6. THE BACK-CALCULATION OF FISH GROWTH FROM OTOLITHS

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1   Introduction

> There is nothing more practical than a good theory.
> **Leonid Ilich Brezhnev**, *quoted in Nature, 1977, 270, pp 470–1*

The earliest example of using earbones or otoliths to provide estimates of fish ages dates back to at least 1899 (Reibisch, cited in Jones 1992). Back-calculation to reconstruct growth patterns from hard parts of fish (otoliths, bones and scales) followed soon after (Lea 1910). The approach involves using measurements made on these bony structures to infer, or back-calculate, body length at ages prior to capture. Back-calculation has been used to generate individual growth histories of fishes for almost a century (Francis 1990) and has proved to be an invaluable tool for fisheries scientists and fish ecologists.

Otoliths can show annual, and for younger fish, daily patterns of growth (Pannella 1971, 1974). The analysis of daily increments within otoliths has been very popular over the last 30 years due to the ability of the technique to provide growth data during the larval and juvenile phases of the life history, when growth rates are critical to year-class success and, ultimately, population size (Stevenson & Campana 1992). Typically, growth data can be generated from otoliths in 2 ways. When sample sizes are large and the fish collected by a study encompass a wide range of age and length, then ageing fish may suffice to estimate growth curves for a population. In many cases, however, the use of growth back-calculation techniques to estimate fish growth is not a choice, it is a necessity. Sample sizes are often small, due to rarity in multi-species assemblages (a particular issue in species-rich tropical habitats such as coral reefs) or because of the diversity of catches in some fisheries. The difficulty of collecting particular life history stages of interest to researchers, such as pelagic larvae or juveniles, may also result in small sample sizes. Back-calculation may be required where cohorts or populations must be sampled sequentially, as in studies of size selective mortality (Hovenkamp 1992, Meekan & Fortier 1996). Only back-calculation of histories held within otoliths will allow the reconstruction of growth trajectories in such situations, both increasing sample sizes and filling gaps in life history information at earlier ages.

Back-calculation of daily or annual growth from otoliths requires firstly that the rate of deposition of increments in otoliths does not vary. This usually can be verified experimentally (Geffen 1992). Secondly, it assumes that these increments can be read

with accuracy and precision (Campana 1992). Thirdly, all back-calculation models assume that there is a relationship between the growth of the otolith (increment width) and the somatic growth, usually length, of the fish. Evidence for this phenomenon usually is derived from strong correlations between the size of the otolith and body size of fish. There have been relatively few studies that have shown a complete lack of correlation between fish and otolith size and most of those that have done so have been confounded by problems in regression analysis and sample selection (Meekan 1997, Meekan et al. 1998, but see Bang & Gronkjaer 2005). Somatic and otolith growth can be uncoupled at least in the short term, however, (Mosegaard et al. 1988, Secor & Dean 1989, Wright et al. 1990, Fey 2006) and this is thought to result from two causes: (i) a "growth effect" whereby otoliths from slow-growing fish at a given size are larger than those of fast-growing fish of the same size (Templeman & Squires 1956); and (ii) an "age effect" where some constant or proportional amount of calcification occurs onto the otolith despite daily fluctuations in somatic growth rate (Secor & Dean 1992). Otolith growth is a conservative daily process (Mugiya 1987, 1990) and it is easiest to describe an age effect in the extreme case where somatic growth ceases but otolith growth continues. Age effects, however, can be important during negative, static, and positive somatic growth phases (Secor & Dean 1992). Because growth varies over time, particularly between ontogenetic stages, growth effects may also vary through time, introducing curvature into individual fish – otolith size trajectories (Campana 1990). Furthermore, the increase in otolith size in non-growing fish is consistent with disproportionately larger otoliths in slower-growing individuals and so the results of growth and age effects may be similar and both can therefore induce a bias into the otolith – fish length relationship upon which back-calculation procedures are based.

        Some authors have recently opted to analyse otolith radius at age in order to generate data sets of growth rate, rather than back-calculated fish length at age, to avoid the potential errors associated with back-calculation or in cases where age and growth effects might be a problem (Hare & Cowen 1995). The principal argument for this approach is that there is no reason to bother with back-calculation because otolith radius is a proxy of fish length. Furthermore, it is thought to avoid bias due to the use of complex back-calculation models (Campana 1990, Francis 1990) and the effect of selective mortality on fish-otolith size relationships (Ricker 1969, Gleason & Bengston 1996, Grimes & Isley 1996). In reality, these points are arguable. Figure 1 compares the growth trajectories and fish length (L) – otolith radius (R) relationships of three individuals. In this example, the three fish have the same growth characteristics, but fish 2 has a slightly different L – R relationship (i.e. morphology) than the others. When comparing individuals of very different ages (e.g. 1 vs 2 or 3), then otolith radius will indeed be a good proxy of length and the same conclusion will hold whether individuals are compared on the basis of otolith radius or body length: individual 1 is smaller than 2 and 3, primarily because it is much younger. When comparing fish of about the same age, however, fish with larger otoliths at age might not necessarily be larger in size (e.g. 2 vs 3). In our example, fish 3 always has a larger otolith radius at age than fish 2, despite both having exactly the same body length at age (growth trajectory). This demonstrates that otolith radius is a poor proxy of fish length when comparing size-at-age. The confounding effect of morphology may be relevant not only when comparing otolith radii among individuals but also when comparing among populations or samples with different L – R relationships.
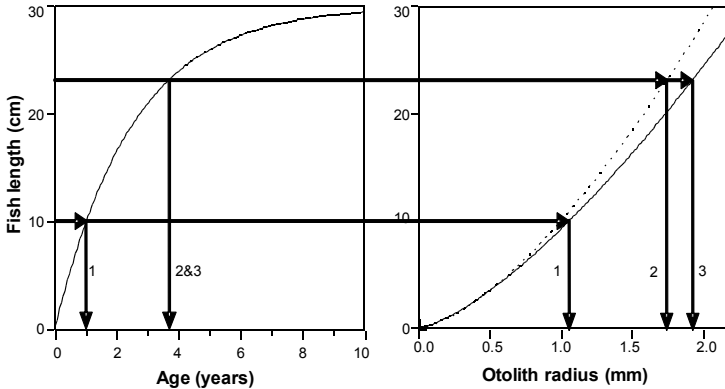
Figure 1. The growth trajectories and fish length (L) – otolith radius (R) relationships
of three fish from a simulated data set. The plot of age vs size (*left*) shows that
the three individuals have the same growth curve. The plot of otolith vs fish
size on the right, however, shows fish 2 has a slightly different L – R
relationship (i.e. morphology) than the others. The lines overlaid on the plots
show that otolith radius will be a good proxy of length when fish are of
different ages but fish with larger otoliths at age might not necessarily be
larger in size than other fish of the same age.

It is also important to recognise that the use of otolith radii to generate growth
data does not in fact avoid back-calculation. Rather, the approach inherently assumes
that the simplest and most primitive back-calculation model applies ($L_i = aR_i$, where "a"
is a constant and L_i and R_i are fish length and otolith radius at age i, respectively).
Because "a" is the same for all fish, the approach is equivalent to back-calculating fish
size by the "simple regression method" (see below), which is not recommended since it
ignores individual differences in L – R relationships (Francis 1990). Effectively, a
century of research and refinement in back-calculation techniques is discarded. Perhaps
the only situation where such an approach could be preferred is in the analysis of fossil
otoliths where there may be no extant populations of the study species and so
relationships between otolith and fish size cannot be constructed empirically.

So, if back-calculation is inevitable even when we try to avoid it, what then is the
best approach? The purpose of this chapter is not to describe all of the various back-
calculation models that have been developed over the last century, these have been
expertly reviewed by Francis (1990, 1995). Rather, we will update the lists provided by
Francis (1990, 1995) and examine the theoretical and experimental evidence for and
against the use of the various models published in the last decade. This is the first aim
of our chapter. We also propose to take the issue of back-calculation one step further
and ask the question: once you have a back-calculated data set of size at age, how do
you go about analysing it? Chambers and Miller's review in 1995 recognised the unique
nature of these data sets, which are longitudinal, auto-correlated and invariably
unbalanced. These features provide some challenges for statistical analysis that recent
developments in software have addressed, principally through the use of mixed-effects
models (Pinheiro & Bates 2000). A description of the use of these models for analysis
of back-calculated data is the second aim of our chapter.

2 Selection of a back-calculation model

2.1 BACK-CALCULATION METHODS

The back-calculation of fish length (L_i) from otolith radius (R_i) at age i requires the development of a back-calculation model, which is a two-step procedure. Firstly, the shape (linear or curvilinear) of the relationship between fish length (L) and otolith radius (R) must be determined. This can be described by two functions, "f" and "g" where R = f(L) and L = g(R) (Francis 1990). It is important to realise that f and g define families of lines (that may be straight or curved) and that any given line is defined by a unique set of function parameters. Because back-calculation for a particular fish will involve just one line from the family for a given function, the second step of model development will involve determining which particular line (i.e., the set of function parameters) should be used for a given individual. This can be done using three different methods. Paradoxically, the first method considers that all fish follow the same L − R line calculated by regression of fish length on otolith radius for the function g. Size-at-age estimated by this "simple regression" method (*sensu* Secor & Dean 1992) is given by the following back-calculation model (Francis 1990):

$$L_i = g(R_i) \tag{1}$$

This "simple regression" method is insensitive to changes in individual growth histories due to its averaging effect (Secor et al. 1989) and will often produce biased size-at-age estimates. It is hard to see any reason for its use (Francis 1990). The second and third methods both consider that each individual has a unique L − R line that passes through otolith and fish size at capture (R_{cpt}, L_{cpt}), but differ in how the parameters of individual lines are estimated. The second method back-calculates size by assuming proportionality between measurements at individual and population levels. Two hypotheses of proportionality exist. Whitney and Carlander (1956) stated these as: "if the otolith of one individual was 10% larger at capture than the mean size of otoliths from a group of fish of the same length, then its otolith had also been 10% larger than the average throughout life"; and "if one individual was 10% larger in length at capture than the average length for fish with the same otolith radius, then this individual had also been 10% larger than the average throughout life". These are known as the "Scale Proportional Hypothesis" (SPH) and "Body Proportional Hypothesis" (BPH) (*sensu* Francis 1990) respectively. Size-at-age estimated by "proportionality" methods are given by the following back-calculation models:

$$\text{SPH} \qquad f(L_i) = \frac{R_i}{R_{cpt}} f(L_{cpt}) \tag{2}$$

$$\text{BPH} \qquad L_i = \frac{L_{cpt}}{g(R_{cpt})} g(R_i) \tag{3}$$

"Proportionality" methods can be applied to any function f or g of any mathematical form, providing that the parameters of f are estimated by R-on-L regression and those of g by L-on-R regression.

The third back-calculation method constrains individual L – R lines to go through one or more known points such as otolith size and body length at capture (R_{cpt}, L_{cpt}) or the origin of the growth curve (R_0, L_0, usually at hatching), which can be estimated by regression or fixed at a known biological intercept (BI, Campana 1990). This "Constraint" method thus requires as many known points as there are function parameters, and so can be difficult to apply for complex functions.

2.2   EXISTING BACK-CALCULATION MODELS

The use of "constraint" or "proportionality" methods coupled with back-calculation functions f and g lead to the development of different back-calculation models (BCMs). In his review of the subject, Francis (1990) compiled 6 back-calculation functions, to which two others have subsequently been added by Tremblay and Giguère (1992) and by Morita and Matsuishi (2001). Because most back-calculation functions can be inverted (i.e., L = g(R) ↔ R = f(L)), a total of 8 f and 8 g functions exist that are appropriate for use in back-calculation. A total of 22 back-calculation models have been derived from these functions (Appendix 1).

Differences between "constraint" and "proportionality" models are often subtle (some may say artificial, because models are derived from the same f and g functions), or even non-existent when functions f or g have a single parameter. For example, the Dahl-Lea back-calculation model (BCM 1 "DALE", Appendix 1) can be obtained by constraining the function L = bR to pass through the point at capture (R_{cpt}, L_{cpt}) or by applying a BPH to the function L = bR or a SPH to the function R = L/b. When an intercept is added to the linear function (i.e., L = a + bR), then the BCMs will differ in the intercept value calculated for each individual. For example, the Fraser-Lee model (BCM 2 "FRALE", Appendix 1) is obtained when individual L – R lines are all constrained to pass through the intercept (R=0, L=a) with "a" estimated by L-on-R regression. Similarly, the linear biological intercept model (BCM 3 "BI", Appendix 1) results in individual L – R lines passing through biologically-determined intercepts

(R=0, $L = L_{cpt} - R_{cpt} \dfrac{(L_{cpt} - L_{0p})}{(R_{cpt} - R_{0p})}$),   although all lines cross at ($R = R_{0p}$,

$L = L_{0p}$), whereas the linear BPH (BCM 4 "LBPH", Appendix 1) results in individual

L – R lines passing through intercepts (R=0, $L = \dfrac{aL_{cpt}}{(a + bR_{cpt})}$) where "a" and "b" are

estimated by L-on-R regression. These differences are subtle but important as they can render back-calculation models more or less sensitive to bias induced by growth or age effects.

Campana (1990) showed that growth effects will always result in linear regressions between L and R that overestimate slopes and underestimate intercepts, so that regression-based Fraser-Lee (BCM 2) and linear BPH or SPH (BCM 4 & 6) will inevitably produce biased size-at-age estimates. The linear biological intercept model (BCM 3) developed by Campana (1990) constrained a linear function of back-

calculation to pass through biologically-determined intercepts of otolith and fish size to reduce the influence of variable growth rates in the population (i.e., the growth effect). The model was still sensitive to non-linear effects, however, and in particular those induced by growth rate variations through time (i.e., a time-varying growth effect). This can introduce curvature into individual fish – otolith size trajectories (Campana 1990). The Time-Varying Growth model (BCM 5 "TVG", Sirois et al. 1998, Appendix 1) was developed to address non-linear fish – otolith relationships formed because of time-varying growth rates by incorporating a growth effect into the structure of the linear biological intercept model. This was done by weighting the contribution of individual increments in the length back-calculation. The TVG model still assumes the underlying relationship between fish and otolith size to be linear, however, and so may not be appropriate when the relationship is non-linear for reasons other than growth effects.

Vigliola et al. (2000) took a different approach to non-linearity. They constrained an allometric L – R function to go through a biological intercept so their modified Fry model (BCM 14 "MF", Appendix 1) assumed non-linearity and was robust to growth effects. Only the Age Effect (BCM 7 "AE", Appendix 1) model of Morita and Matsuishi (2001), however, was designed to remove bias due to age effects. That model was developed on the assumption that otolith radius was a linear combination of both fish length and age. A SPH was then applied to the 3 dimensional back-calculation function that linked otolith radius, fish length and age to obtain the AE model.

2.3   SELECTION OF A ROUTINE BACK-CALCULATION MODEL

The plethora of models now available for back-calculation creates a dilemma for any researcher intending to back-calculate fish size from otoliths: which of these is the most appropriate to use? The first step for model selection consists of determining which function(s) should be used to describe the relationship between fish length and otolith radius. Back-calculation functions f and g technically could be of any mathematical form (Francis 1990, 1995) but back-calculation usually assumes that there is a proportional relationship between the growth of the otolith and the somatic growth of the fish (Campana 1990, Hare & Cowen 1995, Sirois et al. 1998, Vigliola et al. 2000, Morita & Matsuishi 2001). Thus, we define a function f or g as appropriate for use in back-calculation if it complies with the assumption of proportionality between otolith and somatic growth. Of the 16 functions listed in Appendix 1, this criterion allowed the immediate elimination of functions (f3), (g3), (f6), (g6), (f7), (g7), (f8) and (g8). The remaining functions were all derived from the assumption of proportionality between the relative growth rates of the fish and the otolith and could be written as:

$$\frac{d(L - a)}{(L - a)dt} = c\frac{dR}{Rdt} \qquad (4)$$

where "a" is the body length of an individual at otolith formation (Fraser 1916, Lee 1920) and "c" is the proportionality coefficient.

Solution of this differential equation resulted in $L = a + bR^c$ (g5), a generalisation of the well-known function for an allometric relationship between two body parts of an organism (Ricker 1975, 1979). When isometry existed (i.e., $c = 1$),

function (g5) became identical to L = bR (g1) if a = 0 and to L = a + bR (g2) if a ≠ 0. Furthermore, function $L = bR^c$ (g4) was a specific case of (g5) if a = 0. It seems likely that "a" will be greater than 0 in most cases (Francis 1990) since the otoliths of many species form in the period just prior to hatching (Geffen 1992). Thus, only functions (g2) and (g5) (and their inverse f2 and f5) remained appropriate to model the relationship between the length of a fish and the radius of its otolith.

After some back-calculation functions have been selected on the criterion of a proportionality relationship between otolith and somatic growth (Equation 4), it must be determined which of the "constraint" or BPH or SPH "proportionality" methods (Equations 2 & 3) are most appropriate for model development. Models derived by BPH or SPH are sensitive to growth effects because they cannot be constrained to go through a biological intercept. Practically, this means that BPH and SPH proportionality methods can generate unrealistic estimates of fish size, in particular when back-calculating fish size outside the age range of the sample from which the parameters of the back-calculation formulae were estimated by regression (see below, Vigliola et al. 2000). Consequently, a criterion of accuracy in back-calculated size-at-age (i.e., the BCM output) eliminated all models based on proportionality hypotheses SPH and BPH as well as other models that did not contain a biological intercept.

Application of our first (proportionality of otolith – fish growth) and second (to generate realistic size-at-age data) selection criteria to the list of 22 existing back-calculation models thus eliminated all but the linear Biological Intercept model ("BI", BCM 3), the Time-Varying Growth model ("TVG", BCM 5) and the Modified Fry model ("MF", BCM 14). The applicability of these models can be determined by examining the evidence from the few recent studies that have attempted to assess their accuracy and precision as descriptors of fish growth in the field and laboratory.

2.4   RECENT FIELD AND EXPERIMENTAL EVIDENCE OF THE ACCURACY OF BACK-CALCULATION MODELS

Vigliola et al. (2000) compared the outputs of BI, TVG, ABPH (the L – R relationship was allometric in the 3 study species) and MF models to field estimates of size of newly settled individuals of 3 species of sparid (*Diplodus sargus, D. vulgaris* and *D. puntazzo*) from the Mediterranean Sea. Field estimates of size were obtained by underwater visual survey. The error associated with estimating size by this method was determined by comparing visual estimates with the actual sizes of fish after capture and was always <±3.5 mm (Macpherson 1998). Growth curves derived from visual survey were extrapolated using published values for length-at-age of planktonic larvae to include the pre-settlement stage of the life history of the three species. Overall, Vigliola et al. (2000) found that the MF model produced growth curves that were the most similar to those derived from direct observations of juvenile *Diplodus* (Figure 2). As predicted, the ABPH model gave unrealistic estimates of size-at-age of very young fish, as it was not constrained to go through a biological intercept and was sensitive to growth effects. Both the BI and TVG curves consistently overestimated size-at-age in comparison with curves estimated directly by visual survey. These models produced very similar estimates despite the presence of an allometric L – R relationship. This result was consistent with the findings of Sirois et al. (1998) study of rainbow smelt (*Osmerus*

mordax) larvae, where the TVG model was originally proposed. Vigliola et al. (2000) concluded that further comparisons were required in situations where the growth rates were highly variable to distinguish between the MF and TVG models.

Wilson et al. (2008) recently completed such comparisons. They collected 2 species of newly settled tropical gobies (*Elacatinus evelynae* and *E. prochilos*) from reefs in the Florida Keys and maintained them in aquaria for 2 months. Fish were marked externally by the subcutaneous injection of coloured paint (Wilson & Osenberg 2002) to identify individuals and measured (standard length, SL) every 2 weeks. Otoliths were tagged at the start of the study and then by regular immersion (every 4 weeks) in Alizarin Red to validate daily increment formation and monitor otolith growth. Growth rates of fishes were manipulated by altering ration so that individuals received high, medium, low or variable (2 weeks high, 2 weeks low) levels of food. Growth curves generated by back-calculation were compared with directly measured longitudinal size-at-tag data by linear mixed effect modelling (LME, Pinheiro & Bates 2000) that included food regime as a fixed factor in the models. Smaller negative log-likelihood in the LME models indicated greater precision in predicted size, whereas slopes of 1 in LME models indicated unbiased predictions (greater accuracy).

Both species examined by Wilson et al. (2008) had linear $L - R$ relationships. Furthermore, analyses showed that age effects, growth effects and time-varying growth effects were present in the dataset. Surprisingly, however, growth curves generated by the MF model gave a better fit to directly measured growth than the LBPH, BI, TVG and AE models. This result was consistent for both species and there was no significant effect of food regime (Table 1). With the exception of the AE model, all back-calculation models yielded lower negative log-likelihoods (i.e., better fits to directly measured data) than the use of otolith radius in the analysis (Table 1), confirming that back-calculation generates a better proxy of fish size than does otolith radius. Most models gave reasonably precise predictions of fish size, although all overestimated size at age, though the AE model was an exception which yielded negative log-likelihoods twice as large as those obtained with other methods (i.e., a poor fit to directly measured data). The biases in these predictions were very small, again with the exception of the AE model. The smallest overestimation (i.e., slope closest to 1) was made by the MF model, with a slope of 0.95 for both species, giving a relatively trivial 5% average overestimation in size (Figure 3). Predictions from the MF model averaged within 0.9 mm of measured size and yielded a maximum error of 4.5 mm for fish ranging in size from 8.5 to 26.6 mm standard length between the start and end of the experiment. Other models gave similar, although slightly larger errors in prediction of up to 5.7 mm greater than measured values. Very large errors in predicted size were often produced by the AE model (36 mm average error and 657 mm maximum error), presumably because of collinearity problems among the variables of fish length, otolith radius and fish age in the multiple linear regression model.
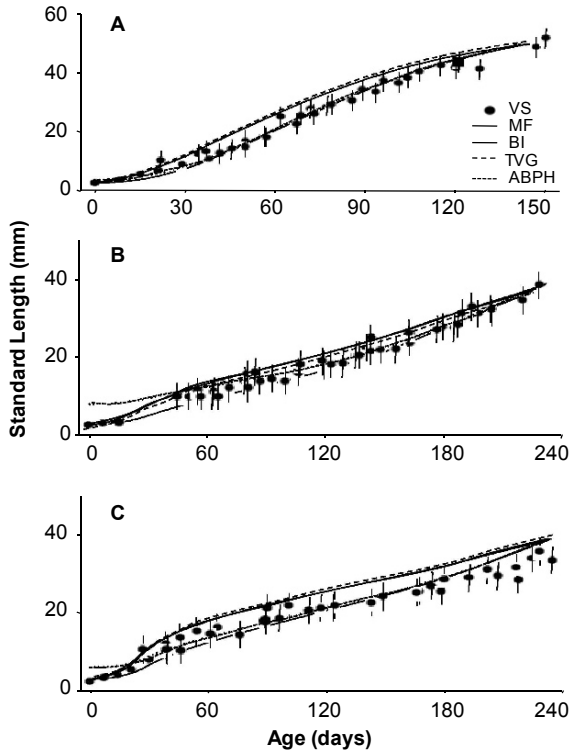
Figure 2. Growth curves back-calculated from otoliths and estimated directly in the field for *Diplodus sargus* (**A**), *D. vulgaris* (**B**) and *D. puntazzo* (**C**). Field size-at-age data are shown as points with error bars of fixed size (±3.5 mm), the maximum error when fish sizes were estimated underwater by divers (Macpherson 1998). Sizes of 30–40 specimens of each species were estimated visually and the specimens then captured and measured using a ruler. All differences between mean estimated and measured sizes were <3.5 mm. Conversion of survey date to age was as follows. First, the average size of all individuals recorded during the last survey was calculated. An age was then assigned to this size by averaging all increment counts of otoliths from fish of this size. Dates of sampling for all surveys were known, so ages could be assigned to average sizes from those surveys. Size-at-age was extrapolated to the planktonic larval stage using growth curves provided by other studies. These points are shown without error bars. Four models of back-calculation were used to generate growth curves from otoliths: the modified Fry (MF), biological intercept (BI), time-varying growth (TVG) and allometric body proportional hypothesis (ABPH) models. After Vigliola et al. (2000).

Table 1. Linear mixed effect (LME) model between longitudinal observations of fish length at tag and otolith radius at tag (R) or between longitudinal observations of fish length at tag and predicted fish length at tag using different back-calculation models for two species of gobies *Elacatinus evelynae* and *E. prochilos* experiencing different food treatments. Food regime is entered as a fixed factor in the LME models. LBPH: linear body proportional hypothesis, BI: biological intercept, MF: modified Fry, AE: age effect, TVG: time varying growth, p: probability, SE: standard error. Lowest Akaike information criterion (AIC), Bayesian information criterion (BIC) or negative log likelihood (Neg-Log-Lik) indicate models generating the most precise size estimate (in bold). Slope of models is tested against 1 (no bias in size predictions) and slopes smaller than 1 indicate size overestimation (observed size at tag smaller than size-at-tag predicted by back-calculation). Bold font indicates model with smallest bias. NR: non-relevant test. From Wilson et al. (2008).

Species	Statistics	R	LBPH	BI	MF	AE	TVG
E. prochylos	AIC	463	405	426	**390**	752	433
	BIC	479	422	442	**406**	768	450
	Neg-Log-Lik	225	197	207	**189**	370	211
	p Food effect	0.55	0.83	0.88	0.90	0.59	0.85
	Slope	0.050	0.948	0.937	**0.949**	0.610	0.915
	SE slope	0.001	0.007	0.008	0.007	0.022	0.008
	p slope=1	NR	<0.001	<0.001	<0.001	<0.001	<0.001
E. evelynae	AIC	251	208	207	**200**	497	210
	BIC	264	221	220	**212**	509	223
	Neg-Log-Lik	120	98	98	**94**	242	99
	p Food effect	0.39	0.60	0.60	0.50	0.74	0.62
	Slope	0.051	0.944	0.942	**0.948**	-0.019	0.917
	SE slope	0.003	0.011	0.011	0.011	0.008	0.011
	p slope=1	NR	<0.001	<0.001	<0.001	<0.001	<0.001

Morita and Matsuishi (2001) compared the outputs of LSPH, LBPH, MONA-SPH, MONA-BPH, BI, Fraser-Lee, MF and AE models to directly measured size and growth rates of individually tagged white-spotted char (*Salvelinus leucomaenis*). The TVG model was not included in the comparison because the growth effect factor was negligible and not statistically significant. Only the AE model generated unbiased estimates of fish length and growth, but it was also the least precise model, giving lower r^2 values between predicted and observed lengths than any of the other models. It seems possible that the increased dispersion of size estimates produced by the AE model occurred in a relatively random manner, which may have disguised any inherent bias. For example, the AE model predicted a size of ~90–140 mm for a 110 mm fish, while in comparison the MF model narrowed the predicted size to 90–110 mm (see Figure 2 in Morita & Matsuishi 2001). The results of Wilson et al. (2008) confirm that very large errors in predicted size are often produced by the AE model. This result was expected since the AE model is vulnerable to growth effects (as is the case for all proportionality models) and very sensitive to the accuracy and precision of the regression calculated between fish length, otolith radius and fish age. Further illustration of the latter point is

provided by Finstad's (2003) study of Arctic charr (*Salvelinus alpinus*). This author included a length by age interaction term in the fish length, otolith radius and age multiple regression. Although this was not a conceptual change to the original AE model, predictions of fish length with and without the interaction term could differ by up to 40% on average (and occasionally much more given a S.D. of ~ 20% around this mean; see Figure 1 of Finstad 2003).

Other studies that have attempted to assess the accuracy and precision of back-calculation models over the last decade (summarised in Table 2) found that biological intercept methods generally performed better or similar to other models (Escot & Granado-Lorencio 1999, Klumb et al. 2001). These studies also highlighted the importance of the shape of the relationship between fish length and otolith radius (particularly where there was curvilinearity) to the relative accuracy and precision of the different models (Smedstad & Holm 1996, Schirripa 2002).
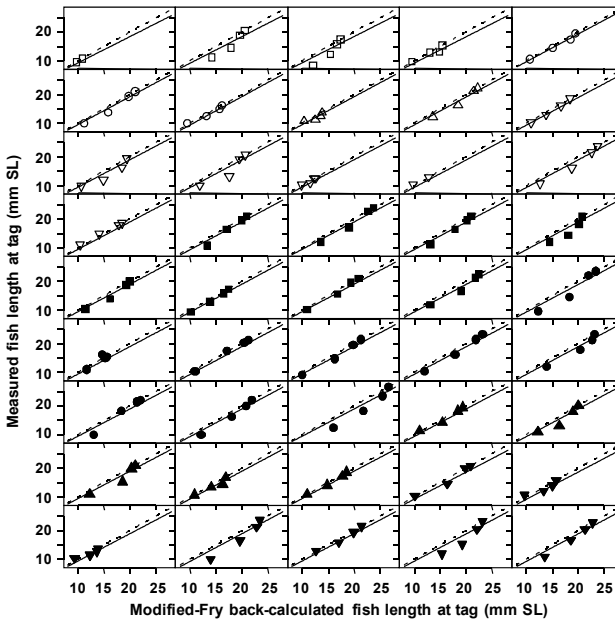

Figure 3. Relationships between observed (multiple internal and external tagging) and predicted (Modified Fry back-calculation model) length-at-age fitted by linear mixed-effects model for two species of tropical gobies. Each plot represents one individual with *Elacatinus evelynae* in open symbols (○) and *E. prochilos* in closed symbols (●). Food regime was entered as a fixed factor in the LME models and treatments are represented as: square = medium food, circle = high food, triangle up = low food, triangle down = varying food. The *solid lines* represent the linear relationships at individual level (i.e., fixed and random effects combined) while the *dashed lines* represent the 1:1 line. *Solid lines* "below" *dashed lines* indicate that observed lengths were smaller than predicted lengths, that is, the back-calculation model overestimated fish length. After Wilson et al. (2008).

Table 2. Summary of studies published in the last decade that sought to identify the most appropriate model for back-calculation. See Appendix 1 for model acronyms.

Study	Models compared	Method	Comparison	Conclusion
Smedstad and Holm (1996)	Fraser-Lee, regression of L on R (BCM 2), Fraser-Lee, symmetrical regression line (modification of BCM 2), MONA-SPH (with log(R) on log(L) regression), MONA-BPH (with log(L) on log(R) regression), LSPH, LBPH	Predictions versus observations from individually tagged fish reared in laboratory and measured repeatedly	Validation at individual level	MONA-BPH (Monarstyrsky-BPH) gave best results
Sirois et al. (1998)	BI, TVG	Predictions versus observations of reared larvae of known age	Validation at population level	Similar results from TVG & BI but TVG slightly more precise
Vigliola et al. (2000)	ABPH, BI, MF, TVG	Predictions vs observations from underwater visual surveys with extrapolated lengths of planktonic larvae.	Validation at population level	MF better, TVG & BI similar, ABPH poor at young ages
Escot and Granado-Lorencio (1999)	Monastyrsky (log(L) on log(R) regression, with intercept), Fraser-Lee (after log₁₀ transformation; thus a "non-linear" Fraser-Lee), BI (after log₁₀ transformation; thus a "non-linear" BI), simple regression of linear function (after log₁₀ transformation; thus a nonlinear model)	Comparisons of back-calculated size at last increment with size at capture	Not a validation	(non-linear) BI gave best results
Morita and Matsuishi (2001)	LSPH, LBPH, MONA-SPH, MONA-BPH, BI, Fraser-Lee, MF and AE	Predictions versus observations from tag-recaptured fish	Validation at individual level	Only AE gave unbiased estimates but AE was less precise than any other model.

Study	Models compared	Method	Comparison	Conclusion
Klumb et al. (2001)	Dahl-Lea, BI, Weisberg[1]	Predictions vs observations from multiple internal tag on reared fish	Validation at population level	Dahl-Lea and BI produced best size estimates
Finstad (2003)	AE, AE with a length by age interaction term	Comparison of models without observation of size	Not a validation	Major differences in predicted size, dependent on interaction term in AE model.
Schirripa (2002)	10 back-calculation models were compared: Dahl-Lea, 5 linear and nonlinear simple regression models, 2 Fraser-Lee (least-square and functional regression), Monarstyrsky (after log transformation), and a derivation of the Weibull function (not described in this chapter)	Data simulated by individual-based bioenergetics model using 4 shapes of fish length – otolith radius relations	Not a validation but a simulation at individual level	Accuracy of the estimated length-at-age directly related to how well the model fitted the otolith radius - fish length relation, implying the L – R function is at least as important as back-calculation model selection.
Wilson et al. (2008)	LBPH, BI, MF, TVG, AE	Predictions versus observations from multiple measurements on reared fish internally and externally tagged	Validation with longitudinal data at individual level	MF best, AE unrealistic, TVG and BI similar; output of all models except AE better length proxies than otolith radius.

1 The Weisberg model (Weisberg & Frie 1987, Weisberg 1993) partitions fish growth into two main effects, growth due to age of the fish and growth due to environmental effects in a given year, in a general additive ANOVA model. This model is rather complex and is not widely used.

2.5   WHICH MODEL?

The studies summarised above confirm that the lack of constraint to biological intercepts by BPH and SPH models results in unrealistic estimates of size, particularly at young ages. Similarly, application of the AE model (an SPH model) produced estimates of size-at-age that varied markedly from reality, although the addition of age in back-calculation models to correct for age effects is a promising avenue for the future improvement of predictions of fish length from otoliths. Thus, the remaining models that can be considered for use based on the evidence above are the MF, TVG and BI. Both Vigliola et al. (2000) and Wilson et al. (2008) found that the MF model tended to produce slightly more precise estimates of size-at-age than the TVG and BI models, and that the outputs of the latter two were essentially equivalent, even where growth rates varied. Given this result, most researchers would be inclined to use the BI rather than the TVG model, since the latter is more complex to compute.

Selection between the modified Fry (MF) and the linear biological intercept (BI) models is rather more difficult, for two reasons. Firstly, the BI model has been used widely for back-calculation since its development by Campana in the early 1990s and most researchers will be comfortable with the calculations involved. The MF model, however, is more recent and there are few published examples of its use. Secondly, calculation of the MF model is more complex than the BI model, and the ideal model should be as simple as possible. There are good reasons, however, to use the MF instead of the BI model in many situations. Vigliola et al. (2000) showed that where there is an allometric $L - R$ relationship, the MF model will produce more precise results than the BI model. The outputs of the MF and BI models will be identical where this relationship is linear, since the BI is a special case of the MF model.

The appropriateness of the 2 models will thus depend on the degree to which the $L - R$ relationship for a species is allometric. Vigliola et al. (2000) concluded that in case of isometry (a linear $L - R$), the BI model should be used for simplicity, while in case of allometry (a curvilinear $L - R$) they suggested choosing the MF model due to precision. The results of Wilson et al. (2008) show that this recommendation should be revised. They were able to record the shape of the $L - R$ relationship at an individual level for their study species by repeatedly tagging fish internally (immersion in Alizarin solution) and externally (subcutaneous injection). No allometry was detected using a population level approach (regression analysis of single measurements of L_{capt} and R_{capt} from each fish), and the relationship between these variables appeared isometric for both goby species (Figure 4A). The longitudinal data sets available for each fish, however, showed that on an individual basis there was an allometric $L - R$ relationship (Figure 4B). This result demonstrates that allometric $L - R$ relationships may be undetectable in population analyses (in this case due to low sample sizes), and explains why the MF model performed better despite an apparently linear $L - R$ relationship at population level. It is unlikely that individual longitudinal $L - R$ data will be available in most situations, however, since fish are usually killed when collected. Hence, the most conservative approach will be to use the MF model for routine back-calculations rather than the BI, despite its more complex form.
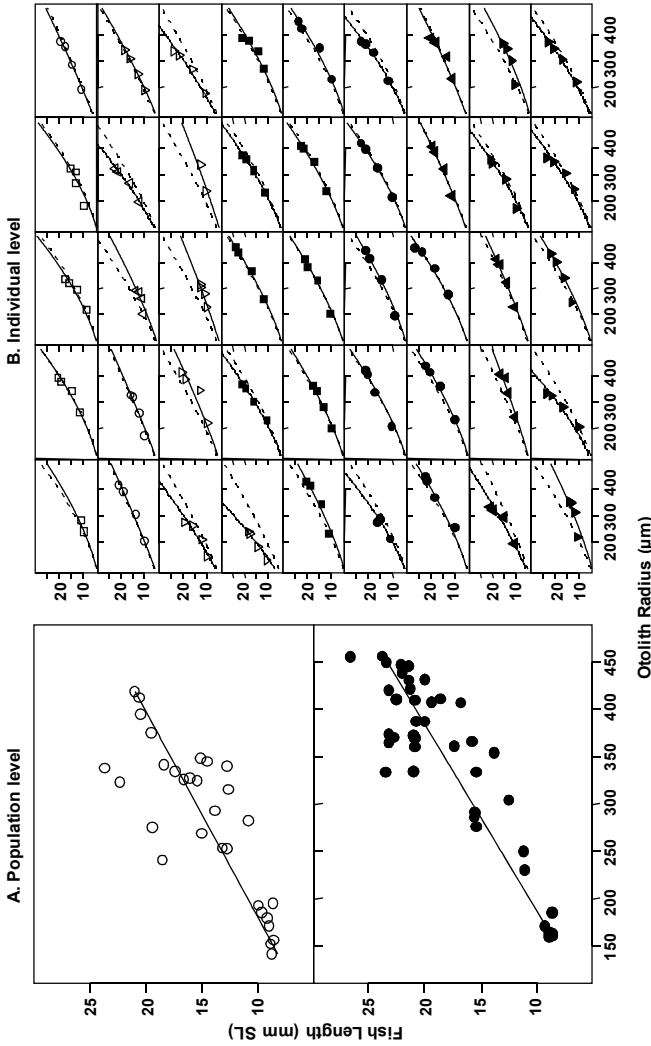
Figure 4. Population (*left*) and individual (*right*) relationships between fish length and otolith radius. *Left panel* (population level): Linear fish length – otolith radius relationship at capture for two species of tropical gobies, *Elacatinus evelynae* (○) and *E. prochilos* (●). *Right panel* (individual level): Allometric fish length – otolith radius relationships fitted by non-linear mixed-effects (NLME) model for the two species of gobies (Each plot represents one individual). Food regime was a fixed factor in the NLME models. Treatments are represented as: ■ □ = medium food, ● ○ = high food, ▲ △ = low food, ▼ ▽ = varying food. *Solid lines* represent allometric relationships at individual level (fixed & random effects combined); *dashed lines* represent allometric relationships at population level (fixed effect). After Wilson et al. (2008).

One of the most desirable features of the BI model is that it does not rely on parameter estimation from a sample of the population. Indeed, back-calculations can be made from an individual fish in the absence of any other fish from the population (Campana 1990). Unfortunately, this is not the case for the MF model where estimates of fish size "a" at otolith formation are required. This parameter will always be very close to fish size at hatching (the biological intercept L_{0p}), however, since otoliths usually form just prior to hatching. For example, average values of body length at otolith formation were a=2.39, 2.42 and 2.45 mm for the three species studied by Vigliola et al. (2000) with a common average length at hatching of $L_{0p} = 2.5$ mm, which gives ratios a/L_{0p} of 0.96, 0.97 and 0.98, respectively. Vigliola et al. (unpublished data) calculated this ratio for 53 species, mostly coral reef fishes from Florida Keys. They found that 22 (40%) of the species had a significant allometric L – R relationship at the population level and that the average ratio was 0.75, with 95% confidence interval of 0.69–0.80 (range 0.22–0.99). Campana (1990) indicated that back-calculation accuracy of the BI model was relatively insensitive to normal variation around the intercept value, largely because of the small values involved in L_{0p}. This is likely also the case for "a", which is smaller than L_{0p}, so it would be possible to replace "a" in the original MF model by its average value of $0.75L_{0p}$, allowing MF back-calculations from an individual fish in the absence of any other fish from the population. This "experimental MF" model should only be used when there is no data to estimate "a" and can be written as follows:

$$
L_i \;=\; 0.75L_{0p} \;+\; \exp\!\left(\frac{ \ln(L_{0p} - 0.75L_{0p}) \;+\; [\ln(L_{cpt} - 0.75L_{0p}) - \ln(L_{0p} - 0.75L_{0p})][\ln(R_i) - \ln(R_{0p})] }{ [\ln(R_{cpt}) - \ln(R_{0p})] } \right) \qquad (5)
$$

Whenever there are linear or allometric L – R relationships, as is the case for most fishes, we recommend using the original MF model or its experimental form (5). This will likely increase precision and reduce bias in back-calculated size-at-age. Furthermore, the MF model is derived from the assumption of proportionality between otolith and somatic growth, a basic assumption that considers back-calculation within the more general context of allometry (Hare & Cowen 1995) for which a large body of literature exists (for reviews see Cock 1966, Gould 1966). When the L – R relationship is not a straight line or an allometric curve (see the study of Tremblay & Giguère 1992 for an example), however, then there is little choice other than using SPH or BPH. In these cases, we recommend including a biological intercept in the f or g function prior to the regression, for example by replacing the intercept a_0 by L_{0p} and R by ($R–R_{0p}$) in the polynomial g7 function of BCM 19 (Appendix 1). These complex SPH or BPH models should be used with extreme caution, however, and validated using longitudinal data at individual level. A detailed example of the recommended back-calculation procedure is provided in Box 1.

Box 1: Recommended back-calculation procedure

Most species display either a linear or an allometric relationship between otolith radius, R, and fish length, L. Where there is insufficient data to examine the shape of the L – R relationship, theoretical considerations, field, and experimental data all indicate that best size estimates are likely from the Modified Fry back-calculation model (MF) (Vigliola et al. 2000). Where L – R relationships are best described by complex functions (e.g., polynomial), we recommend including a biological intercept into the function prior to fitting to data at capture and applying a proportionality hypothesis (SPH or BPH). The MF back-calculation procedure is as follows.

1. Obtain an estimate of fish size at first increment formation (usually hatching) for the study species. This parameter is the biological intercept L_{0p} of the model.

2. Using all samples, calculate the mean radius of first increment in the otolith. This parameter is the otolith radius R_{0p} at the biological intercept.

3. If there is not enough data to examine the shape of the L – R relationship (i.e., a single fish or many fish of similar age and size), then for each individual, back-calculate fish size at age i (L_i) from the longitudinal records of otolith radius at age i (R_i), records of fish length and otolith radius at capture (L_{cpt}, R_{cpt}), knowledge of biological intercept (R_{0p}, L_{0p}, steps 1 & 2) using the experimental MF back-calculation model (otherwise go to step 4):

$$L_i = 0.75L_{0p} + \exp\left(\frac{\ln(L_{0p} - 0.75L_{0p}) + [\ln(L_{cpt} - 0.75L_{0p}) - \ln(L_{0p} - 0.75L_{0p})][\ln(R_i) - \ln(R_{0p})]}{[\ln(R_{cpt}) - \ln(R_{0p})]} \right)$$

4. Using all samples, estimate b, c, d by fitting the following by NL regression:

$$L_{cpt} = L_{0p} - bR_{0p}^c + bR_{cpt}^c \qquad \text{and} \qquad L_{cpt} = L_{0p} - dR_{0p} + dR_{cpt}$$

   where L_{cpt} is fish length at capture and R_{cpt} is otolith radius at capture.

5. Test c against 1 using estimates of c and standard error SE_c given by non-linear regression (step 4) and calculate $|c - 1|/SE_c$ which follows a t-distribution with n-2 df. If c is significantly different from 1 (allometry) then using estimates of b and c (step 4), calculate $a = L_{0p} - bR_{0p}^c$. If c is not significantly different from 1 (isometry) then using estimate of d (step 4), calculate $a = L_{0p} - dR_{op}$

   In the original MF model (BCM 14 of Table 1), "a" was calculated from "a_1" and "a_2" obtained from L-on-R and R-on-L regression, respectively. However, Vigliola et al. (2000) showed that differences between a_1 and a_2 were so small (0.08%) that they may be ignored.

6. For each individual, back-calculate fish size at age i (L_i) from the longitudinal records of otolith radius at age i (R_i), records of fish length and otolith radius at capture (L_{cpt}, R_{cpt}), knowledge of biological intercept (R_{0p}, L_{0p}, steps 1 & 2) and estimate of a (steps 4 & 5) using the MF model:

$$L_i = a + \exp\left(\ln(L_{0p} - a) + \frac{[\ln(L_{cpt} - a) - \ln(L_{0p} - a)][\ln(R_i) - \ln(R_{0p})]}{[\ln(R_{cpt}) - \ln(R_{0p})]} \right)$$

3   Analysis of back-calculated data

Datasets generated from growth back-calculations are longitudinal and autocorrelated, characteristics that make them unsuitable for many statistical analyses (Chambers & Miller 1995). In particular, data that originate from multiple observations per otolith cannot be assumed to be independent. Thus, the longitudinal nature of back-calculated size-at-age data, with repeated measures on each individual, violates the basic assumption of independence that underlies traditional analyses such as univariate regression or ANOVA (Chambers and Miller 1995). Moreover, because statistical hypotheses are accepted or rejected based on the magnitude of the test statistic and the degrees of freedom (df), calculating the df from the total number of non-independent size-at-age data is inappropriate. Chambers and Miller (1995) gave a numerical example for this latter problem with 20 fish collected from each of two populations and 10 increment radii measured on each fish. A traditional regression approach would treat these 200 measurements per population as 200 independent size-at-age observations. Consequently, the hypothesis of a population effect on growth rate would be tested from an F-statistic based on 1,397 df whereas only 40 fish were included in the comparison. This would significantly increase the likelihood of committing a Type 1 error of falsely rejecting the null hypothesis of no difference in size-at-age among populations.

The lack of independence of longitudinal data implies that analysis should occur at the level of individuals, rather than the population. This poses two problems. First, there is often not enough information to fit a growth model by regression to each individual, in addition to the longitudinal and autocorrelated nature of within-individual size-at-age data. Second, it may be unclear how to obtain growth estimates of the population (typically the variable of central interest) from individual analyses, particularly when the growth model is non-linear. We illustrate the issues in Figure 5 where we generated size-at-age data for 10 fish sampled from a simulated population that followed a von Bertalanffy growth model (Box 2, Figure 5A). Individual regressions of a von Bertalanffy growth model could not be calculated for 6 fish as they were too young, with only 2–3 points available for analysis (Figure 5B). Additionally, outcomes of the individual regressions were suspect for at least two other fish. Fish 7 had a growth trajectory unexpectedly higher while fish 4 had a trajectory far lower than the parent population (Figure 5B, L_∞ original population = 30 cm; L_∞ fish 7 = 56 cm; L_∞ fish 4 = 20 cm). A common but flawed approach to obtain an estimate of the growth of the population would be to average growth parameters from individual analyses. Figure 5 shows that this will be unreliable, as the average growth of the 4 fish for which individual regressions could be calculated departed strongly from the trajectory of the original population (Figure 5C). Another common approach would consist of pooling back-calculated size-at-age data of all individuals to collectively derive a mean growth curve by regression. Again, this would be inappropriate as indicated by strong departure of the pooled regression curve from the true population growth curve (Figure 5C). A further risk in the pooling of back-calculated data is that the growth patterns of the oldest individuals will contribute disproportionately, as these older fish will add more points to the data set than young fish. Finally, using only the 10 records of size-at-capture in the regression would not give an appropriate estimate of the true population growth curve (Figure 5C), presumably due to low sample size and the presence of only one old fish in the sample, which is "pooling the trend" for L_∞.

Pinheiro and Bates (2000) recently proposed the use of mixed effects models to analyse longitudinal, autocorrelated, unbalanced data such as those generated by back-calculation. This method fits any linear (linear mixed-effects, LME) or non-linear (NLME) model to longitudinal data with great flexibility in modelling the within-group correlation often present in such data. The term "mixed effects" refers to the fact that both fixed and random effects are modelled. Fixed effects correspond to a population (or factor) level estimate of parameters, while random effects correspond to variability of parameters among individuals relative to the population. Thus, mixed-effects models generate estimates of model parameters at both the individual and population level. Our example of Figure 5 is used to illustrate this point. We fitted a von Bertalanffy growth model using a non-linear mixed-effects model (NLME) for the sample of 10 fish. NLME provided accurate estimates of growth parameters at both the population (Figure 5C) and individual (Figure 5D) levels (see Box 3 for computation of NLME).
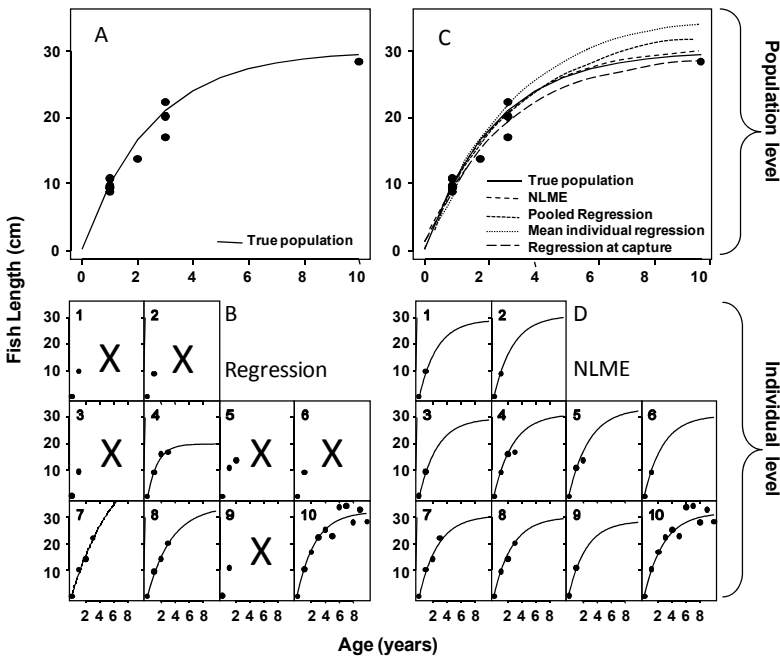

Figure 5. Comparison of regression and mixed effects models. **A**. Ten fish (*points*) sampled from a simulated population of known von Bertanlanffy growth trajectory (*solid line*). Size-at-age was generated for each individual (Box 2) and longitudinal data analysed by non-linear regression (**B**) and non-linear mixed-effects models (NLME, D, Box 3). X indicates individuals for which regressions were not possible due to lack of data. Heteroscedasticity was modelled in NLME by a variance function that was a power of the absolute value of the variance covariate. Plot C compares population level estimates of the growth trajectory by NLME, regression based on pooling size-at-age data of the 10 fish, average individual regression, and regression of size at age of capture of the 10 fish with known growth. Individual ID numbers appear at top of each plot in the lower graphs.

Box 2: Algorithm used to simulate size-at-age data

1. Define a growth function (von Bertalanffy, exponential etc), e.g., $L = L_0 * \exp(K * Age)$, where L = fish length-at-age, L_0 = fish length at hatching, K is the exponential growth rate.

2. Set true population growth parameters μ_p, e.g., $L_0 = 2$ mm and $K = 0.06$ %.d^{-1}.

3. Randomly generate growth parameters for each individual j by: (i) assuming normal distribution of growth parameters with mean μ_p and standard deviation $0.1*\mu_p$ (hence a coefficient of variation of 10%); and (ii) adding a random uniform noise within 2% of the expected value ($\pm 0.02*\mu_p$, which is 5 times smaller than standard deviation). For example:

$L_{0j} \sim N(\mu=2, \sigma=2*0.1)+U(\min=-2*0.02, \max=2*0.02)$ across j fish;

$K_j \sim N(\mu=0.06, \sigma=0.06*0.1)+U(\min=-0.06*0.02, \max=0.06*0.02)$ across j fish.

4. Generate size-at-age data for each individual j at each age i. To do this, we first estimated size-at-age from individual growth parameters (points 1–3 above), then introduced a random normal error (mean=0, sd=0.1*size) and a uniform random noise (min=-0.02*size, max=0.02*size). In this example, size of individual j at age i is given by:

$$L_{ji}= L_{0j}*\exp(K_j*i) + \varepsilon_{ji}$$

   With:

$\varepsilon_{ji} \sim N(\mu=0, \sigma=0.1*L_{0j}*\exp(K_j*i)) + U(\min=-0.02*L_{0j}*\exp(K_j*i), \max=0.02*L_{0j}*\exp(K_j*i))$

5. Further constraints such as $L_{ij} > 0$ were added in the simulation code. Simulated size-at-age data were analysed by NLME (Box 3).

Box 3: Analysing size-at-age data by NLME: An example in R

1. Set the workspace

Install R software from the internet (http://www.r-project.org/) including required libraries. Create a data file (e.g., in MS Excel™), and save it in .txt format. In the examples below, the data file is named "mydata.txt" & has at least 3 columns: fish identification (id), length-at-age (L) and incremental age (Age). Add further columns for factors such as population (Pop) or time of sampling (Time). *Caution,* R is case sensitive. Comments are preceded by # and ignored.

```
# load required libraries
library(grid)
library(lattice)
library(stats)
library(nlme)
```

2. Import data and declare growth functions

```
#Import the data into R under the object name "datgr"
datgr=groupedData(L~Age|id, data=read.table("mydata.txt",header=TRUE, sep="\t"),
labels=list(x="Age",y="Size"), units=list(x="(Years or Days)",y="(cm or mm)"))
#visualise the data
plot(datgr)
#Declare growth functions
LVB=function(x,t0,Lmax,K) {
y=Lmax*(1-exp(-K*(x-t0)))
y
}
EXP=function(x,L0,K) {
y=L0*exp(K*x)
y
}
```

Box 3 (Continued)

3. Fit growth function by NLME

```
# fit a LVB or an exponential growth model by NLME. If the model does not converge, try
changing starting values (bold).
LVB.nlme=nlme(L~LVB(Age,t0,Lmax,K),data=datgr,
fixed=list(t0~1,Lmax~1,K~1),
random= t0+Lmax+K~1,
start=list(fixed=c(t0=-0.02,Lmax=30,K=0.4)))
EXP.nlme=nlme(L~EXP(age,L0,K),data=datgr,
fixed=list(L0~1,K~1),
random=L0+K~1,
start=list(fixed=c(L0=2,K=0.06)))
#to see results and plots:
summary(LVB.nlme)
intervals(LVB.nlme)
anova(LVB.nlme)
plot(LVB.nlme)
plot(augPred(LVB.nlme,level=0:1))
coef(LVB.nlme)
```

4. Comparing populations (or levels of other factors)

```
# Compare LVB growth models of two (or more) populations. There must be as many
starting values as levels of factors (in this case 2 levels: population 1 and 2). The two
models below are inherently identical (type anova(LVB1.nlme,LVBbis.nlme) to verify this).
The first model includes an intercept, which is convenient to test the effect of factor
population, whereas there is no intercept in the second model, which is convenient to
estimate growth parameters for each level of the factor population.
LVB1.nlme=nlme(L~LVB(Age,t0,Lmax,K),data=datgr,
fixed=list(t0~Pop,Lmax~Pop,K~Pop),
random= t0+Lmax+K~1,
start=list(fixed=c(-0.02,0,25,0,0.4,0)))
anova(LVB1.nlme)
LVB1bis.nlme=nlme(L~LVB(Age,t0,Lmax,K),data=datgr,
fixed=list(t0~Pop-1,Lmax~Pop-1,K~Pop-1),
random= t0+Lmax+K~1,
start=list(fixed=c(-0.02,-0.02,30,20,0.4,0.4)))
summary(LVB1bis.nlme)
```

5. Comparing sequential samples (size-selective mortality)

```
#Highlight size-selective mortality effect on exponential growth parameter K with 3
sequential samples (there must be as many starting values as there are samples or levels for
factor Time).
#selection on both L0 and K
EXP1.nlme=nlme(L~EXP(age,L0,K),data=datgrl,
fixed=list(L0~Time,K~Time),
random=L0+K~1,
start=list(fixed=c(2,0,0,0.06,0,0)))
#selection on K only
EXP2.nlme=nlme(L~EXP(age,L0,K),data=datgrl,
fixed=list(L0~1,K~Time),
random=L0+K~1,
start=list(fixed=c(2,0.06,0,0)))
```

Box 3 (Continued)

```
#no selection
EXP3.nlme=nlme(L~EXP(age,L0,K),data=datgrl,
fixed=list(L0~1,K~1),
random=L0+K~1,
start=list(fixed=c(2,0.06)))
#most parsimonious model
anova(EXP1.nlme,EXP2.nlme,EXP3.nlme)
#results
EXP2bis.nlme=nlme(L~EXP(age,L0,K),data=datgrl,
fixed=list(L0~1,K~Time-1),
random=L0+K~1,
start=list(fixed=c(2,0.06,0.065,0.075)))
summary(EXP2bis.nlme)
plot(augPred(EXP2bis.nlme,level=0:1))
```

6. Modelling heteroscedastic and/or correlated within-group variance

#The NLME library provides a set of classes of variance functions, the varFunc classes, and a set of classes of correlation structures, the corStruct classes. The former are used to specify within-group variance and thus model heteroscedasticity whereas the latter are used to specify within-group correlation and thus model dependence among observations (see Pinheiro & Bates 2000 for details). For example, heteroscedasticity was modelled by a variance function that is a power of the absolute value of the variance covariate in all simulated examples given in this chapter (Figures 5, 6 & 7). For the size-selection example shown in Figure 7, this gives:

```
EXP2bis.final.nlme=nlme(L~EXP(age,L0,K),data=datgrl,
fixed=list(L0~1,K~Time-1),
random=L0+K~1,
start=list(fixed=c(2,0.06,0.065,0.075)),
weights=varPower())
```

The aim in most studies is not only to estimate growth, but also to compare the growth of 2 or more populations. Mixed-effects models can perform this task easily by incorporating a qualitative co-variable (or factor). Importantly, LME and NLME allow different variances for each level of a factor and hence can be used to flexibly model heteroscedasticity of the within-error group. Figure 6 shows how NLME can estimate the von Bertalanffy growth parameters of two simulated populations that differ only in L_∞ (Pop 1 L_∞ = 30; Pop 2 L_∞ = 20 cm). A von Bertalanffy growth model was fitted by NLME to simulated size-at-age of 10 fish sampled from each of two populations (Boxes 2 and 3). The technique provided not only accurate estimates of growth parameters at both individual and population levels, but also correctly identified that the two populations differed significantly in L_∞.

In the same way that the qualitative factor "population" was inserted into the mixed-effects model in the example above, any other factor (e.g. diet, site, year, time, etc.) or combination of factors (e.g. "diet × population") could also be added to the model. Of all factors whose influence on growth trajectories is likely to be of interest to a researcher, "age" (or "time") is one of the most difficult to analyse. For example, size-selective mortality is a phenomenon that typically removes the smaller, slower growing fish as a cohort ages, so that sequential samples from the same cohort display different

growth trajectories (Meekan & Fortier 1996, Sogard 1997, Vigliola & Meekan 2002). Generally, size-at-age data back-calculated from sequential samples are analysed by repeated-measures MANOVA (Chambers & Miller 1995). Although this analysis can successfully demonstrate size-selection, it does not provide any estimate of growth rates, as no growth model is fitted to the data. Furthermore, it restricts the analysis to the youngest fish of each sequential sample. For example, Vigliola and Meekan (2002) were forced to limit the comparison of growth trajectories of settlement stage, 1, 2 and 3 months post-settlement fish to 12 d (a repeated factor at levels 0, 3, 6, 9, 12 d) since the youngest fish collected in the samples was 12 d of age. In contrast, Vigliola et al. (2007) analysed the entire dataset in a NLME model and used individual growth estimates to demonstrate a proximal link between phenotypic and genetic selection.
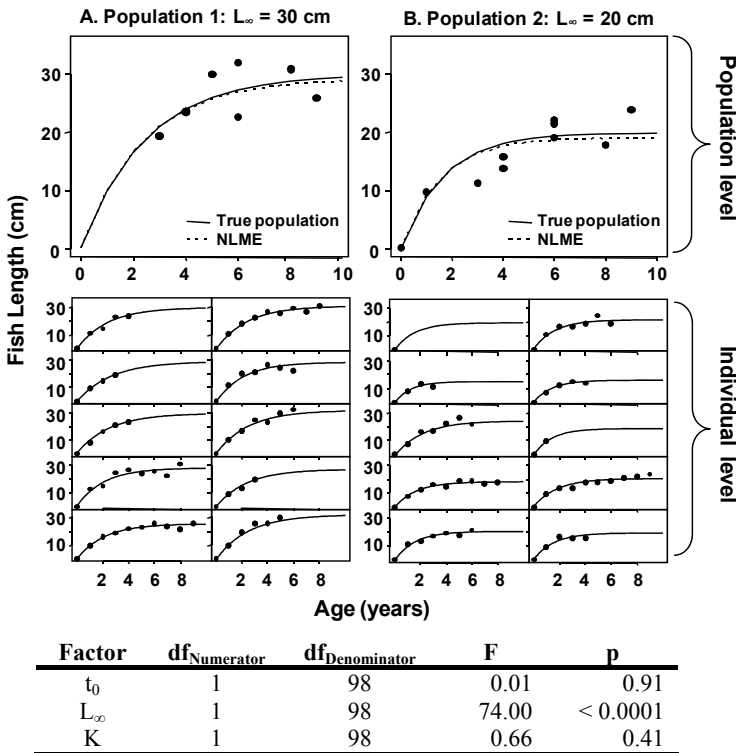

Factor	$df_{Numerator}$	$df_{Denominator}$	F	p
t_0	1	98	0.01	0.91
L_∞	1	98	74.00	< 0.0001
K	1	98	0.66	0.41

Figure 6. Data from two populations of known von Bertanlanffy growth trajectories. The two populations differed only in the value of parameter L_∞. Ten fish were sampled from each population and simulated size-at-age (Box 2) analysed by non-linear mixed-effects models with "population" entered as a fixed factor (Box 3). Heteroscedasticity was modelled by a variance function as a power of the absolute value of the variance covariate. NLME not only provided accurate estimates of the von Bertallanfy model at both population (*upper panels*) and individual levels (*lower panels*), but also provided statistics (*table*) indicating that the two populations only differed in parameter L_∞.

NLME can be a very powerful alternative to repeated-measures MANOVA to study size-selective mortality, while estimating growth parameters and enabling a researcher to determine the growth parameters on which selection acts (Figure 7). A simulated cohort with an exponential growth trajectory ($L = L_0 \exp(K \text{ age})$ with $L_0 = 2$ mm and $K = 0.06 \%.d^{-1}$) was sampled so that 10 fish were randomly removed at 10, 20 and 30 days after hatching. No size-selection occurred in the cohort from 0–10d after hatching, but only fish with a growth rate (K) bigger than the 50% quantile survived to 20 d, and only fish with a K bigger than the 90% quantile survived to 30 d. Quantiles were calculated from the random generation of 10,000 individuals using the general algorithm described in Box 2. No selection acted on the size at hatching parameter L_0. Size-at-age data were simulated for the 30 sequentially sampled fish (Box 2) and an exponential growth model fitted by NLME to the entire dataset with "time of sampling" added as a factor (Box 3). NLME analysis successfully estimated growth parameters of each individual and of each sequential sample, while indicating that strong size-selective mortality occurred in this population where fish with higher K survived (Figure 7). This latter result was obtained by identifying the most parsimonious among three possible NLME models. The first included an effect of the factor Time on both L_0 and K; the second only included an effect of Time on K, while the third did not include Time as a covariate (Box 3). The analysis indicated that the second model was the most parsimonious, thus demonstrating that size-selective mortality had indeed occurred and had only acted on growth rate K.

In summary, mixed-effects model are a powerful tool to analyse data from growth back-calculation as they:

- can accommodate longitudinal, unbalanced, auto-correlated data;
- account for individual effects while also estimating group (or factor) effects;
- allow the within-error group to be heteroscedastic and/or correlated;
- are efficient even with small sample sizes;
- can easily integrate factors and covariates; and
- allow researchers to analyse, explore and interpret complex designs.

LME and NLME are programmed in the language S (also used in the software package R). Examples of basic NLME modelling of fish growth are provided in Box 3. Details of LME and NLME can be found in Pinheiro and Bates (2000) and a guideline to programming in S can be found in Venables and Ripley (2002). Implementations of S are available in S-PLUS, a commercial system (http://www.insightful.com), and R, an open source software package (http://www.r-project.org).
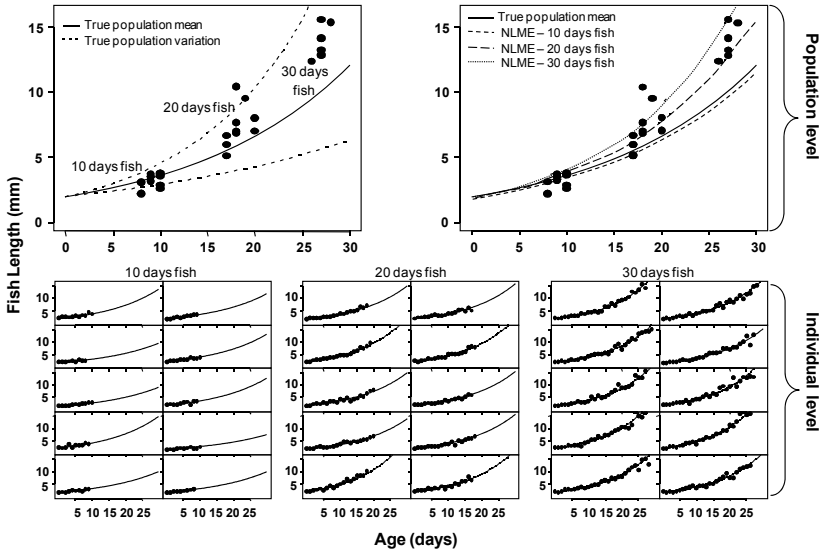
Effect	Model	df	AIC	BIC	L-L	Test	L-L Ratio	p
Selection on K & L_0	1	11	774	822	-376			
Selection on K	2	9	774	814	-378	1 vs 2	3.9	0.1426
No Selection	3	7	794	824	-389	2 vs 3	23.2	< 0.0001

Figure 7. A simulated cohort with an exponential growth trajectory ($L = L_0 \exp(K\, age)$, $L_0 = 2$ mm, $K = 0.06$ %.d^{-1}) was sampled so that 10 fish were randomly removed at 10, 20 and 30 days after hatching. No size-selection occurred from 0–10 d after hatching, but only fish with a growth rate (K) bigger than the 50% quantile survived to 20 d, and only fish with a K bigger than the 90% quantile survived to 30 d. No selection acted on the size at hatching parameter L_0. Quantiles were calculated from the random generation of 10,000 individuals using the general algorithm described in Box 2. Size-at-age data were generated from the 30 sequentially sampled fish (see Box 2) and an exponential growth model fitted by NLME to the entire dataset with "Time of sampling" added as a fixed factor (see Box 3). Heteroscedasticity was modelled in the NLME by a variance function, which is a power of the absolute value of the variance covariate. NLME analysis successfully estimated growth parameters of each individual and of each sequential sample. Log likelihood ratio tests (Table embedded in figure) indicated that a model with an effect of the factor Time on both L_0 and K was not better than a model only including an effect of Time on K (p=0.1426), while the latter was significantly better (p<0.0001) than a third model that did not include Time as a covariable, hence demonstrating that size-selective mortality had indeed occurred and had only acted on growth rate.

4   Conclusions and future directions

The discovery of daily increments within otoliths by Panella in the 1970s has led to a major proliferation in use of back-calculation techniques over the last 30 years. Jones (1992) noted that the number of published studies using daily increments (many of which involved back-calculation) was increasing exponentially. The trend has not abated, so that today even a cursory search of online databases yields hundreds of otolith back-calculation studies published in the last decade. It is now well recognised that the assumptions underlying the technique (periodicity of increment deposition, reliability of ageing and proportionality between otolith and somatic growth) require validation and more than 200 studies have addressed the issue of age validation in the last 10 years. These contrast with the studies that have sought to validate and identify the most appropriate model for back-calculation over the same period: a total of 9 in peer-reviewed journals of which we are aware (Table 2). Of these, 6 attempted a proper validation of the models by comparing direct observations of fish length with estimates provided by back-calculation, while only 3 validated at the individual level. Only one study was based on longitudinal data collected at the individual level under conditions of variable growth.

Is it necessary to validate the output of a back-calculation model every time it is used? Although relative accuracy can only be determined by a comparison of model outputs against independent data sets of growth, this will be logistically difficult to achieve in most situations and virtually impossible for some life history stages such as pelagic larvae. Validation of back-calculation, therefore, will involve considerable effort and will probably only occur in studies designed with this as a central aim. We have shown here, however, that simple criteria can be used to reduce the number of potential back-calculation models to a more manageable short-list. Two recent studies that attempted comprehensive comparisons of these remaining models (Vigliola et al. 2000, Wilson et al. 2008) found that the MF model performed better than others, while one other (Morita & Matsuishi 2001) suggested that the AE model produced the most accurate estimates of size from back-calculation. Experimental studies (Wilson et al. 2008) show that the AE model can generate unrealistic estimates of fish size (as is the case for all proportionality hypothesis and other regression-based models), so we do not recommend use of this model without thorough validation.

Wilson et al. (2008) found that allometric L – R relationships may be undetectable in population level analyses, implying that it may be very difficult to choose between BI and MF models, even when evidence from regression analyses suggests that L – R relationships are isometric (linear). We thus recommend the use of the MF model as a conservative approach in routine back-calculations of fish size at age from otoliths (Box 1).

The development of back-calculation models that can accommodate both isometric and allometric L – R relationships is major advance in the analysis of fish growth from otoliths and other hard parts. These models now need to be validated under a range of environmental conditions and with a variety of life history stages of fish. In particular, validation studies need to focus on the transitions between ontogenetic stages and environments, for two reasons: Firstly, this is where L – R relationships often break down or change; Secondly, transitions such as those between pelagic and reef

environments, or between juvenile and adult habitats, are "critical periods" where fish undergo intense periods of mortality (e.g., Doherty et al. 2004, Webster & Almany 2006), and thus are of great interest to ecologists and fisheries biologists due to their influence on population regulation.

Recent advances in statistical methods and the greater availability of NLME models in software packages gives researchers powerful new tools to analyse back-calculated data sets of growth. Like other methods such as repeated-measures MANOVA, these offer a means to include a range of covariates or factors into the model analysis while providing results than can be interpreted in a simple manner. Notably, these models are able to detect processes such as size-selective mortality and to incorporate analysis of the abiotic and biotic correlates of growth within and among different populations. Unlike other methods, however, NLME models account for individual effects while also estimating group (or factor) effects, and can explicitly model heteroscedastic and correlated within-group error. It is important to remember, however, that quality of the output of these new techniques will be determined by the quality of the back-calculated data that is analysed and the growth function that is fitted to data sets. Examples of basic NLME modelling of fish growth are provided in Box 3.

Our call for more validation studies is not new and echoes that of Francis (1990, 1995) and Brothers (1995). In the last decade many more back-calculation models have been proposed, but relatively few studies have attempted to comprehensively assess the ability of these to reproduce real patterns of fish growth. Back-calculation models are theoretical constructs that provide us with a practical means of generating a wealth of useful data, but without rigorous validation we have no idea if they accurately reflect reality. For the most part, this validation remains to be completed.

Appendix 1. Summary of back-calculation models. R: otolith radius; L: fish length; R_i, L_i: radius and length at age i; R_{cpt}, L_{cpt}: radius and length at time when fish were sacrificed; R_0, L_0: radius and length at origin (usually hatching, age=0); R_{0p}, L_{0p}: biologically-determined radius and length at origin ("biological intercept" BI, usually at hatching); f: function such as R=f(L); g: function such as L=g(R); a, b, …: function parameters; BCF: back-calculation function (f or g).

Model & Calculation Details	Abbreviations
Model: $\quad L_i = \dfrac{R_i}{R_{cpt}} L_{cpt}$	**BCM 1**
Name: Dahl-Lea (Lea 1910),	**DALE**
Function: Linear w/o intercept; L = bR (or R = L/b)	(g1 or f1)
Hypothesis: "the scale grows in exact proportion to the length of the individual"; $\quad L_i / R_i = \text{constant} = b$	
Geometry: A straight line passing through (R=0, L=0) & (R_{cpt}; L_{cpt}).	
Computation: Only the directly measured (L_i; R_i; R_{cpt}; L_{cpt}) are required to compute BCM 1. Note that BPH applied on function g1 or SPH applied on function f1 will also result in BCM 1. BCM 3 is identical to BCM 1 if (R_{0p}=0; L_{0p}=0), which may be reasonable when back-calculating size-at-age of adult fish from annual increments.	

Model & Calculation Details	Abbreviations

Model: $$L_i = a + (L_{cpt} - a)\frac{R_i}{R_{cpt}}$$

BCM 2

Name: Fraser-Lee (Fraser 1916, Lee 1920),   **FRALE**

Function: Linear w/ intercept; $L = a + bR$ (g2)

Hypothesis: "the growth increment of the scale is, on average…, a constant

proportion of the growth increment of the fish"; $\dfrac{(L_i - L_{i-1})}{(R_i - R_{i-1})} = \text{constant} = b$

Geometry: A straight line passing through (R=0; L=a) & (R_{cpt}; L_{cpt}).

Computation: Fraser (1916) and Lee (1920) originally described "a" as the length of
the fish at the time of scale formation (and assumed this length was the same
for all scales from all fish in a population). Current practice is to set "a" as the
intercept of g2 function fitted by "L-on-R" regression (Francis 1990):
    1. Fit $L = a + bR$ by L-on-R linear regression to estimate a.
    2. Apply BCM 2.

Model: $$L_i = L_{cpt} + (R_i - R_{cpt})\frac{(L_{cpt} - L_{0p})}{(R_{cpt} - R_{0p})}$$

BCM 3

Name: Biological Intercept (Campana 1990), or Linear Biological Intercept,   **BI, LBI**

Function: Linear w/ intercept; $L = a + bR$ (g2)

Hypothesis: Modification of BCM 2 with replacement of statistically estimated
intercept (R=0; L=a) by biologically determined intercept (R_{0p}; L_{0p}).

Geometry: A straight line passing through (R_{0p}; L_{0p}) & (R_{cpt}; L_{cpt}).

Computation: "Generally, I would define the biological intercept of a fish-otolith
trajectory as the fish and otolith length corresponding to the initiation of
proportionality between fish and otolith growth. …In many cases, the
biological intercept could be determined by simple measurements of otolith
and fish size in newly-hatched larvae in the laboratory" (Campana 1990).
    1. Obtain an estimate of mean fish size at first increment formation (usually
      size at hatching) for the species. This parameter is the biological intercept
      L_{0p} of the model
    2. Obtain estimate of R_{0p}, mean radius of first otolith increment for the species.
      R_{0p} may be calculated from the study sample.
    3. Apply BCM 3.

Model: $$L_i = (a + bR_i)\frac{L_{cpt}}{(a + bR_{cpt})}$$

BCM 4

Name: Linear Body Proportional Hypothesis,   **LBPH**

Function: Linear w/ intercept; $L = a + bR$ (g2)

Hypothesis: The BCF is linear and there is "constant proportional deviation from the
mean body size" (BPH, Francis 1990).

Geometry: A straight line passing through (R_{cpt}; L_{cpt}) & preserving a constant
proportional distance to regression line g2.

Computation:
    1. Fit $L = a + bR$ by L-on-R linear regression to estimate a & b.
    2. Apply BCM 4.

Model & Calculation Details	Abbreviations

Model: $L_i = L_{0p} + \sum_{j=1}^{i}(W_j + G_e(W_j - W))\dfrac{(L_{cpt} - L_{0p})}{(R_{cpt} - R_{0p})}$ **BCM 5**

Name: Time-Varying Growth (Sirois et al. 1998),   **TVG**

Function: Linear w/ intercept; $L = a + bR$ (g2)

Hypothesis: Modification of the linear biological intercept model (BCM 3) with inclusion of a growth effect factor in the structure of the model.

Geometry: Complex curve not necessarily passing through $(R_{0p}; L_{0p})$ or $(R_{cpt}; L_{cpt})$.

Computation: W_i is the otolith increment width at age i and W is a stage-specific mean increment width. Values of W are calculated for each fish at each developmental stage, e.g., Sirois et al. (1998) calculated W at yolk-sac, preflexion and post-flexion stages for each fish.

  1. Obtain a biological intercept $(R_{0p}; L_{0p})$ as for BCM 3.

  2. Define developmental stages and calculate average otolith increment for each fish at each stage (one value per fish per stage).

  3. Compute the estimated slope of the fish size – otolith size relationship for each fish,: $S = \dfrac{(L_{cpt} - L_{0p})}{(R_{cpt} - R_{0p})}$.

  4. Compute linear growth rate in length for each fish: $G = \dfrac{(L_{cpt} - L_{0p})}{Age_{cpt}}$.

  5. Calculate growth effect factor G_e by S-on-G linear regression, $S = d + G_e G$ using each fish as an independent observation.

  6. Apply BCM 5. Note there is one value of W per fish per stage. W should be replaced by W_k for k developmental stage in BCM 5.

Model: $L_i = \dfrac{(\frac{R_i}{R_{cpt}}(A + BL_{cpt}) - A)}{B}$ **BCM 6**

Name: Linear Scale Proportional Hypothesis,   **LSPH**

Function: Linear w/ intercept; $R = \dfrac{(L - a)}{b} = A + BL$ (f2)

Hypothesis: The BCF is linear and there is "constant proportional deviation from the mean scale size" (SPH, Francis 1990).

Geometry: A straight line passing through $(R_{cpt}; L_{cpt})$ & preserving a constant proportional distance to regression line f2.

Computation:

  1. Fit $R = A + BL$ by R-on-L linear regression to estimate A & B.

  2. Apply BCM 6.

Model & Calculation Details	Abbreviations

Model: $L_i = -\dfrac{a}{b} + \left(L_{cpt} + \dfrac{a}{b} + \dfrac{c}{b}age_{cpt}\right)\dfrac{R_i}{R_{cpt}} - \dfrac{c}{b}age_i$ 　　**BCM 7**

Name: Age Effect (Morita & Matsuishi 2001), or Age Effect Scale Proportional
　　　Hypothesis,　　　　　　　　　　　　　　　　　　　　　　　　　　**AE, AESPH**

Function: Plane w/ intercept; $R = a + bL + c \times age$　　　　　　　　　(f3)

Hypothesis: "otolith increases with both fish body length and age following f3"
　　　and "the deviation of the otolith length of a fish from the average for that
　　　fish length and age is relatively the same throughout the life of a fish
　　　(SPH)".

Geometry: A complex trajectory passing through $(R_{cpt}; L_{cpt})$ and included in a
　　　plane passing through $(R_{cpt}; L_{cpt}; Age_{cpt})$ and preserving a constant
　　　proportional distance to regression plane f3.

Computation:
　　　1. Fit $R = a + bL + c \times age$ by R-on-L-and-age multiple linear regression
　　　　to estimate a, b, c.
　　　2. Apply BCM 7

Model: $L_i = (A + BR_i + C \times age_i)\dfrac{L_{cpt}}{(A + BR_{cpt} + C \times age_{cpt})}$　　**BCM 8**

Name: Age Effect Body Proportional Hypothesis,　　　　　　　　　　　**AEBPH**

Function: Plane w/ intercept; $L = \dfrac{R - a - c \times age}{b} = A + BR + C \times age$　　(g3)

Hypothesis: Same as BCM 7 but a BPH rather than a SPH is applied.

Geometry: A complex trajectory passing through $(R_{cpt}; L_{cpt})$ and included in a plane
　　　passing through $(R_{cpt}; L_{cpt}; Age_{cpt})$ and preserving a constant proportional
　　　distance to regression plane g3.

Computation:
　　　1. Fit $L = A + BR + C \times age$ by L-on-R-and-age multiple linear regression to
　　　　estimate A, B, C.
　　　2. Apply BCM 8.

Model: $L_i = \left(\dfrac{R_i}{R_{cpt}}\right)^c L_{cpt}$　　　　　　　　　　　　　　　**BCM 9**

Name: Monastyrsky (Bagenal & Tesch 1978),　　　　　　　　　　　**MONA**

Function: Allometric w/o intercept; $L = bR^c$　　(g4)

Hypothesis: The model assumes the BCF is described by an allometric function
　　　passing through the origin and the point at capture.

Geometry: An allometric curve passing through (R=0; L=0) & $(R_{cpt}; L_{cpt})$.

Computation: This model requires an estimate for c. Originally, it seems that c may
　　　have been estimated from fit of function g4 by eye (Francis 1990). However,
　　　common practice may be to estimate c from L-on-R linear regression of
　　　$\ln(L) = B + c \times \ln(R)$ with intercept $B = \ln(b)$.

　　　1. Fit $L = bR^c$ by eye (or regression) to estimate c
　　　2. Apply BCM 9

Model & Calculation Details	Abbreviations

Model: $L_i = \dfrac{bR_i^c}{bR_{cpt}^c} L_{cpt} = \left(\dfrac{R_i}{R_{cpt}}\right)^c L_{cpt}$ — **BCM 10**

Name: Monastyrsky Body Proportional Hypothesis, — **MONA-BPH**

Function: Allometric w/o intercept; $L = bR^c$ (g4)

Hypothesis: Modification of the Monastyrsky's model (BCM 9) with application of BPH. Morita and Matsuishi (2001) described BCM 10 as a "nonlinear BPH".

Geometry: Allometric curve through (R=0; L=0) & $(R_{cpt}; L_{cpt})$.

Computation: Note an estimate of "b" is not required to compute BCM 10.
   1. Fit $L = bR^c$ by L-on-R non-linear regression to estimate c.
   2. Apply BCM 10.

Model: $L_i = \left(\dfrac{\left(\dfrac{R_i}{R_{cpt}} BL_{cpt}^c\right)^{\frac{1}{c}}}{B}\right) = \left(\dfrac{R_i}{R_{cpt}}\right)^{\frac{1}{c}} L_{cpt}$ — **BCM 11**

Name: Monastyrsky Scale Proportional Hypothesis, — **MONA-SPH**

Function: Allometric w/o intercept; $R = \left(\dfrac{L}{b}\right)^{\frac{1}{c}} = \left(\dfrac{1}{b}\right)^{\frac{1}{c}} L^{\frac{1}{c}} = BL^C$ (f4)

Hypothesis: Modification of BCM 9 with application of SPH. Morita and Matsuishi (2001) described BCM 11 as a "nonlinear SPH".

Geometry: Allometric curve passing through (R=0; L=0) & $(R_{cpt}; L_{cpt})$.

Computation: Note that an estimate of B is not required to compute BCM 11.
   1. Fit $R = BL^c$ by R-on-L non-linear regression to estimate C.
   2. Apply BCM 11

Model: $L_i = \exp\left(\ln(L_{0p}) + \dfrac{[\ln(L_{cpt}) - \ln(L_{0p})][\ln(R_i) - \ln(R_0)]}{[\ln(R_{cpt}) - \ln(R_0)]}\right)$ — **BCM 12**

Name: Watanabe and Kuroki (1997), — **WAKU**

Function: Allometric w/o intercept; $L = bR^c$ (g4)

Hypothesis: Watanabe and Kuroki (1997) stated that "we assumed that the relationship of i-th otolith ring radius and L on the day of i-th ring formation (L_i) can be expressed by an allometric formula for individual larvae" and "the allometric parameters 'b' and 'c' were calculated for each larvae by solving the two equations; $L_{0p} = bR_0^c$ and $L_{cpt} = bR_{cpt}^c$, where …R_0 is the measured radius of the first daily ring".

Geometry: Allometric curve passing through (R=0; L=0) & $(R_{cpt}; L_{cpt})$.

Computation:
   1. Obtain a biological intercept L_{0p} as for BCM 3.
   2. Apply BCM 12 with different measured R_0 (1st increment) per fish.

Model & Calculation Details	Abbreviations

Model: $L_i = a + \exp\left(\ln(L_0 - a) + \dfrac{[\ln(L_{cpt} - a) - \ln(L_0 - a)][\ln(R_i) - \ln(R_0)]}{[\ln(R_{cpt}) - \ln(R_0)]}\right)$ **BCM 13**

Name: Fry (1943),   **FRY**

Function: Allometric w/ intercept; $L = a + bR^c$ (g5)

Hypothesis: Assumes the BCF is an allometric function through (R=0; L=a), $(R_0; L_0)$ and $(R_{cpt}; L_{cpt})$ (but see Francis 1990). Vigliola et al. (2000) showed both allometric and linear BCF were consistent with proportionality between relative growth rates of fish and otoliths".

Geometry: An allometric curve through (R=0; L=a), $(R_0; L_0)$ & $(R_{cpt}; L_{cpt})$.

Computation: Requires estimates for a, R_0 & L_0. Originally, a was chosen to linearize the plot of $\ln(L-a)$ against $\ln(R)$, and $(R_0; L_0)$ was a point arbitrarily chosen on the curve g5 (Francis 1990).

1. Find a, b, c so that the line $\ln(L - a) = \ln(b) + c \times \ln(R)$ is as close as possible to data, e.g., by numerical optimisation. The function "optim" of R (http://www.r-project.org/) can be used to find the best set of a, b, c that minimise the function $[\ln(L-a) - \ln(b) - c \times \ln(R)]^2$.

2. Select (arbitrarily) point $(R_0; L_0)$ on the $L = a + bR^c$ curve.

3. Apply BCM 13.

Model: $L_i = a + \exp\left(\ln(L_{0p} - a) + \dfrac{[\ln(L_{cpt} - a) - \ln(L_{0p} - a)][\ln(R_i) - \ln(R_{0p})]}{[\ln(R_{cpt}) - \ln(R_{0p})]}\right)$ **BCM 14**

Name: Modified Fry (Vigliola et al. 2000), or Allometric Biological Intercept),   **MF, ABI**

Function: Allometric w/ intercept; $L = a + bR^c$ or $R = \left(\dfrac{L-a}{b}\right)^{\frac{1}{c}}$ (g5 or f5)

Hypothesis: Proportionality between relative growth rates of the fish and the otolith (Vigliola et al. 2000). Modification of BCM 13 with biologically-constrained but statistically-estimated "a" and biologically determined intercept $(R_{0p}; L_{0p})$ instead of $(R_0; L_0)$.

Geometry: Allometric curve through (R=0; L=a), $(R_{0p}; L_{0p})$ & $(R_{cpt}; L_{cpt})$.

Computation: Requires an estimate for "a", "R_{0p}" and "L_{0p}".

1. Obtain a biological intercept $(R_{0p}; L_{0p})$ as for BCM 3.

2. Fit $L = L_{0p} - b_1 R_{0p}^{c_1} + b_1 R^{c_1}$ by L-on-R non-linear regression to estimate "b_1" and "c_1". Then, calculate $a_1 = L_{0p} - b_1 R_{0p}^{c_1}$.

3. Fit $R = \left(\dfrac{L - L_{0p} + b_2 R_{0p}^{c_2}}{b_2}\right)^{\frac{1}{c_2}}$ by R-on-L non-linear regression to estimate b_2 and c_2. Then, calculate $a_2 = L_{0p} - b_2 R_{0p}^{c_2}$.

4. Calculate $a = \dfrac{a_1 + a_2}{2}$.

5. Apply BCM 14.

Model & Calculation Details	Abbreviations
Model: $L_i = (a + bR_i^c) \dfrac{L_{cpt}}{(a + bR_{cpt}^c)}$	**BCM 15**
Name: Fry Body Proportional Hypothesis, or Allometric Body Proportional Hypothesis,	**FRY-BPH, ABPH**
Function: $L = a + bR^c$	(g5)
Hypothesis: Modification of the Fry's model (BCM 13) with application of BPH	
Geometry: An allometric curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve g5.	
Computation:	
1. Fit $L = a + bR^c$ by L-on-R non-linear regression to estimate a, b, c.	
2. Apply BCM 15.	
Model: $L_i = a + (L_{cpt} - a)\left(\dfrac{R_i}{R_{cpt}}\right)^c$	**BCM 16**
Name: Fry Scale Proportional Hypothesis, or Allometric Scale Proportional Hypothesis,	**FRY-SPH, ASPH**
Function: $R = \left(\dfrac{L-a}{b}\right)^{\frac{1}{c}}$	(f5)
Hypothesis: Modification of the Fry's model (BCM 13) with application of SPH.	
Geometry: An allometric curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve f5.	
Computation: Note that estimate of b is not required in BCM 16.	
1. Fit $R = \left(\dfrac{L-a}{b}\right)^{\frac{1}{c}}$ by R-on-L non-linear regression to estimate a, c.	
2. Apply BCM 16.	
Model: $L_i = (a + bR_i + cR_i^2)\dfrac{L_{cpt}}{(a + bR_{cpt} + cR_{cpt}^2)}$	**BCM 17**
Name: Quadratic Body Proportional Hypothesis (Francis 1990),	**QBPH**
Function: $L = a + bR + cR^2$	(g6)
Hypothesis: Sherriff (1922) first used a quadratic equation but gave no BCM. Francis (1990) gave the BPH formulation of the quadratic function. BCM 17 assumes a quadratic L-on-R BCF and a BPH.	
Geometry: A 2nd degree polynomial curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve g6.	
Computation:	
1. Fit $L = a + bR + cR^2$ by L-on-R non-linear regression to estimate a, b, c.	
2. Apply BCM 17.	

Model & Calculation Details	Abbreviations
Model: $a + bL_i + cL_i^2 = \dfrac{R_i}{R_{cpt}}(a + bL_{cpt} + cL_{cpt}^2)$	**BCM 18**
Name: Quadratic Scale Proportional Hypothesis,	**QSPH**
Function: $R = a + bL + cL^2$ (f6)	
Hypothesis: Francis (1990) claimed "Thomas (1983) gave a good statement of SPH and applied it with a quadratic body-scale curve" but the BCM was not explicitly developed in Thomas (1983) or in Francis (1990). BCM 18 assumes a quadratic R-on-L BCF and a SPH.	
Geometry: A curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve f6.	
Computation: 1. Fit $R = a + bL + cL^2$ by R-on-L non-linear regression to estimate a, b, c 2. Solve the quadratic equation of BCM 18 by numeric optimisation or using the quadratic solution (for $ax^2 + bx + c = 0$, $x = \dfrac{-b \pm \sqrt{b^2-4ac}}{2a}$).	
Model: $L_i = \dfrac{a_0 + a_1 R_i + a_2 R_i^2 + \cdots + a_n R_i^n}{a_0 + a_1 R_{cpt} + a_2 R_{cpt}^2 + \cdots + a_n R_{cpt}^n} L_{cpt}$	**BCM 19**
Name: Polynomial Body Proportional Hypothesis,	**PBPH**
Function: $L = a_0 + a_1 R + a_2 R^2 + \cdots + a_n R^n$ (g7)	
Hypothesis: Gutreuter (1987) generalized quadratic BCM to a polynomial of arbitrary degree. BCM 19 assumes a polynomial L-on-R BCF and a BPH.	
Geometry: A polynomial curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve g7.	
Computation: 1. Fit $L = a_0 + a_1 R + a_2 R^2 + \cdots + a_n R^n$ by L-on-R non-linear regression to estimate $a_0, a_1 \ldots a_n$. 2. Apply BCM 19.	
Model: $a_0 + a_1 L_i + a_2 L_i^2 + \cdots + a_n L_i^n = \dfrac{R_i}{R_{cpt}}(a_0 + a_1 L_{cpt} + a_2 L_{cpt}^2 + \cdots + a_n L_{cpt}^n)$	**BCM 20**
Name: Polynomial Scale Proportional Hypothesis,	**PSPH**
Function: $R = a_0 + a_1 L + a_2 L^2 + \ldots + a_n L^n$ (f7)	
Hypothesis: BCM 20 assumes a polynomial R-on-L BCF and a SPH.	
Geometry: A curve passing through $(R_{cpt}; L_{cpt})$ and preserving a constant proportional distance to regression curve f7.	
Computation: 1. Fit $R = a_0 + a_1 L + a_2 L^2 + \ldots + a_n L^n$ by R-on-L non-linear regression to estimate $a_0, a_1 \ldots a_n$. 2. Solve BCM 20 (by numeric optimisation or other means).	

Model & Calculation Details	Abbreviations
Model: $L_i = \exp(a + bR_i)\dfrac{L_{cpt}}{\exp(a + bR_{cpt})}$	**BCM 21**
Name: Exponential Body Proportional Hypothesis,	**EBPH**
Function: $L = \exp(a + bR)$	(g8)
Hypothesis: Tremblay and Giguère (1992) used an exponential L-on-R BCF and then used a proportionality method for back-calculation. BCM 21 assumes exponential L-on-R BCF and a BPH.	
Geometry: An exponential curve passing through $(R_{cpt}; L_{cpt})$ & preserving a constant proportional distance to regression curve g8.	
Computation: Tremblay and Giguère (1992) originally fitted g8 after log transformation ($\ln(L) = a + bR$), which is not BPH per se. 1. Fit $L = \exp(a + bR)$ by L-on-R non-linear regression to estimate a & b. 2. Apply BCM 21.	
Model: $L_i = \exp[a + (\ln(L_{cpt}) - a)\dfrac{R_i}{R_{cpt}}]$	**BCM 22**
Name: Exponential Scale Proportional Hypothesis,	**ESPH**
Function: $R = \dfrac{\ln(L) - a}{b}$	(f8)
Hypothesis: BCM 22 assumes logarithmic R-on-L BCF and a SPH.	
Geometry: An exponential curve passing through $(R_{cpt}; L_{cpt})$ & preserving a constant proportional distance to regression curve f8.	
Computation: 1. Fit $R = \dfrac{\ln(L) - a}{b}$ by R-on-L non-linear regression to estimate a, b. 2. Apply BCM 22.	

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