

**Validation of back-calculation methods using otoliths  
to determine the length of anchovy kilka  
(*Clupeonella engrauliformis*)**

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**Abstract**

Age structure of the Caspian Sea anchovy kilka, *Clupeonella engrauliformis*, was estimated for the first time by back-calculation methods. Otolith growth and the rate of increment in anchovy kilka were examined to determine whether otoliths could be used to back calculate body sizes at various life stages. Sampling was carried out on commercial fishing vessels board along the Iranian coast in 2007. The age structure of the samples ranged from 2 to 7 years old which was dominated by the third year class (38.6%). The largest fish measured was 137.2mm fork length. The relationship between fork length (FL) and otolith radius (OR) was described by the following equation:  $FL=13.77+ 82.78*OR$  ( $r^2=0.92$ ). Three proportional back-calculation methods, Fraser-Lee, Whitney & Carlander and Dahl-Lea models, were compared by using data sets of anchovy kilka otoliths, and we validated back calculation by comparing them with observed lengths. Back calculated lengths generally corresponded well with observed lengths in anchovy kilka age classes. Variance of the back calculated length data obtained from three models indicated no significant difference ( $P>0.05$ ).

**Keywords:** *Clupeonella engrauliformis*, Otolith, Back-calculation, Age structure, Caspian Sea

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## Introduction

“Kilka” is a common name referring to three species, viz *Clupeonella engrauliformis* Borodin, 1904 (anchovy kilka), *C. grimmi* Kessler, 1877 (bigeye kilka) and *C. cultriventris caspia* Svetovidov, 1941 (common kilka). These fish are widely distributed in the Caspian Sea and are important commercially. They formed more than 80% of total catch in past decade and are a crucial part of the food chain in the Caspian Sea (Mamedov, 2006).

Growth is an important aspect of the biology and the life history of fish, and quantification of growth is frequently a crucial part of fisheries research and management (Summerfelt & Hall, 1987; Weatherly & Gill, 1987). In particular, knowledge of age and growth in the early life stages is fundamental to point out the effects of environmental changes on growth, and can result in an improved understanding of the factors affecting recruitment (Stevensen & Campana, 1992). Today a number of different techniques are used for age determination (Boehlert, 1985; Fletcher & Blight, 1996; Kalish *et al.*, 1996). The most frequently used method is still simple counting of annuli in the otoliths, as described by Jensen (1965) and Powles (1966).

Back-calculation of lengths from otoliths is a widely used approach for estimating the growth of fish populations (Busacker *et al.*, 1990; Francis, 1990; Vigliola *et al.*, 2000). Back-calculation of lengths from otoliths relies on recognition of annual growth markings (annuli) on otoliths to calculate an estimated body

length associated with each annulus. To back-calculate fish growth, it is necessary to know the periodicity of increment formation and to establish the relationship between otolith size and fish size (Campana & Neilson, 1985). The use of otoliths enables to derive a back-calculation formula to estimate the length at certain ages and stages of life for many species of fishes (Roemer & Oliveira, 2007). Use of otoliths to estimate growth in this way can provide the same information as long-term laboratory experiments and tagging studies without the time and expense of rearing or recapturing fish. However, all back-calculation methods incorporate 2 key assumptions: (1) there is a constant rate of deposition of growth marks (e.g., daily or annual) in the structure being used, and (2) there is a constant or predictable between some measurements of the structure (otolith or scale) and body size (Snover *et al.*, 2007).

There have been no published studies on back-calculation methods to demonstrate the relationship between otolith growth and somatic growth of anchovy kilka. The aims of this study were to demonstrate the relationship between somatic and otolith growth in anchovy kilka, and to compare the reliability of the different equations for back calculation.

## Materials and methods

Sampling areas were located in the Iranian coastal waters of southern Caspian Sea. Specimens were caught by commercial

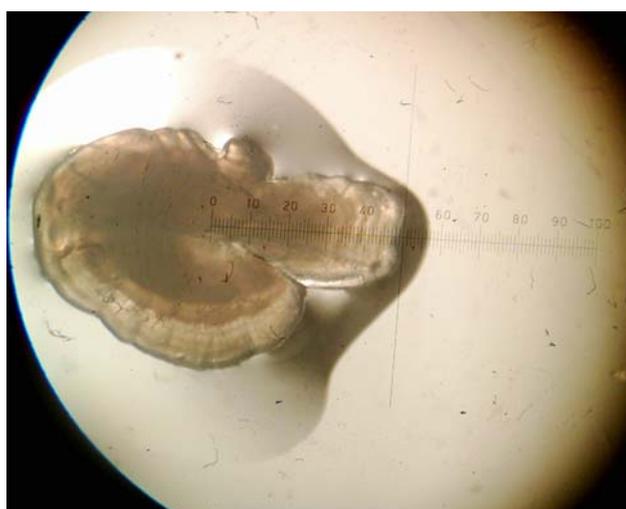
vessel called Val-Fajer equipped with liftnet and under water electric lights. The fish were individually weighed to the nearest 0.01g on an electronic balance and fork length measured to the nearest 1mm, in 2007. The sagitta otoliths were removed and prepared for analysis.

Both pair of sagitta otoliths were prepared according to the technique described by Secor *et al.*, (1992). The whole otoliths were cleaned, dried and immersed in glycerine for 12 hours and observed under a stereo microscope with reflected light against a dark background at 10x magnification.

Growth rate of individual fish was determined by aging and back-calculation of lengths at previous ages from otoliths. Otoliths from 101 fish were viewed without knowledge of age assignments from other structures. Each otolith was read twice by the same reader, first from the centre to the edge and then back from the edge to the

centre following the same growth axis along the longest axis of otolith (Campana,1992), a straight line measurement from nucleus to edge. The following variables were measured on each otolith: 1) the otolith diameter at capture time which corresponds to the maximum length on the anteroposterior axis of the otolith, 2) the otolith radius at capture which corresponds to the distance between the nucleus and the edge along the axis of fastest growth, and 3) the otolith radius at previous ages which corresponds to the distance between the nucleus and the previous ages mark along the axis.

The radius of the *i*th band, distance from the centre of the otolith to the outer margin of the translucent ring, and the radius of the otolith at capture, distance from the centre of the otolith to the periphery, were measured (Fig.1). Measurements were always made along the longest axis of the otolith.



**Figure 1: Sagitta otolith of *Clupeonella engrauliformis* in the Caspian Sea showing the diagram of the variable measured and the reading axis used in reading otolith**

Using the data obtained from the individual body lengths, otolith radii and body lengths at previous ages, a back-calculation was carried out in three primary proportional back-calculation methods, reviewed by Francis (1990) and the impact of using an alternative body length–otolith regression as advocated was investigated (Ricker, 1992). To generate body length–otolith radius relationship, all age groups, excluding 7 years old fish, were used in order to avoid potential error. Since 7 years old fish were relatively few, they were excluded from this calculation. Back-calculated size of each fish at the time of formation of each annulus was determined by substituting the measurement to each annulus into a body proportional equation (Francis, 1990). Back-calculation was applied by using three Back-calculation models, Dahl-Lea (Equation 1), Fraser-Lee (Equation 2) and Whitney & Carlander (Equation 3), as described in Francis (1990) as followings:

$$L_i = L_c * (R_i / R_c) \quad (\text{Equation 1})$$

$$L_i = a + (L_c - a) * (R_i / R_c) \quad (\text{Equation 2})$$

$$L_i = L_c * [(a + bR_i) / (a + bR_c)] \quad (\text{Equation 3})$$

In these equations,  $L_i$  is the fork length (mm) of the fish at the time of annulus “i” formation, and  $L_c$  is the fork length (mm) of the fish at the time of otolith removed.  $R_i$  is otolith radius (mm) from nucleus to the annulus “i”, and  $R_c$  is the total radius (mm) of the otolith. In the equation 2 and 3, the

estimates of intercept were obtained from the linear regression of the otolith radius versus the body length. The otolith radii and fork lengths were fitted to linear.

The back-calculated lengths from different methods were compared with observed lengths for individual fish as the preferred method of validation. One-way ANOVAs were used to test significant differences between back-calculated and observed body lengths.

## Results

Marginal increment analysis demonstrated existence of one annulus, consisting of one opaque zone analysis. It also showed presence of hyaline deposition, which was representative of discontinuous or slow growth; coincide with the spawning season of the anchovy kilka.

While interpreting microstructures in anchovy kilka otoliths, four types of problems were encountered: 1) difficulty in interpreting microstructures in the otolith region that corresponded to first growth stages, especially near the core and first annual ring; 2) difficulty in having to switch the reading axis; 3) difficulty in reading some zones; and 4) difficulty in identifying microstructures near the outer edge of otolith especially in old fish.

The values of the fork length and age of the specimens, were presented in Table 1. Fork lengths and ages ranged from 86.2 to 137.2mm and 2 to 7 years old, respectively.

**Table 1: The fork length and age of specimens of *Clupeonella engrauliformis* in the present study**

	N	Minimum	Maximum	Mean±S.E.
Fork length (mm)	101	86.2	137.2	114.4±1.1
Age (year)	101	2	7	4.19±0.13

The concentric pattern of opaque and translucent rings were visible and readily distinguishable on otoliths, and easily interpreted. Age 3 was the largest age group and represented 38.6% of the samples (Table 2).

Radial measurements along the axis of fastest growth in different age groups presented in table 3.

The relationship between fish length and otolith length was determined by establishing the regression of otolith radius and the fork length at capture time (Fig. 2). There were

statistically significant relationships between the values for back-calculated and observed lengths ( $P < 0.001$ ) (Table 4).

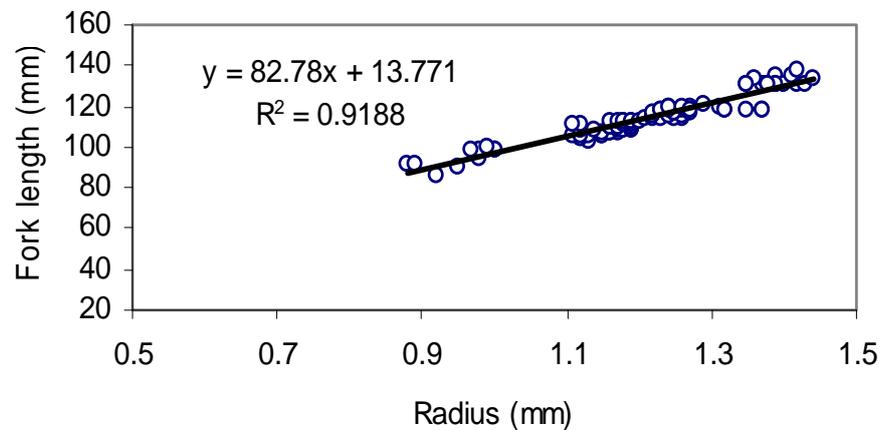
Mean body lengths estimated by the three back-calculation methods showing no significant difference between the estimates and the observed lengths of *Clupeonella engrauliformis* (Table. 4). The closest estimate of the measured  $L_2$  (length at age 2) came from the Dahl–Lea equation, showed no significant difference ( $P = 0.89$ ) between the estimates and the initial lengths of the *C. engrauliformis* (Table 4).

**Table 2: Lengths-at-age in various age groups in the present study**

Age	2	3	4	5	6	7
Number	5	39	15	16	25	1
Minimum (mm)	86.2	98.0	112.0	117.1	118.0	-----
Maximum (mm)	94.1	112.0	116.0	120.4	135.0	-----
Mean (mm)	90.7	106.6	113.8	118.6	128.2	137.2
Standard error	1.27	0.64	0.29	0.24	1.18	-----
% of total (N)	5	38.6	14.9	15.8	24.8	1

**Table 3: Initial radius otoliths in *Clupeonella engrauliformis* in the present study**

Age	1	2	3	4	5	6	7
Number	101	101	96	60	42	26	1
Minimum (mm)	0.60	0.76	0.85	1.24	1.31	1.36	1.60
Maximum (mm)	0.88	1.15	1.32	1.40	1.60	1.63	1.60
Mean (mm)	0.74	1.03	1.21	1.32	1.41	1.48	1.60
Standard error	0.08	0.10	0.10	0.04	0.08	0.08	-----



**Figure 2:** Relationship between fork length and otolith radius observed for *Clupeonella engrauliformis* in the Caspian Sea.

**Table 4:** Comparison of observed lengths and back calculated lengths, summary of variables and ANOVA for testing the results from different models for *Clupeonella engrauliformis*

Age	Observed	Fraser-Lee			Dahl-Lea			Whitney & Carlander		
	L	L	S.E.	sig.	L	S.E.	sig.	L	S.E.	sig.
1	-----	74.2	-----	-----	68.8	-----	-----	74.2	-----	-----
2	90.7	93.1	3.02	0.42	90.3	3.02	0.89	93.1	3.02	0.42
3	106.6	106.3	0.83	0.75	105.1	0.83	0.08	106.3	0.83	0.76
4	113.8	113.4	0.97	0.69	112.4	0.97	0.15	113.4	0.97	0.69
5	118.6	120.3	1.22	0.17	119.7	1.22	0.35	120.3	1.22	0.17
6	128.2	127.2	1.62	0.54	127.0	1.62	0.48	127.2	1.62	0.54
7	137.2	-----	-----	-----	-----	-----	-----	-----	-----	-----

L = Mean fork length (mm), S.E = Standard error, sig. = Significant level

For an individual fish, differences among back-calculated body lengths by the three methods for a given age typically varied by 3mm or less. Back-calculated body lengths corresponded well with observed body lengths in most cases (Table 4). Observed lengths averaged either slightly higher than back-calculated body lengths (except for 2 and 5 age classes).

## Discussion

Fisheries scientists use measures of growth, mortality, and age structure to describe fish populations and evaluate management actions. Accurate age data are required to determine these statistics (Schramm & Doerzbacher, 1982). Aging in fishes is complicated due to the phenomenon of stacking of growth rings towards the otolith margin, particularly in older fish. Although age determination in many clupeid species is difficult, in the case of

the *C. engrauliformis* the translucency of the otoliths allows age determination with relative ease. The annuli formation pattern of the anchovy kilka otoliths closely resembled those observed in other teleosts, with hyaline zones alternated by opaque zones laid down around an opaque nucleus, whose thickness progressively decreased towards the otolith margin.

Evidence of the annual basis of ring formation is an integral component of any age and growth study using calcareous structures such as otoliths to determine age. The formation of alternating translucent and opaque rings of the otoliths has been associated with a variety of factors including seasonal variations in water temperature, feeding and reproduction (Manickchand Heileman & Phillip, 2000). While the mechanisms of growth increment formation are poorly understood, the deposition of the opaque zone in temperate species generally occurs during the summer in association with periods of accelerated growth, whereas the translucent zone is formed when there is reduced metabolic activity (Beckman & Wilson, 1995). Fowler and Doherty (1992) pointed out that the physiology of otolith formation is independent of the other somatic and reproductive processes taking place within the fish; it is an independent physiological response to environmental variations. Difficulties in identifying the otolith first annual ring and restrictions in the applications of the model progression analysis for the continuous spawning species (Morales-Nin & Aldebert, 1997;

Morales-Nin *et al.*, 1998) make the analysis of otolith increments the most accurate growth estimations in the early phases of life.

Back calculated lengths at age were in close agreement with the lengths estimated with otolith readings. The results obtained with the back calculation method were very satisfactory in a sense that they showed the consistency in the interpretation of the sequence of growth increments. That was mainly due to the regular pattern of ring formation which allowed the otoliths be used for age determination as well as the close correlation between the fish length and otolith size which was valid enough to permit the use of measurements to previously formed marks to back calculated the growth history (Francis, 1990).

The Dahl-Lea equation provided the closest estimate of the previous length of the anchovy kilka. However, because otolith formation occurred during the early egg stage (Hare & Cowen, 1994), it would be problematic to get an accurate mean length at time of formation. The oldest fish found in this study was 7 years old. The distribution of ages among our sample was certainly not reflective of the age distribution of the anchovy kilka population in the Caspian Sea. Ages 0-1 specimens have been largely unavailable to our sampling effort owing to minimum size limits applied to the fishery. So, the low proportion of young age classes in our sampling was due to logic regulation applied to fisheries management. Mean lengths at ages 2, 3, 4, 5 and 6 were 90.7,

106.6, 113.8, 118.6 and 128.2mm, respectively. Growth rates were relatively rapid for the first 2 years of life (growth increments 15.9 and 7.2mm), then slowed considerably through ages 4-5 (4.8mm) increments. These mean length values reported by Burani *et al.*, (2008), 77.7, 95.3, 99.3, 102.9, 106.8 and 110.3 for 1 to 6 years old, respectively. Also, according to Fazli *et al.*, (2007), mean length of anchovy kilka during the years 2001-2005, was 64.8, 85.4, 93.1, 105.7, 113.9, 121.5 and 128.9, respectively for 1 to 7 years old.

As shown in our study, underestimation of back calculated fish length by Dahl–Lea model corresponded more to otolith growth rates compared with fish growth rates. These aspects need to discuss in light of the relationship between the overestimation of fish length, and the evidence of uncoupling between fish and otolith growth rates (Mosegaard *et al.*, 1988; Reznick *et al.*, 1989; Secor & Dean, 1989). In conclusion, the model developed by Whitney and Carlander represented a valid model for studies in the field because it considers individual variability in the relationship of fish length to otolith length but further work is needed to validate the use of other Back-calculation models.

Bradford and Geen (1987) advised caution while back calculating fish length because otolith growth seems to be more conservative than fish growth. Otolith growth rates followed fish growth rates within a certain range (Panfili & Tomas, 2001). When fish growth decreased below a certain limit, the otolith continued to growth (Panfili & Tomas, 2001). This

finding confirmed that the rate of growth in otoliths is conservative compared with the rate of somatic growth.

This study was the first attempt and unique at estimating previous length by back calculation method in the *C.engrauliformis*. We believe our results shed light upon two important questions regarding back calculation. The first is "Does back calculation estimate growth history accurately?" Our comparisons of back calculated body lengths with observed body lengths addressed this question. Secondly, "which back calculation method is the best?" For the proportional methods that we evaluated, our comparisons tested for differences among back calculation methods and for correspondence with observed body lengths. Previous synthetic reviews of back calculation methods (Francis, 1990; Ricker, 1992) focused largely on theoretical analyses of various methods. Strengths and weaknesses inferred on theoretical grounds were then illustrated with data sets exhibiting much more variability. Our results suggested that the Fraser – Lee, Dahl - Lea and Whitney & Carlander methods all gave equivalent results when based on body length – otolith relationships that are linear. Although our back calculated body lengths generally corresponded well with observed body lengths, with few exceptions.

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