely to be a function of assumptions used within the present meta-analysis, as analyses were lenient in favour of allocation to size-independent feeding. Dispersion was set at 0.3% , about 0.1% lower than the mean SD found in trophic fractionation from reviewed controlled feeding experiments on fish (Sweeting et al. 2007a,b). These analyses also assumed a uniform sampling distribution across ΔL_{\max} . However, unless sample collection is specifically aimed to maintain uniform sampling, large and very small size classes will be under-represented, a result of catchability. This reduced the effective ΔL_{\max} and thus statistical power.

Given the inadequacies of current investigations allocating fishes to either significant or non-significant trends, it is recommended that either hypothesis testing be taken against some ecologically meaningful threshold or trend slopes are used regardless of significance. The former may be used to separate size independent feeding behaviour from inadequate tests. The latter was used by Jennings et al. (2002) to investigate fishing impacts on North Sea food webs. Results of the present study support this technique assuming

sample size is adequate for the accurate determination of slope.

The main application of power analysis in experimental design is to understand how many samples are needed to accept the outcome of an insignificant statistical result with a particular level of confidence. This study highlights that sample size, ΔL_{\max} , threshold effect size (ΔTP) and σ_ε are all important determinants of whether a study has the statistical power to detect size-based feeding of marine fish. It illustrates that power diminishes rapidly when sampling is conducted over less than 40% ΔL_{\max} and is approximately linearly related to dispersion. The study highlights that simply collecting more samples may not be the most efficient technique to improve power of ecologically motivated

questions relating to size driven trophodynamics or feeding patterns of marine fish.

The aforementioned information has considerable potential for refinement of testing true ontogenetic and growth-based feeding patterns. Ontogenetic trends in foraging behaviour and diet switching can be pronounced when individuals develop from larval stages to juveniles (Wells et al. 2008). These are often accompanied by a switch in habitat utilisation (Nagelkerken & van der Velde 2004). The combination of a diet switch and fast turnover can result in very abrupt changes in stable isotope values over a very narrow size range (Graham et al. 2007, Wells et al. 2008). Within this scenario the potential size range is constrained to be small even if the effect is often large.

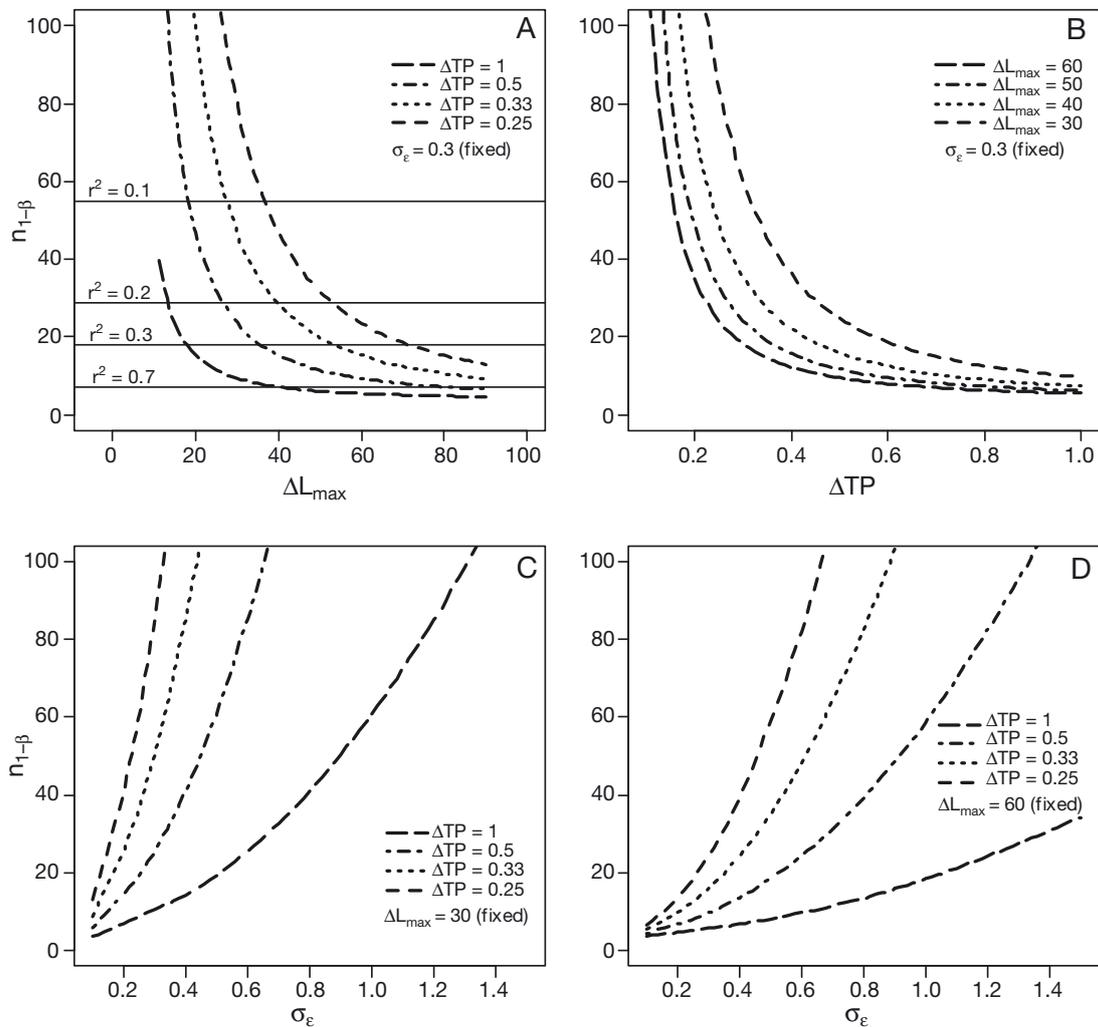


Fig. 2. (A) Sample size required to achieve statistical power of 0.8 ($n_{1-\beta}$) when sampling was conducted over different body size ranges (proportional length range: ΔL_{\max}). Lines represent the power curve assuming a fixed degree of dispersion ($\sigma_\varepsilon = 0.3$) and different threshold effects (change in trophic position: ΔTP). (B) Sample size required to achieve power of 0.8 given a range of threshold effects. Lines represent the power curve assuming $\sigma_\varepsilon = 0.3$ and different ranges of L_{\max} (ΔL_{\max}). (C, D) Sample size required to achieve power of 0.8 when sampling was conducted over different dispersions. Lines represent the power curve for different threshold effects when ΔL_{\max} was (C) 30% and (D) 60%

Sufficient statistical power is therefore usually achieved by greater sampling effort.

Power increased dramatically with an increase in ΔL_{\max} up to between 40 and 60%—the higher ranges being necessary to detect smaller threshold effects. Based on data reviewed in the present study, the median ΔL_{\max} used to date was 30% and is below the 40% ΔL_{\max} where power begins to asymptote, suggesting undersampling of ΔL_{\max} is common. It also highlights that where adults and post-metamorphic juveniles are found in different locations (Cocheret de la Morinière et al. 2003), it may be difficult to obtain a large enough size range. Instead, the robustness of hypothesis testing is related to either extensive sampling or the ability to ameliorate the confounding effects of different isotopic baselines among sites and the isotopic disequilibrium period. The latter is induced by lagged isotopic turnover that occurs when individuals move to a location with a different isotopic baseline.

The ability to detect ecologically meaningful trends will improve if a true reflection of dispersion throughout ΔL_{\max} can be obtained. Variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ has been proposed as indicators of trophic niche width (Bearhop et al. 2004, Layman et al. 2007). High variance in $\delta^{15}\text{N}$ among individuals could be expected for generalist feeders, while low variance may be observed in specialist or generalist feeders feeding on a uniform food supply (Bearhop et al. 2004). When dispersion is high and reflective of real feeding patterns (Layman et al. 2007), then it is even more important to increase sample size to assess how dispersion changes with size. Particular care is required at the extreme ends of L_{\max} where individuals may appear as outliers. Understanding the influence of these points on the regression coefficients is an important part of the data analysis, and there are many good text books that cover this topic (e.g. Zuur et al. 2007). Without an understanding of true dispersion as a result of feeding patterns and good data exploration practices, it may further impact the ability to detect size-related trends between body size and TP.

The ability to detect ecologically meaningful trends will improve if dispersion resulting from experimental, methodological and analytical error can be reduced. Analytical improvements are gained when analysis is restricted to a single machine reducing inter-machine biases (Mill et al. 2008) or by running duplicates. Isotopic baselines are variable in space (Jennings & Warr 2003, Barnes et al. 2009) and time (Jennings et al. 2008b). Modifications in experimental design performed to reduce dispersion may be achieved by either constraining sampling in space and time or by obtaining accurate baseline data integrated at appropriate scales by isotopic turnover sampling frequency. Similarly, tissues of fish on a constant diet differ in their variability (Sweeting et al.

2005). Utilisation of low variability tissues, e.g. muscle, is recommended. Statistical improvements may be gained by incorporation of other explanatory variables, e.g. gender or morph. Methodological improvements may include lipid extraction or acidification to reduce variation induced by variable lipid contents (Sweeting et al. 2006, Post et al. 2007) or carbonate (Bunn et al. 1995). Accounting for lipids or carbonate effects should be considered even if their influence is not systematic. However, care should be taken with these techniques, as additional processing may also increase variation (Bunn et al. 1995) and cause shifts in $\delta^{15}\text{N}$ (Murry et al. 2006).

Machine analytical precision and naturally occurring variation in fish fed on a single diet are similar for all 3 most commonly used stable isotopes in ecology (carbon; $\delta^{13}\text{C}$, nitrogen; $\delta^{15}\text{N}$, and sulphur; $\delta^{34}\text{S}$). Precision of analysis is commonly accepted as ± 1 SD and equates to 0.2‰ for both carbon and nitrogen isotopes. For sulphur isotope analysis, precision is lower at ~ 0.4 ‰. However, observations of variation in tissue $\delta^{34}\text{S}$ from fishes equilibrated with experimental diets (Hesslein et al. 1993, Barnes & Jennings 2007) are within mean ranges observed of carbon (± 0.42 ‰) and nitrogen (± 0.38 ‰) for trophic fractionation estimates reviewed by Sweeting et al (2007a,b). Experimental power also varied with effect size; in this case the threshold slope defined is important. Interpretation of an ecologically meaningful slope will vary with user and application. For example, a 0.25 TP increase has a greater relative impact on food chain length in shorter food chains (e.g.

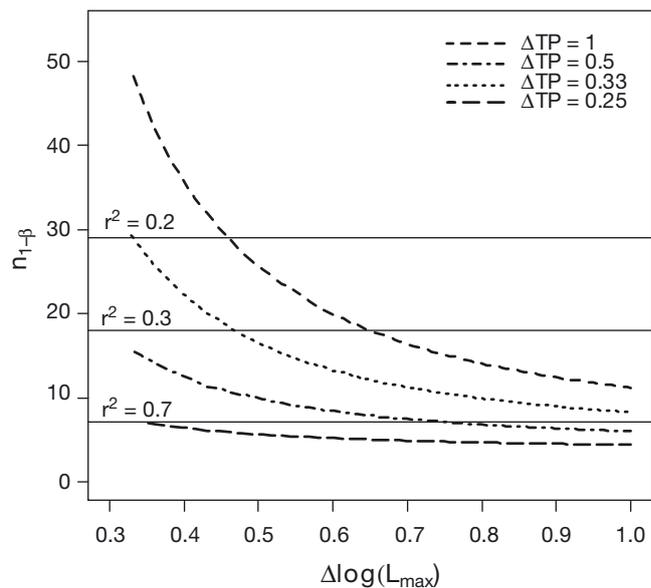


Fig. 3. Sample size required to achieve a statistical power of 0.8 ($n_{1-\beta}$) when sampling was conducted over different size ranges ($\Delta \log L_{\max}$). Fish lengths varied from 10% $< L_{\max} < 21$ % to 10% $< L_{\max} < 100$ %. Lines represent the power curve assuming $\sigma_e = 0.3$ and different threshold effects (ΔTP)

3 TP) of upwelling systems compared to open ocean systems with 5 TP (Ryther 1969). It will also vary among isotopes. Isotopic shifts induced by trophic step fractionation of carbon or sulphur are small, but primary production sources may differ dramatically. Therefore, size-based shifts for $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ will be small when driven by trophic fractionation alone, but may be very large where changes in source production occur.

Log-linear relationships between fish size and TP are a common pattern in nature (Jennings et al. 2002). In this case, the relationship between the required minimum sample size and the ΔL_{max} sampled is different. For example, on a \log_{10} scale the isotopic change between 20 and 50 % of L_{max} ($\Delta L_{\text{max}} = 30\%$) is the same as that between 40 and 100 % ($\Delta L_{\text{max}} = 60\%$) on a linear scale. The latter example shows the importance of an intensive sampling regime over the younger life stages to accurately define the relationship within log-linear trends (Fig. 3).

Another important point to note is that under log-linear relationships the minimum sample size required to achieve a statistical power of 0.8 is smaller than under linear relationships if sampling younger life stages and greater if sampling older life stages (Fig. 3). For example, assuming $\Delta\text{TP} = 0.25$ and $\sigma_{\epsilon} = 0.3$, the $n_{1-\beta}$ required to achieve power of 0.8 is 32 if $\Delta L_{\text{max}} = 50\%$ under linear conditions, while it is 22 for smaller fish (20 to 70 % L_{max} , $\Delta\log L_{\text{max}} = 0.544$) and 46 for larger fish (40 to 90 % L_{max} , $\Delta\log L_{\text{max}} = 0.352$) under log-linear conditions.

Statistical power curves in Fig. 2 were based on assessing the power of linear body size–isotope relationships, the most frequent trend type in the studies reviewed. However, should one desire to assess the statistical power under log-linear conditions, it is possible to estimate the respective minimum sample size $n_{1-\beta}$ under log-linear regressions from this figure using Eq. (3):

$$b_1\Delta L_{\text{max}} = b_2\Delta\log L_{\text{max}} \quad (3)$$

where b_1 represents the threshold effect of the linear relationship, b_2 the threshold effect of the log-linear relationship, $\Delta\log L_{\text{max}}$ the difference between the log values of the maximum and the minimum fish length sampled and ΔL_{max} the value that gives an equivalent power under the same sample size. This then assumes equal sampling effort with size on a log scale rather than a linear scale.

The above discussion provides some guidance on experimental design. Methodologies developed here were principally designed for the post hoc examination of existing tests where dispersion and sampling distribution within ΔL_{max} are unknown. If authors follow uniform sampling, the R code in Supplement 2 may be

used with observed levels of dispersion to assess the statistical power for detecting threshold effects. However, deviations from the assumption of uniform sampling make determinations increasingly inaccurate.

Even under lenient assumptions of below-average dispersion, and uniform sampling across the observed sampling range, less than 10 % of non-significant trends within this data set would have been classified by statistical methods that had the necessary power to detect isotopic shifts of $\leq 1\%$ in $\delta^{15}\text{N}$ throughout life. This suggests that no conclusion may be safely drawn from the majority of non-significant body size $\delta^{15}\text{N}$ trends. The present study provides several recommendations for improvement. First, improvements in power can be achieved by sampling wider size ranges. Where there is an inability to do so, additional sampling effort will be needed. However, be careful when trying to expand ΔL_{max} and make sure regression diagnostics are checked to see whether any higher length or weights, relative to the rest of the data set, are influencing the regression coefficients. Second, the explicit consideration and, where possible, reduction of variance is worthwhile for robust analysis. Finally, when describing non-significant size-based feeding of fishes the correlation coefficient should be defined and the power of the test should be assessed in order to detect ecologically meaningful shifts.

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