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Validation of Different Backcalculation Methods by Using Scales, Opercula and Cleithra of Three Co-existing Cyprinid Species

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Abstract: We compared three proportional backcalculation methods [body proportional (BPH), scale proportional hypothesis (SPH) and Fraser-Lee] for three cyprinid species (*Leuciscus cephalus* (Linnaeus, 1758), *Petroleuciscus borysthenicus* (Kessler, 1859), *Chalcalburnus chalcoides* (Güldenstädt, 1772)). We validated backcalculation by comparing them with observed lengths at time of annulus formation. For *L. cephalus* and *C. chalcoides* scales, opercula and cleithra were used, while *P. borysthenicus* only scales was used. Body-scale, body-opercula and body-cleithra relationships was best described by a power function for *C. chalcoides* and *P. borysthenicus* while same relationships was best described by linear relationship for *L. cephalus.* Our result suggested that in the backcalculation of the lengths of *C. chalcoides* and *P. borysthenicus*, BPH method gives the most reliable results. However, Fraser-Lee method was the best for *L. cephalus*. For all studied fishes, the statistical comparisons showed clearly that the choice of the backcalculation method became less significant with increasing age. These results demonstrated that choice of backcalculation method should be taken into account in the growth studies of fish and the validation of the methods should be made separately for each fish species.

Key words: Cyprinid, backcalculation, SPH, BPH, fraser-lee, scales, opercula, cleithra

Introduction

Growth is an important aspect of the ecology and life history of fish and quantification of growth is frequently a crucial part of fisheries research and management (Summerfelt and Hall, 1987; Weatherley and Gill, 1987). Length of fishes in the past can be backcalculated based on the relationship between fish growth and the growth of calcified structures (e.g., scales, otoliths, opercula, cleithra, otolith). Back-calculation is based on the assumption that the growth of fish proportional to the growth of its bony structures. The yearly marks in the chosen structure are used in calculating the lengths of fish in previous years.

Ages of fish can be determined from several structures, for example, from scales, otoliths, cleithra and opercula (Bagenal and Tesch, 1978; Casselman, 1990; Campana, 1992). The relationship between the chosen structure and the body length of fish may be described with various linear or non-linear equations (Francis, 1990; Secor and Dean, 1992). Finally, for backcalculation of lengths, more than 100

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methods and modifications are available (Everhart, 1950; Libosvársky, 1956; Whitney and Carlander, 1956; Penaz, 1962; Francis, 1990; Smedstad and Holm, 1996; Horppila and Nyberg, 1999; Klumb *et al*., 2001; Morita and Matsuishi, 2001). Backcalculation methodology has been very diverse due to several possibilities in each step.

The linear equation of Fraser and Lee (Fraser, 1916; Lee, 1920; Bagenal and Tesch, 1978) is popular and widely used but has been criticised because it follows no clear hypothesis on the body-scale relationship (Whitney and Carlander, 1956; Francis, 1990). Backcalculation of fish lengths can be based on two different hypothesis: (1) the deviation of the lengths of a fish from the average for fish with that size of scale is relatively the same throughout the life of a fish (body proportional hypothesis, BPH) or (2) that the deviation of the scale radius (or that of some other hard structure) of a fish from the average for that size of fish is relatively the same throughout the life of a fish (scale proportional hypothesis, SPH) (Whitney and Carlander, 1956). Both hypotheses are plausible, but may result in different backcalculated lengths, while the Fraser-Lee equation follows neither (Francis, 1990).

The purpose of this study was to empirically compare several backcalculation methods for scales, opercula and cleithra of three co-existing cyprinid fishes applied to common data sets and to validate backcalculations by comparing them with observed lengths at time of annulus formation.

Materials and Methods

European chub *Leuciscus cephalus*, Dnieper chub *Petroleuciscus borysthenicus* and shemaya *Chalcalburnus chalcoides* were captured from Omerli Reservoir (northwest of Turkey) between March 2003 and May 2004 with gill-netting (10-42 mm mesh sizes, 2.5 m height and 50 m length) and electrofishing (Elektracatch, WFC911). The sample size and size ranges of the species are following; *L. cephalus*; n = 63, 97-257 mm; *C. chalcoides*; n = 248, 130-260 mm; *P. borysthenicus*; n = 88, 88-175 mm. All fishes were weighed and measured to the nearest mm and their ages determined from scales, opercula and cleithra. Otoliths were not used for age determination in cyprinids due to their relatively smaller size and irregular shape. As the opercula and cleithra shape was also not suitable in ageing of *P. borysthenicus*, only scales were used for this species. The scales were taken between the lateral line and dorsal fins. The annual increments of scales were measured from the focus to the posterior edge along the anteroposterior axis using 48 x magnification and microfilm viewer. In opercula and cleithra the annual increments were measured with 5 x magnification from the origin to the posterior edge.

Linear and non-linear models were fitted to determine what equations best describe the relationship between body length and scale radius, opercula length and cleithra length. The backcalculation models used in this study are based on the following formulae;

$$S = a + bL \quad (1)$$

$$L = c + dS \quad (2)$$

$$S = uL^v \text{ or } \ln S = \ln u + v \ln L \quad (3)$$

$$L = wS^k \text{ or } \ln L = \ln w + k \ln S \quad (4)$$

where, S is scale (or some other structure), L is fish body length and a, b, c, d, u, v, w and k are constants obtained from regression analyses. The proposed backcalculation models are as follows;

Scale proportional hypothesis (SPH):

$$L_t = -(a/b) + (L_t + (a/b))S_t/S_T \quad (5)$$

Body proportional hypothesis (BPH):

$$L_t = (c + dS_t/c + dS_T)L_T \quad (6)$$

Fraser-Lee model:

$$L_t = c + (L_T - c)(S_t/S_T) \quad (7)$$

Nonlinear scale proportional hypothesis (nonlinear SPH):

$$L_t = (S_t/S_T)^{1/v}L_T \quad (8)$$

Nonlinear body proportional hypothesis (nonlinear BPH):

$$L_t = (S_t/S_T)^k L_T \quad (9)$$

In these models, L_t is the backcalculated fish body length at age t, L_T is the fish body length at the time of capture T, S_t is scale (or some other structure) length at annulus t, S_T is scale (or some other structure) length at the time of capture T, a and b are constants as in Eq. 1, c and d are constants as in Eq. 2, v is a constant as in Eq. 3 and k is a constant as in Eq. 4.

The validity of BPH, SPH and Fraser-Lee methods were then explored in three ways. To get an overall view on the similarity or dissimilarity of the results given by different methods, the average lengths of each species were backcalculated with each method using the datasets. Results were calculated as a percentage difference between backcalculated lengths and observed lengths. The lengths obtained with different methods were compared using Analysis of Variance (ANOVA) for all species.

Results

Body-scale, body-cleithrum and body-operculum relationships of *L. cephalus* were described best by a linear function with r^2 value of 0.90, while the same relationships *C. chalcoides* and body-scale relationship of *P.borysthenicus* were described best by a power function with r^2 value of 0.73 and 0.79, respectively (Fig. 1-3).

For *L. cephalus*, the results by Fraser-Lee, BPH and SPH were considerably affected by the choice of structure. When the lengths were backcalculated using the whole dataset for all species, the results from Fraser-Lee with cleithra were most consistent with the observed lengths (Table 1). According to Fraser Lee the average length at age of 1 year was 127 mm with cleithra while BPH and SPH gave values 117 and 107 mm (Table 1). Towards older ages the differences were less.

In the two youngest ages of *C. chalcoides*, the lengths backcalculated with BPH differed 3-7% from the observed body when scales were used and 2-12% and 6-11% when opercula and cleithra were

Table 1: The backcalculated mean lengths (mm) of three studied fishes at ages 1-5 obtained with different methods; Standard deviations are given in parantheses (s, scales; o, opercula; c, cleithra)

Leuciscus cephalus	Observed length	BPH(s)	BPH(o)	BPH(c)	SPH(s)	SPH(o)	SPH(c)	Fraser-Lee (s)	Fraser-Lee (o)	Fraser-Lee (c)
Age										
1	138 (12)	93 (11)	112 (18)	117 (21)	87 (13)	103 (20)	107 (27)	95 (11)	121 (16)	127 (19)
2	175 (9)	151 (16)	166 (15)	167 (16)	147 (18)	160 (17)	161 (19)	152 (16)	171 (14)	174 (15)
3	198 (16)	198 (13)	198 (14)	198 (17)	198 (13)	197 (14)	197 (17)	198 (13)	200 (14)	200 (17)
4	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)	236 (18)
Chalcalburnus chalcoides										
Age										
1	130 (12)	126 (10)	127 (17)	122 (16)	103 (9)	116 (17)	111 (16)	137 (8)	135 (15)	127 (15)
2	162 (11)	174 (11)	182 (16)	181 (13)	158 (11)	176 (17)	175 (14)	177 (10)	185 (15)	183 (13)
3	213 (13)	211 (13)	214 (14)	213 (13)	205 (14)	212 (14)	212 (13)	211 (12)	214 (14)	214 (13)
4	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)	244 (10)
Petroleuciscus borysthenicus										
Age										
1	108 (10)	82 (10)	-	-	73 (12)	-	-	85 (9)	-	-
2	115 (10)	107 (12)	-	-	102 (12)	-	-	108 (11)	-	-
3	135 (9)	134 (11)	-	-	131 (11)	-	-	133 (11)	-	-
4	145 (4)	151 (13)	-	-	150 (12)	-	-	151 (12)	-	-
5	170 (7)	170 (7)	-	-	170 (7)	-	-	170 (7)	-	-

Table 2: Results from ANOVA for backcalculated lengths at age of the studied fishes obtained with BPH, SPH and Fraser-Lee methods

Fish species	1	2	3	4	5
Leuciscus cephalus	x	x	o	o	
Chalcalburnus chalcoides	x	x	o	o	
Petroleuciscus borysthenicus	x	o	o	o	o

x = $p<0.001$, o = $p>0.05$

Table 3: Mean differences of backcalculated lengths of the studied fishes at each age given by BPH, SPH and Fraser-Lee lengths, expressed as percentages of observed lengths (s, scales; o, opercula; c, cleithra)

Leuciscus cephalus	1	2	3	4	5
BPH(s)	32.61	15.89	0	0	
BPH(o)	18.84	5.96	0	0	
BPH(c)	15.22	5.30	0	0	
SPH(s)	36.96	18.54	0	0	
SPH(o)	25.36	9.94	0.51	0	
SPH(c)	22.46	9.27	0.51	0	
Fraser-Lee(s)	31.16	15.23	0	0	
Fraser-Lee(o)	12.32	2.65	1.01	0	
Fraser-Lee(c)	7.97	0.66	1.01	0	
Chalcalburnus chalcoides					
BPH(s)	3.08	7.41	13.53	0	
BPH(o)	2.30	12.35	12.30	0	
BPH(c)	6.15	11.72	12.71	0	
SPH(s)	20.77	2.47	15.98	0	
SPH(o)	10.77	8.64	13.12	0	
SPH(c)	14.62	8.02	13.12	0	
Fraser-Lee(s)	5.38	9.26	13.53	0	
Fraser-Lee(o)	3.85	14.20	12.30	0	
Fraser-Lee(c)	2.31	12.96	12.30	0	
Petroleuciscus borysthenicus					
BPH(s)	24.07	6.96	0.74	4.14	0
SPH(s)	32.41	11.30	2.96	3.45	0
Fraser-Lee(s)	21.29	6.09	1.48	4.14	0

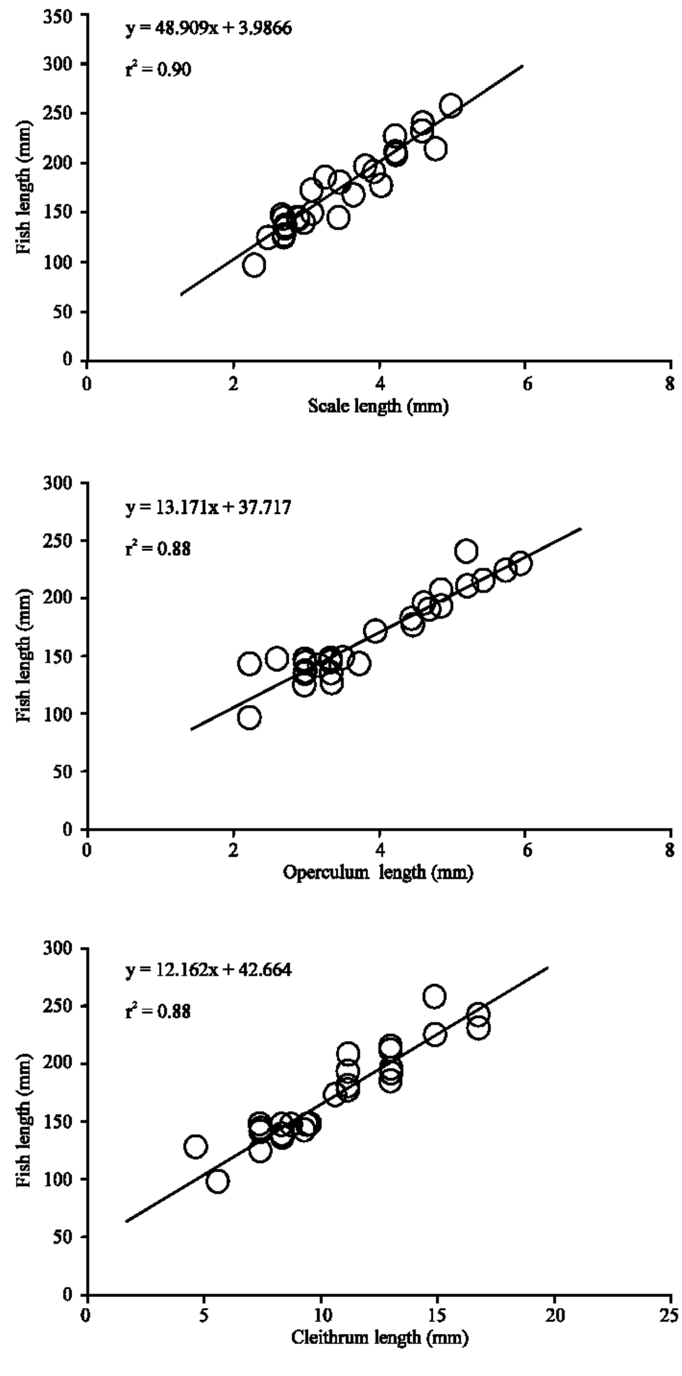

Fig. 1: The body length-scale radius, body length-operculum length and body length-cleithrum length relationships of *L. cephalus* in Ömerli reservoir

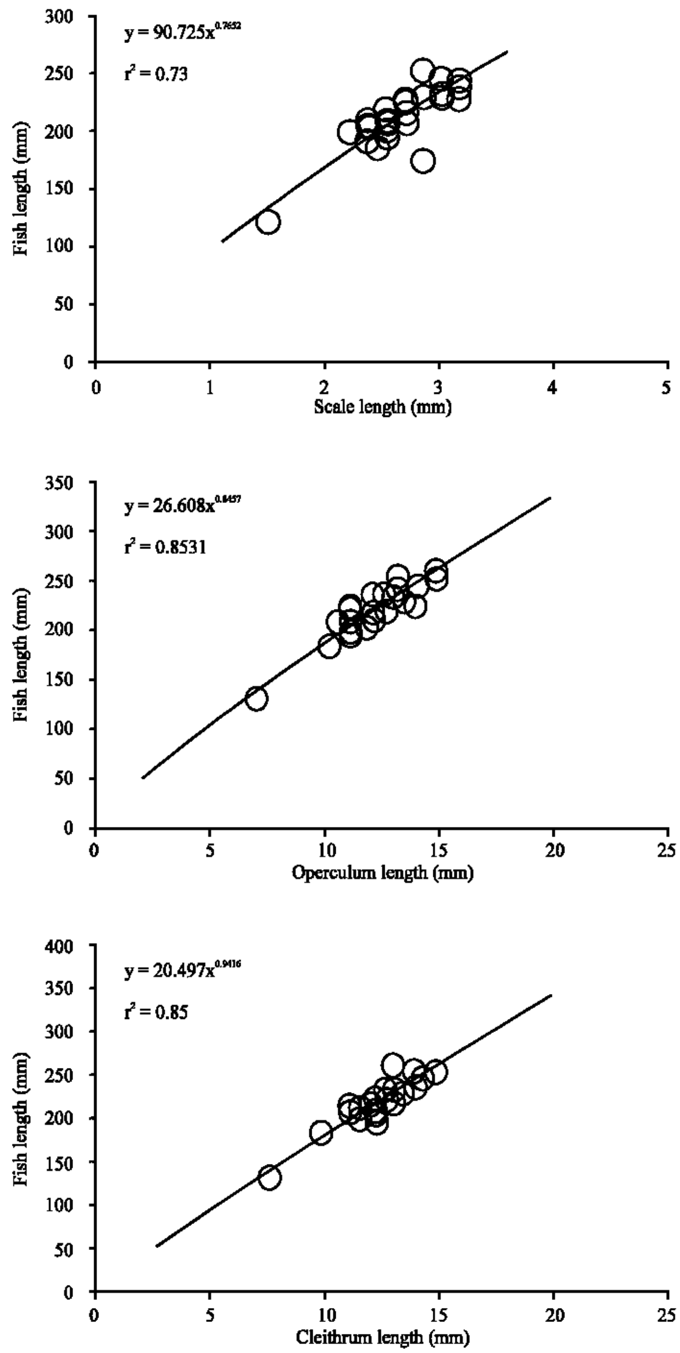

Fig. 2: The body length-scale radius, body length-operculum length and body length-cleithrum length relationships of *C. chalcoides* in Ömerli reservoir

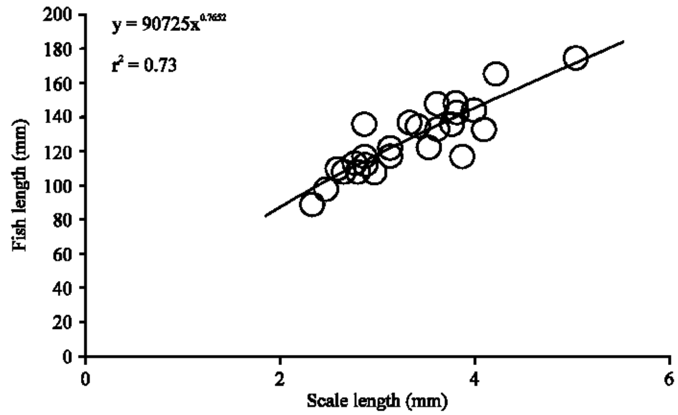

Fig. 3: The body length-scale radius relationships of *P. borysthenicus* in Ömerli reservoir

used (Table 3). With SPH the difference compared with observed lengths somewhat more divergent and values was up to 20% with scales at 1 (Table 3). All the values with BPH and SPH gave underestimated lengths for all age groups. The Fraser-Lee method overestimated the length at age 1 and 2 with scales by 5-9% and opercula by 3-14% while with cleithra it gave values lower than the observed ones (Table 3).

When the ages were backcalculated using only scales of *P.borysthenicus*, backcalculated and observed body lengths matched closely with exception of first age. All backcalculation methods underestimated the lengths at ages 1-3. With SPH, BPH and Fraser-Lee, at age 1 the deviation from the observed length was up to 32, 24 and 21%, respectively (Table 3).

For all the studied fishes, the statistical comparisons showed clearly that the choice of the backcalculation method became less significant with increasing age. At ages 3-5 the results obtained with various methods were not statistically significantly different ($p>0.05$ and Table 2). At ages 1-2, the differences were significantly significant ($p<0.001$ and Table 2).

Discussion

Despite the importance and widespread use of backcalculation in studies fish growth, there remains an abundance of different approaches in common use, considerable confusion surrounding certain methods and little agreement on which method is the best (Francis, 1990; Ricker, 1992). The results of the present study showed that the choice of backcalculation method may have substantial effects on the estimation of the previous lengths of fish. The differences between backcalculated lengths at age 1 accounted for up to 36, 20 and 32% of the lengths of *L. cephalus*, *C. chalcoides* and *P. borysthenicus*, respectively. In older ages the methods gave more consistent results, which was expected, since, for example the lengths backcalculated with BPH and SPH differ most when the difference between the capture length and backcalculated length is greatest (Francis, 1990). This could also be due to low number of samples in the older ages and younger ages.

Francis (1990) described in detail all the proportional methods and attempted to classify them theoretically based on hypotheses implied in their formulation. Francis (1990) concluded that SPH and BPH methods are preferable to others because of their solid theoretical bases and rejected the Fraser-Lee method because of its lack of a clear underlying hypothesis regarding the body-scale relationship.

Ricker (1992) supported the use of Fraser-Lee method, while Pierce *et al*. (1996) found no differences between backcalculated lengths obtained with Fraser-Lee, BPH and SPH methods in pumpkinseeds *Lepomis gibbosus* (L.) and golden shiners *Notemigonus crysoleucas* Mitchill. Pierce *et al*. (1996) concluded that the differences between Fraser-Lee, BPH and SPH are negligible, if calculations are based on precise body-scale relationships. Such relationships can be obtained, if datasets are large, not biased towards certain length-groups and if several scales are measured precisely enough (to the nearest 0.01 mm) from each fish. In practice, however, calculations are often based on more limited datasets. Due to selectivity of sampling methods, body-scale data are typically length-truncated, with smallest and largest fish missing (Smale and Taylor, 1987). Additionally, multiple scale measurements per fish are laborious and the technology necessary for measuring the scales with accuracy suggested by Pierce *et al*. (1996) may not be available. Pierce *et al*. (1996) also suggested that the differences between different methods would also be negligible for other fish species. On the other hand, they stated that the methods give equivalent results, when based on linear body-scale relationships with high r^2 values. Both linear and non-linear equations have been used to describe the relationship between the body length and scale (or some other structures) radius of fish (Mann, 1973; Koutrakis, 2003; Zivkov, 1996). Present result suggested that for scale, cleithra and opercula the relationship was non-linear for both *P. borysthenicus* and *C. chalcoides* while linear for *L. cephalus*. Carlander (1985) mentioned that the observed curvilinearity in the body-scale relationship of fish may often be due to differing body-scale relationships in different year classes. However, when the body-scale plot was constructed, e.g., from the 1989 year class alone, a power function had again the best fit ($r^2 = 0.95$). Body-scale relationships are not species-specific, but may vary between different populations within the same fish species (Hile, 1970).

In this study, BPH method gave results more consistent with the observed lengths for *C. chalcoides* and *P. borysthenicus* when based on body-hard structure relationships that are non-linear with high r^2 values. On the other hand, Fraser-Lee gave most reliable results when based on body-scale relationship that are linear for *L. cephalus*. The results of the present study corroborated the earlier study on the validation of different methods in the backcalculation of the lengths of roach, *Rutilus rutilus* (L., 1758) (Horppila and Nyberg, 1999). They also suggested that BPH method gave the most reliable results. In the present study, cleithra was the most suitable part for the backcalculation. In most previous studies with backcalculation, the lengths of fish were determined from scales and/or opercular bones (L'abee-Lund, 1985; Sinis *et al*., 1999; Gursoy *et al*., 2005). Cleithra have been used rarely in the age determination of fish, but are used commonly in, for example, studies on esocids (Caselman, 1974, 1990). The shape of cleithra makes them more difficult to handle than opercula, which may be the reason for their rarer use. However, cleithra are very useful especially in determining-slow growing cyprinids, the scales of which are often impossible to read.

The most consistent disagreement between observed and backcalculated lengths was age1 and 2 of fish, for which observed lengths were frequently greater than backcalculated lengths. This pattern closely resembles the well known "Lee's phenomenon" (Lee, 1920), which is generally attributed to backcalculation error, differential mortality, biased sampling, or some combination thereof (Ricker, 1969, 1992). Due to several possible explanations for Lee's phenomenon (Bagenal and Tesch, 1978; Duncan, 1980), its presence can not be used as strict criterion for validation of different backcalculation methods (Francis, 1990).

Francis (1990) suggested that since BPH and SPH are equally plausible hypothesis, both methods should be used for each fish population and that the difference between the backcalculated lengths should be taken as a minimum measure of the imprecision of backcalculation. Present result demonstrated that BPH method gives the most reliable results, cleithra giving more accurate results than scales and opercula for *P. borysthenicus* and *C. chalcoides* while Fraser-Lee was the best for *L. cephalus*. It may be concluded that choice of backcalculation method should be taken into account in the growth studies of fish and the validation of the methods should be made separately for each fish species.

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References

Bagenal, T.B. and F.W. Tesch, 1978. Age and Growth. In: Bagenal, T. (Ed.), Methods for Assessment of Fish Production in Fresh Waters. Oxford, Blackwell Scientific Publications, pp: 101-136.

Casselman, J.M., 1974. Analysis of Hard Tissue of Pike *Esox lucius* L. with Special Reference to Age and Growth. In: Bagenal, T. (Ed.), The Ageing of Fish. Farnham Unwin Brothers., pp: 13-27.

Carlander, K.D., 1985. Sampling problems in deriving a body-scale regression for growth calculation from fish scales. Verh. Intl. Ver. Limnol., 22: 2534-2536.

Casselman, J.M., 1990. Growth and relative size of calcified structures of fish. Trans. Am. Fish. Soc., 119: 673-688.

Campana, S.E., 1992. How reliable are growth back-calculation based on otoliths? Can. J. Fish. Aquatic Sci., 47: 2219-2227.

Duncan, K.W., 1980. On the back-calculation of fish lengths; modifications and extensions to the Fraser-Lee equation. J. Fish Biol., 16: 725-730.

Everhart, W., 1950. A critical study of the relation between length and several scale measurements in the smallmouth bass, *Micropterus dolomieu* Lacèpedè. J. Wildlife Manage., 14: 266-276.

Fraser, C., 1916. The growth of the spring salmon. Trans. Am. Fish. Soc., 1915: 29-39.

Francis, R.I.C.C., 1990. Back-calculation of fish length: A critical review. J. Fish Biol., 36: 883-902.

Gursoy, G., A.S. Tarkan, H. Acipinar and O. Gaygusuz, 2005. Validation of the scales for age determination in a cyprinid, *Scardinius erythrophthalmus* (L., 1758). Intl. J. Zoolo. Res., 1: 33-36.

Hile, R., 1970. Body-scale relation and calculation of growth in fishes. Trans. Am. Fish. Soc., 99: 468-474.

Horppila, J. and K. Nyberg, 1999. The validity of different methods in the backcalculation of the lengths of roach-a comparison between scales and cleithra. J. Fish Biol., 54: 489-498.

Klumb, R.A., M.A. Bozek and R.V. Frie, 2001. Validation of three back-calculation models by using multiple oxytetracycline marks formed in the otoliths and scales of bluegill x green sunfish hybrids. Can. J. Fish. Aquatic Sci., 58: 352-364.

Koutrakis, E.T., A.K. Kokkinakis, A.C. Tsikliras and E.A. Eleftheriadis, 2003. Characteristics of the European bitterling *Rhodeus amarus* (Cyprinidae) in the Rihios River. Greece J. Freshwater Ecol., 18: 615-624.

Lee, R.M., 1920. A review of the methods of age and growth determination in fishes by means of scales. Board of Agriculture and Fisheries. Fishery Investigations, Series 2, 4: 32.

L'abee-Lund, J.H., 1985. Age determination and growth in the tench. *Tinca tinca* (L.), in Lake Jorkjenn, South Norway, Fauna Norvegica, Ser. A, 6: 13-17.

Libosvarsky, J., 1956. Growth of the chub and a revision of the two main methods of its determination. Zoologicke Listy, 19: 302-314.

Mann, R.H.K., 1973. Observations on the age, growth, reproduction and food of the roach, *Rutilus rutilus* (L.) in two rivers in southern England. J. Fish Biol., 5: 707-736.

Morita, K. and T. Matsuishi, 2001. A new model of growth back-calculation incorporating age effect based on otoliths. Can. J. Fish. Aquatic Sci., 58: 1805-1811.

Penaz, M., 1962. Critical contribution to the methods of evaluating the body-scale relation of growth in fishes. Zoologickelisty, 11: 77-80.

Pierce, C.L., J.B. Rasmussen and W.C. Leggett, 1996. Back-calculation of fish length from scales: empirical comparison of proportional methods. Trans. Am. Fish. Soc., 125: 889-898.

Ricker, W.E., 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production and yield. J. Fish. Res. Board Canada., 26: 479-541.

Ricker, W.E., 1992. Back-calculation of fish lengths based on proportionality between scale and length increments. Can. J. Fish. Aquatic Sci., 49: 1018-1026.

Summerfelt, R.C. and G.E. Hall, 1987. Age and Growth of Fish. Iowa State University Press. Ames.

Smale, M.A. and W.W. Taylor, 1987. Sources of Back-calculation Error in Estimating Growth of Lake Whitefish. In: Summerfelt, R.C. and G.E. Hall, (Ed.), Age and Growth of Fish. Ames, IA: Iowa State University Press, pp:189-202.

Secor, D.H. and J.M. Dean, 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Can. J. Fish. Aquatic Sci., 49: 1439-1454.

Smedstad, O.M. and J.C. Holm, 1996. Validation of back-calculation formulae for cod otoliths. J. Fish Biol., 49: 973-985.

Sinis, A.I., F.J. Meunier and H. Francillon-Viellot, 1999. Comparison of scales, opercular, bones and vertebrate to determine age and population structure in tench *Tinca tinca* (L. 1758) (Pisces, Teleostei). – Israel J. Zool., 45: 453–465.

Whitney, R.R. and K.D. Carlander, 1956. Interpretation of body-scale regression for computing body length of fish. J. Wildlife Manage., 20: 21-27.

Weatherley, A.H. and H.S. Gill, 1987. The Biology of Fish growth. Academic Press, London.

Zivkov, M., 1996. Critique of proportional hypotheses and methods for back-calculation of fish growth. Environ. Biol. Fishes, 46: 309-320.