A semi-automated method for daily age estimation in larval populations by discriminant function models

J.M. Quintanilla*, L.F. Quintanilla, A. García

Instituto Español de Oceanografía – IEO, Apdo. 285, 29640 Fuengirola, Málaga, Spain

ARTICLE INFO

ABSTRACT

Discriminant analysis including the best age-correlated variables selected by SMLR's to construct a predictive model based on somatic and otolith biometry was applied. Age of anchovy larvae ranging from 10 to 12 mm collected along Western Mediterranean coasts during MEDIAS209 survey were estimated by otolith microstructure analysis and compared with the number of daily increments estimated by the model. The model compound by $Perimeter^2 + Area^2$ was able to estimate correctly the age of the otoliths in 75% of the cases assuming ± 1 day of error increasing to 90% assuming ± 2 days of error with mean values of APE (3.33%) and CV (4.71%) systematically low. The results indicate the precision of the increment estimates. Moreover, no differences between the estimated ages from direct readings and those estimated by the model were observed. The proposed method implies a reduction in the subjectivity factor and the cost/benefit ratio for ageing studies in fish larvae.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Survival at early life stages of fish is crucial for future recruitment success (May, 1974) in which larval growth has a determinant role. Thus, age determination in the early life stages of fish is amongst the most important biological variables because it is essential to estimate larval growth rates and mortality (Campana, 2001). Small variations in growth at early life stages affect larval mortality rates (Houde, 1987). Greater larval mortality may be due to greater prolongation of time spans within vulnerable larval sizes and greater ontogenic stage duration (Chambers and Leggett, 1987; Folkvord and Hunter, 1986; Hare and Cowen, 1995). Low growth rates may be the cause of greater mortalities recorded in field studies (Campana, 1996; Hovenkamp, 1992; Miller et al., 1988; Rice et al., 1993; Wilson and Meekan, 2002). Thus, greater survival probability can occur with faster growth rates by decreasing inanition and predation exposure times as postulated by the growth–mortality hypothesis (Anderson, 1988). In conclusion, it may be assumed that there is a strong relationship between larval growth and recruitment success (Bergenius et al., 2002; García et al., 2003).

Age estimation by otoliths is paramount to fisheries science. Ages of around a million fish are estimated yearly by the interpretation of otoliths (Campana and Thorrold, 2001). Otoliths are the most reliable bony part in fish to estimate age, and particularly in larvae by the analysis of their microstructure (Campana, 1999; Campana and Neilson, 1985; Secor et al., 1995). This implies a meticulous process of extraction and mounting of otoliths. The complexity of the process consumes time and the interpretation of daily increments requires expert qualifications (Megalofonou, 2006). As a result, age determination in larval fish implies a high cost/benefit analysis per otolith (Bedford, 1983; Cardinale and Arrhenius, 2004; Francis and Campana, 2004).

One of the most common sources of error relies on the subjective criteria that an age reader may have. Subjectivity, together with the differences in preparation process of otoliths and the variability of the interpretation of the periodic changes shown in calcified structures are among the major sources of between different age readers' age estimates (Boehlert, 1985; Campana and Moksness, 1991; Cardinale and Arrhenius, 2004). However, otolith measurements show better correlations with age than the somatic measurement variables (Boehlert, 1985). The inclusion of otolith morphometry in age determination can produce more objective and precise age estimates (Doering-Arjes et al., 2008). Although different authors have proposed models for estimating age from otolith morphometry (Fletcher, 1995; Pawson, 1990; Stuart and Ord, 1991; Worthington et al., 1995), the main objection relied on the low proportion of correct age estimates (Francis and Campana, 2004).

Discriminant function analysis allows classifying individuals of unknown origin into groups by using discriminant functions

---

* Corresponding author. Tel.: +34 952 19 87 53; fax: +34 952 463808.
E-mail addresses: jose.quintanilla@ma.ieo.es (J.M. Quintanilla), agarcia@ma.ieo.es (A. García).

generated from a database of information of individuals of known origin (McGarigal et al., 2000). It was first used by Fletcher and Blight (1996) to determine age on the basis of somatic and otolith biometric measurements.

In this study, a database has been assembled to store somatic and biometric information of larvae of determined ages estimated by an expert reader (training sample) which was the source for acquiring discriminant functions that assigned ages to larvae of unknown age (test sample) (Francis and Campana, 2004). This technique allowed us to easily include several variables to attain the discriminant functions, which undoubtedly ameliorated the quality of age estimates (Brander, 1974).

The specific objectives of this study are: (i) to determine which are the best age-correlated variables in anchovy larvae; (ii) to construct a predictive model that allows estimating age with a high percentage of correct assignments based on the somatic and otolith biometry of larvae bringing about an important reduction of subjectivity and increasing the repeatability of age readings.

2. Materials and methods

The anchovy larvae used for this study were collected on board the R/V Cornide de Saavedra during May 24 to 26 June, 2009 in the yearly MEDIAS (Mediterranean Acoustic Survey). Oblique plankton tows were undertaken with Bongo 60 and Bongo 90 ichthyoplankton sampling frames. The area covered by the survey encompassed the whole NW Spanish Mediterranean and part of SW Mediterranean (Alborán Sea).

Anchovy larvae were sorted on board and conserved in liquid nitrogen. The use of different plankton gear towed at different speeds for the Bongo 60 and Bongo 90 nets (2 and 3.5 knots, respectively) and different mesh size (200 and 1000 μm, respectively) assured a wide larval size range.

In the laboratory, the larvae were defrosted at ambient temperature. Standard length (SL) of each larva was measured by means of a calibrated image using the freeware software ImageJ 1.44a (USA National Institute of Health). From each sampling area, a subsample of larval size ranges from 10 to 20 mm was taken. The NW and SW Mediterranean accounted for a total of 91 and 76 anchovy larvae, respectively. In the Catalonian coasts, the selected larvae originate from different sampling stations to avoid age estimation bias of environmental nature. After measuring SL, larvae were freeze-dried for 24 h for posterior weighing with a precision balance (mg).

Shortly afterwards, the larvae were put on a slide where these were rehydrated with distilled water. Otoliths were extracted by means of fine tungsten needles while viewed with an estereoscopic binocular (Nikon SMZ 1500). All impurities originating from extraction were cleaned to obtain a clear vision of the otolith. When the distilled water dried, the otoliths were fixed under a cover of nail lacquer.

The radius, increment widths and increment counts were determined from the analysis of the microstructures observed at 1000× magnification using the Nikon software ACT-U2. Furthermore, a calibrated image of each otolith was taken using the Image-Pro Plus 6.2.0424 software (Media Cybernetics, Inc.) from which all the following otolith biometry was estimated: Area (μm^2), Perimeter (μm), SizeL (μm) (Feret diameter through the major axis of the otolith) and SizeW (μm) (Feret diameter through the minor axis of the otolith).

Larvae from NW Mediterranean (NWM) were then divided into two groups with the sole condition of maintaining in both groups the size distribution of the original population:

NW Mediterranean Training Sample (NWMTR)

$\times$ ($n_{\text{LARVAE}} = 50$; $n_{\text{OTOLITHS}} = 75$)

NW Mediterranean Test Sample (NWMTS)

($n_{\text{LARVAE}} = 41$; $n_{\text{OTOLITHS}} = 71$)

The group NWMTR was used to calculate the discriminant function that predicts otolith increment counts. On the other hand, NWMTS was used to test the model's reliability by analyzing the differences between the predicted increment counts of the model with the increment counts from direct otolith readings.

The SW Mediterranean population (SWM, $n_{\text{LARVAE}} = 76$; $n_{\text{OTOLITHS}} = 130$) was used to examine the applicability of our model to other populations and the influence of the training sample size in the goodness of our results.

Prior to calculating the discriminant functions, the somatic and biometric variables of the otoliths were analyzed to find which variables correlate best with the larval increment counts. To this purpose, a series of stepwise multiple linear regression (SMLR's) was applied after collinearity of the variables included were tested.

The SMLR's were applied to the full model that included all the variables (Size, Weight, Radius, Mean Increment Width, Otolith Area, Otolith Perimeter, SizeL and SizeW) and to a filtered model (Size, Weight, Otolith Area, Otolith Perimeter, SizeL and SizeW) in both directions (forward and backward). The application of the full model allows verifying the goodness of fit of the model predicting increment counts (age). On the other hand, the filtered model provides information on the true predictive capacity of the model to predict increment counts. Although the collinearity assumptions were accounted for to analyze the best increment counts determinations, the SMLR's were repeated for both models with (i) larval weight and (ii) with all the independent variables log linearized (Table 1).

Discriminant functions with the selected variables from the SMLR's, for the different models obtained were calculated. In addition, in the case of the filtered model, selected variables were squared to maximize differences between them in order to improve the assignment capacity by discriminant functions and to obtain better determinations of increment counts.

To determine the model's precision estimating increment counts, the proportion of correct increment counts assignments, the average percentage error (APE), the coefficients of variation means (CV) and the mean error in increment counts were calculated.

All statistical analyses have been done using the Statistica 7.1 Statsoft software package at the significance level $p < 0.05$.

3. Results

Four models of high explicative capacity were obtained from the application of SMLR's to the full model ($R^2 > 96\%$; $p < 0.01$) (Table 1). The range of coincident reader estimated increment counts varied from 38 to 54% and increases to 94% (even up to 99%) allowing an error estimate of ± 1 increment. Less than 1% of the larvae are estimated with an error of ± 2 increment counts, showing APE's lower than al 2.1%, with error means less than 1 increment count and mean CV's less than 2.9% (Table 2).

With respect to the filtered models, three models were obtained ($R^2 > 80\%$; $p < 0.01$) (Table 1). Discriminant functions were applied which provided a percentage of error free assignments of increment counts over 21%. Assuming an error of ± 1 increment count, the accuracy of assignment increases to a minimum of 62% and over 79% for ± 2 days, registering APE's less than 4.1%, mean increment counts around 1.5 days, and mean CV's less than 5.8%. Nonetheless, less than 6% of the larvae were estimated with an error ± 3 increment counts (Table 2).

Table 1
Results from SMLR's analysis for the full and filtered model with explained variance (R^2), significance (** indicates $p < 0.01$) and the variables selected to be included in DDFF analysis.

Variables included in SMLR's	Model	RLM	R^2	p	Variables selected by SMLR's
SL, Dry Weight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL	Full	Forward	97%	**	Mean Inc-W, Radius, Area
		Backward	96%	**	Mean Inc-W, Radius, Area and SizeW
SL, Log Dry Weight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL	Full	Forward	97%	**	Mean Inc-W, Radius, Area and Perimeter
		Backward	96%	**	Mean Inc-W, Radius, Area and SizeW
Log (SL, Dry Weight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL)	Full	Forward	97%	**	Log Mean, Mean Inc-W and Log Radius
		Backward	97%	**	Log Mean, Mean Inc-W and Log Radius
SL, Dry Weight, Area, Perimeter, SizeW and SizeL	Filtered	Forward	81%	**	Perimeter and Area
		Backward	80%	**	Perimeter
SL, Log Dry Weight, Area, Perimeter, SizeW and SizeL	Filtered	Forward	81%	**	Perimeter and Area
		Backward	80%	**	Perimeter
Log (SL, Log Dry Weight, Area, Perimeter, SizeW and SizeL)	Filtered	Forward	81%	**	Log Perimeter
		Backward	81%	**	Log Perimeter
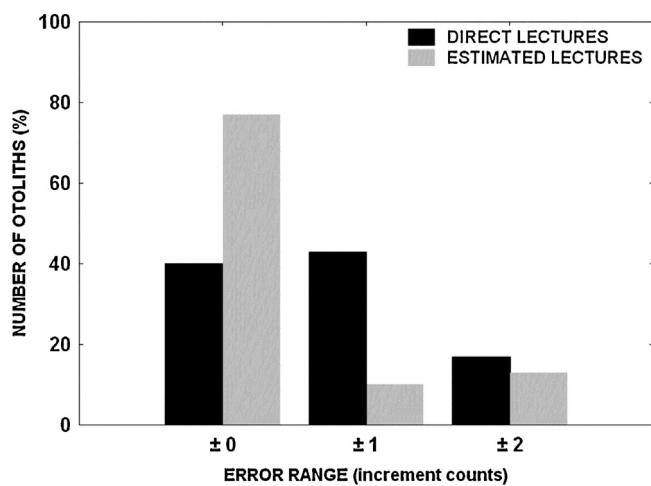
Fig. 1. Comparison between direct (black bars) and estimated by the model Perimeter^2 + Area^2 (grey bars) readings for both otoliths of the same larva (%).

Based on the percentage of correct determinations and its dispersion estimates, we can infer that the best predictive model is that based on otolith Perimeter^2 + Area^2. Fig. 1 shows the comparative increment counts' estimate between the direct age estimate readings and the model estimates for both otoliths of the same individual. The estimates of the model show more precise results than the direct increment counts' readings.

The results have been integrated by individual larvae (Table 3), since two otoliths from the same larvae do not necessarily produce the same increment counts. In this case, the results of the discriminant functions may be presented by either considering otoliths or by larvae (Table 3), ameliorating the results of the model (as shown by APE's and CV's), despite the fact that the test sample database decreased.

In the ANOVA paired comparison between the direct readings and the estimated ones, no significant differences were observed (Table 4). Likewise, no significant differences were observed by ANCOVA in the relationship between increment counts (age) and the somatic variables (Table 4).

Lastly, a test to analyze the model's applicability on other anchovy larval populations, the discriminant functions obtained from the Perimeter^2 + Area^2 with the larval populations sampled in the coasts of Málaga (SWM) were used to compare the direct increment counts's readings with the model's output. Moreover, this test served to estimate the importance of the sample size from which discriminant functions are estimated (training sample) and compare the percentages of increment count assignments from the functions calculated based on NWMTR ($n = 71$) and NWM ($n = 143$) of the Malaga larval data base. In both cases, the comparison of results from the estimated increment counts of our model with the observed estimates of the SWM have shown similar percentages of increment counts assignments and a slight increase in the values of dispersion values (Table 3).

4. Discussion

Since the seventies with the development of computer software developments, semi-automatic methods were designed to estimate the age from otoliths (Manson, 1974). The objective was two-fold: on one hand, to reduce time-consuming tasks in the interpretation of otoliths and on the other part, reduce the subjectivity from age reader's interpretation (Francis and Campana, 2004).

The viability of the system proposed by this study is examined under three different points of view, that is, the percentage of correct increment count assignments within the tolerable error bounds, the degree of precision provided by the dispersion parameters (APE, mean CV and its error in terms of increment counts), and lastly, a cost/benefit analysis comparing the traditional system of

Table 2
Results from DDFF analysis for the full and filtered model with % assignment for each error range; average percent error (%) and coefficient of variation (%).

Variables included in discriminant analysis	Model	% Assignment					APE mean	% CV mean
		±0	±1	±2	±3	>±3		
Mean Inc-W + Radius + Area	Full	48	96	100	100	100	1.68	2.38
Mean Inc-W + Radius + Area + SizeW	Full	54	97	100	100	100	1.46	2.07
Mean Inc-W + Radius + Area + Perimeter	Full	51	99	100	100	100	1.52	2.14
Log Radius + Log Mean Inc-W	Full	38	94	99	99	100	2.03	2.88
Perimeter + Area	Filtered	23	63	83	96	100	3.92	5.54
Perimeter	Filtered	23	66	83	99	100	3.66	5.18
Log Perimeter	Filtered	25	65	87	96	100	3.65	5.16
Perimeter^2 + Area^2	Filtered squared	21	75	90	99	100	3.33	4.71
Perimeter^2	Filtered squared	24	62	79	94	100	4.10	5.79
Log Perimeter^2	Filtered squared	25	63	87	96	100	3.70	5.24

Table 3

Results from DDFF for Perimeter^2 + Area^2 model with % assignment for each error range; average percent error (%) and coefficient of variation (%) taking into account the three scopes of applicability considered for otoliths and larvae.

Application	n training sample	n test sample	% Assignment					APE mean	% CV mean
			±0	±1	±2	±3	>±3		
OTOLITHS (NWMTR in NWMTS)	71	71	21	75	90	99	100	3.33	4.71
LARVAE (NWMTR in NWMTS)	71	41	22	76	90	98	100	3.28	4.64
OTOLITHS (NWMTR in SWM)	71	130	25	67	92	96	100	3.72	5.26
LARVAE (NWMTR in SWM)	71	76	22	76	90	98	100	3.25	4.59
OTOLITHS (NWM in SWM)	143	130	22	68	92	95	100	3.86	5.45
LARVAE (NWM in SWM)	143	76	22	71	93	97	100	3.64	5.15

age readings with the modelling method of estimating increment counts from discriminant functions.

When applying SMLR to the full model, we obtained four models (Table 1) from which discriminant functions were generated. These yielded high percentages of assignments (99% otoliths with an error of ±1 increment count) (Table 2). These results confirm the capability of the proposed method in the estimation of age. However, the full model cannot provide true age-predictive values because it includes variables as Radius and Increment Width that cannot be used to predict age because these variables originate from direct otolith readings. But examining the high percentages of increment count assignments from the full models, we could use them to correct increment counts a posteriori; by for instance, identifying cases which showed strong deviations of observed increment counts from the model estimated increment counts.

The filtered models eliminate Radius and Increment Width from the analysis which accounts for significant decrease in the explicative power of the model, although it may be considered relatively high from the statistical viewpoint ($R^2 > 80\%$). But on the other hand, the removal of these variables increases notably the model's applicability because it exclusively feeds from the biometric measurements that we can get from a calibrated image of each otolith. Thus, this system provides a true predictive capacity because it does not provide any variable originating from the traditional method of direct increment counts' readings.

The filtered model provided three models (Table 1) which in turn provided discriminant functions that generated percentages of assignments over 62% allowing for an error of ±1 increment, and over 79% for errors of ±2 increments. Notwithstanding, no cases are observed where more than 6% of the larvae are estimated with errors ±3 increments (Table 2).

It is important to highlight the fact that the somatic variables were not selected by SMLR's to be included in none of the models of the discriminant function analysis (Table 1), thus corroborating that biometric variables of the otoliths are more correlated with age (Boehlert, 1985; Doering-Arjes et al., 2008). The inherent great variability larval weight or size implies their less ageing predictability.

Precision is defined as the degree of repeatability in the successive measures of a determined structure (Sokal and Rohlf, 1979). It cannot be used as a measure of accuracy which refers to the true value of an estimate. However, the value of precision relies on the repetitive nature of assigning an age to an individual (Campana, 1995; Campana et al., 1990; Campana and Moksness, 1991).

APE and CV are the most utilized precision estimates in the comparison of age readings. There is no pre-established precision value applicable to growth studies. Besides the proper expert reader, the processes involving age estimation are greatly influenced by the species being analyzed and the nature of the structure being used for the estimates. Many studies provide CV's less than 6–7% to an APE of 5.5% (Campana, 2001) while other authors indicate CV's

ranging from 5 to 15% (Kimura and Lyons, 1991) acceptable for growth studies.

In the comparison between the increment counts from direct readings with those obtained from the discriminant functions, the results of all the models implemented presented similar APE's and CV's and in many instances, lower than in our bibliographic consultations (Table 2). Discrepancies between different readers in the increment count estimations may account for even greater CV's, as shown by the results of readings of larval otoliths of anchovy from the same site of this work, which provided a mean CV of 14% among nine readers (ICES, 2013). Such results bear witness to the reliability of the model proposed in this study.

The interpretation of age during the larval phases of development implies an inherent subjectivity on behalf of the reader's interpretation. In addition to the meticulous process of otolith extraction and mounting, the expert reader is faced with his own subjectivity in the distinction of increments. Taking into account that anchovy larvae do not show clear-cut increment bands and that the estimated increment range of this study is rather ample for larvae (14), an error of ±2 increment counts may be considered acceptable since these differences may even occur from direct readings of the otoliths of the same individual.

Among the models with a valid predictive capacity drawn from the filtered model, Perimeter^2 + Area^2 provided the best age estimates, predicting increment counts in 3 out of 4 otoliths considering ±1 increment count error that increases to 9 out of 10 considering ±2 increment count error. Assuming an error of ±2 increment counts, the values of APE (3.33%), mean error of increment counts (1.39 increments) and CV's (4.71%) are considerably low (Table 3; Fig. 2).
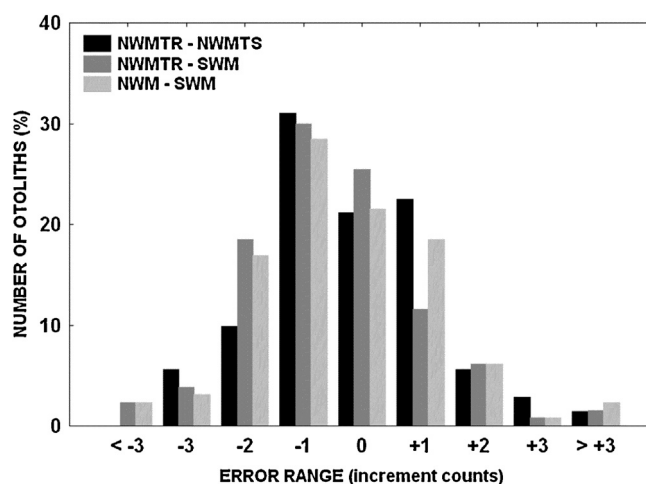
Fig. 2. Differences between increment counts obtained by direct readings and estimated for each otolith in the three scopes of applicability considered (NWMTR in NMWTS with black bars; NWMTR in SWO with dark grey bars and NWM in SWO with light grey bars).

Table 4

Results for analysis comparing direct and estimated readings (ANOVA) and their relation with somatic variables (ANCOVA) taking into account the three scopes of applicability considered for otoliths and larvae.

| | NWMTR in MWMTS | | | | NWMTR in SWO | | | | NWM in SWM | | | |
| | OTOLITH | | LARVAE | | OTOLITH | | LARVAE | | OTOLITH | | LARVAE | |
	F	p	F	p	F	p	F	p	F	p	F	p
ANOVA Increment counts	$F_{(1,140)}=0.11$	NS	$F_{(1,80)}=0.00$	NS	$F_{(1,258)}=3.24$	NS	$F_{(1,150)}=0.89$	NS	$F_{(1,258)}=1.49$	NS	$F_{(1,150)}=0.31$	NS
ANCOVA Length vs. Daily Increments	$F_{(1,139)}=0.39$	NS	$F_{(1,79)}=0.00$	NS	$F_{(1,257)}=1.21$	NS	$F_{(1,149)}=1.63$	NS	$F_{(1,257)}=0.56$	NS	$F_{(1,149)}=0.52$	NS
ANCOVA Weight vs. Daily Increments	$F_{(1,139)}=0.22$	NS	$F_{(1,79)}=0.00$	NS	$F_{(1,257)}=1.20$	NS	$F_{(1,149)}=1.83$	NS	$F_{(1,257)}=0.58$	NS	$F_{(1,149)}=0.58$	NS

Several studies advise on the precautions that one must take into account when applying the results of the functions obtained from a population to other populations (Anderson et al., 1992; Pilling et al., 2003; Worthington et al., 1995). Nonetheless, the results presented in the study show that applying discriminant functions obtained from a NW Mediterranean larval anchovy populations to SWM larvae, the results in the percentages of assignments and precision parameter's estimate remained similar (both for otoliths and for the individual larvae) to the ones obtained from the discriminant functions of larvae of their own population (Table 3; Fig. 2). This result would seem unexpected, bearing in mind that that there are significant larval growth differences between these larval populations (ANCOVA size vs increment counts, $F(164,1) = 5.649$; $p < 0.01$). Thus, the results corroborate the fact that otoliths preserve a better temporal register of ageing (ontogenic development) than do somatic variables.

Another important matter in the modelling application is determining the adequate sample size for calculating the discriminant functions that influence the goodness of fit of increment counts assignments. The results of this analysis have shown that the quality of the estimates are independent of the number of otoliths included in the training sample because the percentages of assignments and the estimators show similar results (see Table 3; Fig. 2).

Another important factor to bear in mind is that no significant differences should occur between direct readings and the model estimated ones that could provoke differences in the somatic variables' relationships with daily increments, and thereby, differences in the growth patterns. Our results (Table 4) show that no significant differences were observed between the direct readings of increments and the model estimated increments (ANOVA), maintaining intact the relationships of the somatic variables with daily increments (ANCOVA). No differences between the larval populations were observed.

Despite the promising results by this semi-automated ageing method, its advantages should be shown through cost/benefit of the application in comparison to the traditional method of direct interpretation of age through the analysis of otolith microstructure (Francis and Campana, 2004).

From this point of view, the costs related with the extraction and mounting of otoliths would remain similar. The difference lies on the comparison between the interpretation of increment counts and the image capture and processing of the otoliths that comprise the test sample. The direct readings imply the formation of ageing expertise which involves learning, discussion and intercalibration exercises between readers to define protocols that represent a time-consuming task. On the other hand, the acquisition of biometric measurements of otoliths from calibrated images is nowadays simple to carry out automatic measurements through developed image software.

Thus, the cost in the time consumption and personnel involved in the age reading process is decreased by the application of the proposed model-based ageing procedure. Furthermore, from a reduced number of larvae it is possible to carry out increment counts of great samples sizes providing these have similar larval size distributions. Undoubtedly, the increase of automated otolith readings by the implementation of discriminant functions would certainly save time and effort. It has been shown that automated increment counts by discriminant functions have been as precise or even ameliorated the results obtained from direct increment counts.

Despite the fact that the discriminant functions are calculated from a database constructed with direct readings of increment counts, and thus cannot be considered objective (Francis and Campana, 2004), we must take into account that the method is exclusively based on the biometric measurement of the otoliths which consequently significantly minimizes the subjective factor of counting increments where the main source of errors of

ageing occur (Boehlert and Yoklavich, 1984; Kimura and Lyons, 1991; Richards et al., 1992).

In conclusion, the proposed semi-automated discriminant function based method for estimating age in larvae has the advantage of counting daily increments based on otolith biometry undertaken through image analysis software. The results of the study have shown acceptable levels of error estimate and great precision estimates. The method offers the advantage is that with a relatively low number of larvae, it can be applied to other larval populations (that may even differ in their growth patterns).

Acknowledgements

References

Anderson, J.T., 1988. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J. Northwest Atl. Fish. Sci. 8, 55–66.

Anderson, J.R., Morison, A.K., Ray, D.J., 1992. Age and growth of Murray cod, *Maccullochella peelii* (Perciformes, Percichthyidae), in the lower Murray–Darling Basin, Australia, from thin-sectioned otoliths. Aust. J. Mar. Freshw. Res. 43, 983–1013.

Bedford, B.C., 1983. A method for preparing sections of large numbers of otoliths embedded in black polyester resin. J. Cons. Int. Explor. Mer. 41, 4–12.

Bergenius, M.A.J., Meekan, M.G., Robertson, D.R., McCormick, M.I., 2002. Larval growth predicts the recruitment success of a coral reef fish. Oecologia 131, 521–525.

Boehlert, G.W., 1985. Using objective criteria and multiple regression models for age determination in fishes. Fish. Bull. US 83, 103–117.

Boehlert, G.W., Yoklavich, M.M., 1984. Variability in age estimates in Sebastes as a function of methodology, different readers, and different laboratories. Calif. Fish Game 70, 210–224.

Brander, K., 1974. The effects of age-reading errors on the statistical reliability of marine fishery modelling. In: Bagenal, T.B. (Ed.), The Ageing of Fish. Unwin Brothers Limited, Surrey, England, pp. 181–191.

Campana, S.E., 1995. Expert Age Determination of 4VW and 4X Haddock Otoliths by National and International Laboratories. Department of Fisheries and Oceans Atlantic Fisheries Research Document 95/120.

Campana, S.E., 1996. Year-class strength and growth rate in young Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. 135, 21–26.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188, 263–297.

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59, 197–242.

Campana, S.E., Zwanenburg, K.C.T., Smith, J.N., 1990. $^{210}Pb/^{226}Ra$ determination of longevity in redfish. Can. J. Fish. Aquat. Sci. 47, 163–165.

Campana, S.E., Moksness, E., 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J. Mar. Sci. 48, 303–316.

Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42, 1014–1032.

Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58, 30–38.

Cardinale, M., Arrhenius, F., 2004. Application of the otolith weight–age relationship to estimate the age-structure of haddock (*Melanogrammus aeglefinus*). J. Appl. Ichthyol. 20, 470–475.

Chambers, R.C., Leggett, W.C., 1987. Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a view of variation in other species. Can. J. Fish. Aquat. Sci. 44, 1936–1947.

Doering-Arjes, P., Cardinale, M., Moseggard, H., 2008. Estimating population age structure using otolith morphometrics: a test with known-age Atlantic cod (*Gadus morhua*) individuals. Can. J. Fish. Aquat. Sci. 65, 2342–2350.

Folkvord, A., Hunter, J.R., 1986. Size specific vulnerability of northern anchovy (*Engraulis mordax*) larvae to predation by fishes. Fish. Bull. US 84, 859–869.

Francis, R., Campana, S.E., 2004. Inferring age from otolith measurements: a review and a new approach. Can. J. Fish. Aquat. Sci. 61, 1269–1284.

Fletcher, W.J., 1995. Application of the otolith weight–age relationship for the pilchard, *Sardinops sagax neopilchardus*. Can. J. Fish. Aquat. Sci. 52, 657–664.

Fletcher, W.J., Blight, S.J., 1996. Validity of using translucent zones of otoliths to age the pilchard *Sardinops sagax neopilchardus* from Albany, Western Australia. Mar. Freshw. Res. 47, 617–624.

García, A., Cortés, D., Ramírez, T., Giráldez, A., Carpena, A., 2003. Contribution of larval growth rate variability to the recruitment of the Bay of Málaga anchovy (SW Mediterranean) during 2000–2001 spawning seasons. Sci. Mar. 67 (4), 477–490.

Hare, J.A., Cowen, R.K., 1995. Effect of age, growth rate, and ontogeny on the otolith size–fish size relationship in blue-fish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. Can. J. Fish Aquat. Sci. 52, 1909–1922.

Houde, E.D., 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2, 17–29.

Hovenkamp, F., 1992. Growth-dependent mortality of larval plaice *Pleuronectes platessa* in the North Sea. Mar. Ecol. Prog. Ser. 82, 95–101.

ICES, 2013. Report of the Workshop on Microincrements Daily Growth in European Anchovy and Sardine (WKIMIAS). 21–25 October 2013, Mazara del Vallo, Italy, ICES CW pending.

Kimura, D.K., Lyons, J.J., 1991. Between-reader bias and variability in the age-determination process. Fish. Bull. US 89, 53–60.

May, R.C., 1974. Larval mortality in marine fishes and critical period concept. In: Blaxter, J.H.S. (Ed.), The Early Life History of Fish. Springer-Verlag, Berlin, pp. 3–19.

Manson, J.E., 1974. A semi-automatic machine for counting and measuring circuli on fish scales. In: Bagenal, T.B. (Ed.), The Ageing of Fish. Unwin Brothers Limited, Surrey, England, pp. 87–102.

McGarigal, K., Cushman, S., Stafford, S., 2000. Multivariate Statistics for Wildlife and Ecology Research. Springer-Verlag, New York.

Megalofonou, P., 2006. Comparison of otolith growth and morphology with somatic growth and age in young-of-the-year bluefin tuna. J. Fish Biol. 68, 1867–1878.

Miller, T.J., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45, 1657–1670.

Pawson, M.G., 1990. Using otolith weight to age fish. J. Fish Biol. 36, 521–531.

Pilling, G.M., Grandcourt, E.M., Kirkwood, G.P., 2003. The utility of otolith weight as a predictor of age in the emperor *Lethrinus mahsena* and other tropical fish species. Fish. Res. 60, 493–506.

Rice, J.A., Miller, T.J., Rose, K.A., Crowder, L.B., Marschall, E.A., Trebitz, A.S., DeAngelis, D.L., 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. 50, 133–142.

Richards, L.J., Schnute, J.T., Kronlund, A.R., Beamish, R.J., 1992. Statistical models for the analysis of ageing error. Can. J. Fish. Aquat. Sci. 49, 1801–1815.

Secor, D.H., Dean, J.M., Campana, S.E., 1995. Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia.

Sokal, R.R., Rohlf, F.J., 1979. Biometría. Principios y métodos estadísticos en la investigación biológica. H. Blume Ediciones, Madrid.

Stuart, A., Ord, J.K., 1991. Kendall's Advanced Theory of Statistics. Classical Inference and Relationship, vol. 2., 5th ed. Charles Griffin and Company Limited, London.

Wilson, D.T., Meekan, M.G., 2002. Growth-related advantages for survival to the point of replenishment in the coral reef fish *Stegastes partitus* (Pomacentridae). Mar. Ecol. Prog. Ser. 231, 247–260.

Worthington, D.G., Doherty, P.J., Fowler, A.J., 1995. Variation in the relationship between otolith weight and age: implications for the estimation of age of two tropical damselfish (*Pomacentrus moluccensis* and *P. wardi*). Can. J. Fish. Aquat. Sci. 52, 233–242.