Marine and Freshwater Resources Institute

Shape analysis and ageing of orange roughy otoliths from the South Tasman Rise.

Final Report to the Australian Fisheries Management Authority

Simon Robertson and
Sonia Talman

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Shape analysis and ageing of orange roughy otoliths from the South Tasman Rise

Principal Investigator: Simon Robertson

Address: Marine and Freshwater Resources Institute
P.O. Box 114
Queenscliff, Vic 3225
Tel: (03) 5258 0111 Fax: (03) 5258 0270
Email: Simon.Robertson@nre.vic.gov.au

Objectives:

1. To analyse otolith morphology for at least 600 orange roughy from the South Tasman Rise (STR). These samples included ones taken during the 1998 and 2001 fishing periods. An additional sample of otoliths was included from outside the Economic Exclusion Zone (Remote Zone) for the 1998 fishing period. The groups used for the Fourier shape analysis are:
   - the South Tasman Rise during the 2001 fishing period,
   - the South Tasman Rise during the 1998 fishing period and
   - the Remote Zone during the 1998 fishing period.
2. To age 1000 samples from the STR which included samples from the 1998 and 2001 fishing periods.
3. Interpret results and prepare a final report outlining the implications of this study for the stock structure of orange roughy in South Tasman Rise.

Summary:

The morphology of orange roughy (*Hoplostethus atlanticus*) otoliths was analysed and compared between two areas during two fishing periods in an attempt to identify individual stocks within the south-east Australian fishery. Three sets of approximately 300 otoliths were compared using harmonic randomisation tests (South Tasman Rise 2001, South Tasman Rise 1998 inside the EEZ and South Tasman Rise 1998 outside the EEZ). The results obtained using these tests suggest that all location/time period groups of orange roughy otoliths differ from each other. Based on a probability of 0.05, the null hypothesis that the groups are comprised of the same stock is rejected.

A total of 1171 otoliths were prepared for age estimation. From these samples, 1091 age estimates were made with 70 failing due to morphological difficulties. The age-class distributions were similar between the 1998 (outside the EEZ) and 2001 samples. The 1998 (inside the EEZ) show younger individuals than the 2001 and 1998 outside samples. This is also reflected in the length frequency and otolith morphology parameter distributions.

Introduction

An important aspect of fisheries management is the identification of stock structure as effects of recruitment and mortality operate independently upon the individual stocks that make up a species. Along with ageing, measuring and comparing variation in characteristics such as body shape or meristic count is one of the traditional methods for discriminating between stocks. A more recent technique measures phenotypic variation in the shape of the otolith. Otolith shape analysis has been effective in identifying orange roughy stocks, both in Australia and New Zealand, and has also been applied to other commercially important fisheries, including smooth and black oreos in New Zealand. This technique is used in the current project to compare groups of orange roughy from different locations within South Tasman Rise area, collected during two fishing periods and two locations within the STR. It is important to understand the structure of orange roughy stocks in this region as separate quotas are being allocated to the different areas.

Ageing information provides the necessary data for the longterm monitoring of orange roughy stocks and is an integral input into the stock assessment of commercially important species.

Methods

Samples

Samples from the South Tasman Rise in the 2001 fishing period were received from Rudy Kloser and registered with the Central Ageing Facility (CAF) in September 2001. Otoliths were supplied in envelopes together with biological, trip, station and date of capture information. Historical samples from the STR, collected during 1998, were also included in the analysis. Samples were allocated batch numbers of 112 (STR 2001), 62 (STR 1998) and 63 (Remote Zone). Individual samples were allocated a sequential fish number from 1 to *n*, where *n* was the count of the samples in each batch

Shape analysis

Preparation

To prepare samples for shape analysis, a greyscale image of each pair of otoliths was collected using the customised image analysis system developed by the CAF (Morison *et al*. 1998), and saved in Joint Photographic Effect Group file format (JPEG) for subsequent analysis (Figure 1). Images were collected at a magnification of 2.54x (0.63x primary objective, 6.4x magnification and 0.63x secondary objective). All images were written to network drives and backed up on compact disk. The nomenclature of each image was constructed from species code, batch code and CAF fish number (all three digit) giving a unique image name, e.g. 707062001.

Otoliths were weighed to the nearest milligram. Biological data, otolith weight and image details, including name and path were combined in a Microsoft (MS) Excel spreadsheets.

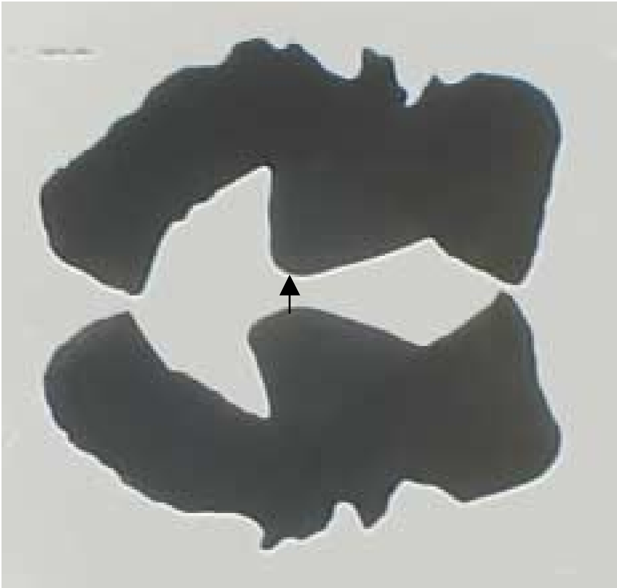

Figure 1. Image of orange roughy otoliths (JPEG format). Automatic tracings started at landmark location marked with an arrow.

Data collection

Shape and meristic analysis of the orange roughy otoliths was undertaken using the shape analysis programs developed by the CAF for the purpose of stock discrimination. Each image was opened in Optimas™ and three automated pixel gradient tracings of the left otolith were performed. The first, second and third trace collected perimeter data, area data and x-y coordinate data for the Fourier series respectively. Circularity was calculated as the perimeter squared over the area of the otolith. The data used to compute the Fast Fourier Transform (FFT) was collected as 128 equidistant points around the outline of the otolith. The FFT was calculated as a Cartesian FFT using the x-y coordinates as a complex number ($a+b_i$). Each of the automated tracings started at an arbitrary landmark point on the otolith, which was the dorsal lobe adjacent to the sulcus (Figure 1). All tracings were counter-clockwise in direction around the silhouette of the otolith.

All data collected from the images were exported to an MS Excel spreadsheet. Other data written to the spreadsheet included species, batch number, fish number and image name. These data were transferred to MS Excel via Dynamic Data Exchange (DDE).

Fourier Analysis

The FFT was calculated in Optimas and the resultant array of 128 complex numbers (Fourier descriptors) saved for later analysis. The 0^{th} descriptor was used to normalize for differences in otolith position in the image and the 1^{st} descriptor to normalize for size and rotation of the otolith. All data normalisation of the FFT was performed in MS Excel. The remaining Fourier descriptors represented the otolith shape independent of its position, size and rotation.

Determination of the appropriate number of Fourier descriptors

The appropriate number of Fourier descriptors for orange roughy otoliths was calculated from a previous stock discrimination project (Smith *et al*. 2001). The technique is described below:

As the number of Fourier descriptors increases, the resultant reconstructed shape converges to the original shape. To determine the number of Fourier descriptors used for later analysis, a range finding procedure was performed. An otolith was randomly selected and the Fourier series calculated. The descriptors were normalized for position by setting the 0^{th} descriptor to $0+0i$. The shape was then reconstructed using the first (and last) descriptor. The number of descriptors was then increased by one and the shape reconstructed. This was repeated until 30 descriptors from each end of the complex FFT were used. The maximum error of 100 percent was defined as the difference between the inverse FFT reconstruction of the otolith outline using the full array of Fourier descriptors, and the reconstruction using only the second and last elements of the original Fourier series. These distances were squared and summed, and expressed as a percentage (percentage reconstruction error). This test determined the relationship between the number of descriptors and the accuracy of the reconstruction.

Based on the results of the range finding test, nine samples from each area were randomly selected and the inverse FFT calculated from the 12^{th} to (and including) the 22^{nd} Fourier descriptor and the percentage error calculated. These samples were used to estimate the number of Fourier descriptors needed for the mean otolith reconstruction to be less than 5% of the original shape.

The relationship between number of Fourier descriptors and percent reconstruction error showed that the complex shape of the orange roughy otolith required twenty two Fourier descriptors to reduce the mean reconstruction error to below five percent. Because the Cartesian Fourier Transform is asymmetric around the middle frequency, both the 2^{nd} to the 22^{nd} descriptors and the 106^{th} to the 127^{th} descriptors from the original Fourier series were used for subsequent harmonic analyses.

Test of differences between groups

The reduced set of twenty two Fourier descriptors from each end of the series was used to compare the shape of otoliths from each of the three location/time-period groups. The absolute value (harmonic) of each of the Fourier descriptors in the shape vector was calculated as :

Harmonic = $(a+b_j)^{0.5}$

Where :

a = real component of complex number

b_j = imaginary component of complex number

The mean harmonic distance between each of the groups was then calculated. The estimator used to test differences between groups was the square root of the sum of the squared differences in the mean harmonics. This was calculated as:

$$DH_{jk} = \sqrt{\sum_{i=2}^{22}(\overline{H}_{ij} - \overline{H}_{ik})^2 + \sum_{i=106}^{127}(\overline{H}_{ij} - \overline{H}_{ik})^2}$$

Where :

$DH_{jk} =$ Observed harmonic distance between group *j* and group *k*

$\overline{H}_{ij} =$ i^{th} mean harmonic from group *j* and $\overline{H}_{ik} = i^{th}$ mean harmonic from group k

The matrix of Fourier descriptors for samples from the two groups being compared was thus reduced to one harmonic distance value. The observed harmonic distance was calculated. This was repeated for each of the group comparisons.

A randomisation test was then applied to estimate the probability that the observed harmonic distance would occur by chance alone. Samples were randomly allocated to groups and a new harmonic distance calculated. This process was repeated 5,000 times and a distribution of values for randomised harmonic distance was obtained. The probability of obtaining the observed harmonic distance by chance was estimated as the proportion of randomisations for which the harmonic distance was greater than or equal to the observed harmonic distance.

Each group was pair-wise tested against each of the groups giving a total of three tests for the three groups.

All programs for conducting randomisation tests were written in Visual Basic for Applications (VBA) in MS Excel.

Morphometric Analysis

The significance of differences between areas within the STR and fishing periods for the morphometric data (otolith area, perimeter and circularity) was tested using analysis of variance (ANOVA) in MS Excel. Where a significant difference occurred, Student Newman - Kuels multiple range tests (SNK) were used to determine area groupings (Zar 1974).

Age estimation

Preparation

To prepare samples for age estimation, one otolith from each pair was sectioned using a three stage process; embedding, sectioning and mounting. To embed the otoliths, a thin layer of clear polyester casting resin was poured on to the base of a silicon mould and left to partially cure. Otoliths were arranged in two rows of five. Resin blocks were labelled and coated with another layer of resin. Blocks were then oven cured at 55°C for 24 hours.

Otolith sections were cut using a Gemmasta™ lapidary saw fitted with a diamond impregnated blade. From each otolith, up to three sections were taken (approximately 350µm in thickness) to ensure the primordium of each otolith was included. All otoliths were longitudinally sectioned. Sections were cleaned using alcohol and stored in vials. For identification, each vial contained a sample identification label.

A small amount of resin was poured on to a glass slide (50 x 75 mm). Otolith sections were immersed in the resin and the identification label placed at the top of the slide. Once the resin had semi-cured, further resin was applied to the preparations and coverslipped. Slides were oven cured at 30°C for 3 hours.

All of the prepared slides were randomised and assigned an index number. This allows each sample was examined without reference to area within the STR or fishing year.

Reading protocol

Sections were viewed with transmitted light and the age estimated by counting the number of completed zones (translucent – opaque sequence). Two counts were were generally made, one from the primordium (biological center of the otolith) to the last major zone, and a second count from the last major zone to the edge. Where the otolith section showed no observable transition between the last major zone, a single count was made. The samples were examined under a compund microscope at magnifications of 40 or 100 x depending on the area of the otolith being examined, or the clarity of the zones. These zone count data were recorded in Microsoft Excel, along with comments and index number.

Other information recorded in the Excel file was a readability score. This is a subjective measure of the sample's readability (Table 1). To avoid potential bias, all counts were made without knowledge of fish size or otolith weight.

Table 1. Interpretation of readability scores.

Score	Interpretation
1	Sample has excellent readability
2	Sample is clear, but may differ by less than one percent of the initial age estimate
3	Sample may differ by up to five percent of the orginal estimate
4	Sample is subject to multiple interpretations
5	Sample is unreadable

Comparison of age estimates

Repeated readings of the same otoliths provide a measure of intra-reader and inter-reader variability. They do not validate the assigned ages but provide an indication of the size of the error to be expected with a set of age estimates, due to variation in otolith interpretation. Beamish and Fournier (1987) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right]$$

Where N is the number of fish aged, R is the number of times fish are aged, X_{ij} is the ith determination for the jth fish, and X_j is the average estimated age of the jth fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson *et al.* 1992).

To establish confidence intervals to these estimates of precision, a bootstrap technique was employed on the individual error estimates following methods described by Efron and Tibshirani (1993). Five thousand samples of error estimates (each the same size as the original) were randomly taken with replacement from the original and repeat reading pairs, and a new IAPE calculated for each. The mean of these replicate IAPE's is the mean bootstrap IAPE and the standard deviation is the standard error of the mean. The bootstrap procedure exaggerates any bias present in the original estimate, so it is necessary to correct for this by adding the difference between the original statistic and the bootstrap mean, to the original estimate. The bias-corrected bootstrapped IAPE is calculated thus:

Bias-corrected IAPE = Original IAPE + (Original IAPE- Mean Bootstrap IAPE)

The 95% confidence interval was calculated as:

95 % C.I. = Bias-corrected IAPE ± (1.96 Standard deviation of Bootstrap IAPE)*

According to CAF protocol (Morison *et al.* 1998), a minimum of 25% of samples were re-read by the same reader.

Results

Shape analysis

Samples

The number of samples that were processed for each location/time period group is shown in Table 2.

Table 2. Batch number, group, and number of samples processed for shape analysis.

Batch	Location and fishing period	Samples
62	STR 1998	350
63	Remote Zone 1998	328
112	STR 2001	361
Total		1039

Fourier Analysis

The observed harmonic distances represent the harmonic distance between the samples before the randomisation tests. These provide the estimator using the randomisation test to determine the probability that the distance between the mean otolith shape from the sampled groups occurred through chance. The observed harmonic distance from the FFT harmonic analysis between each of the groups is presented in Table 3. The probability that the otoliths were sampled from the same stock is presented in Table 4. The results from the analyses of otolith shape show that all the groups differ from each other ($p<0.05$). The distributions of the randomised harmonic differences are presented in Appendix 1, Figure 2 (Randomised harmonic differences between 1998 South Tasman Rise and the 1998 Remote Zone fishing area / periods); Figure 3. (Randomised harmonic differences between 1998 South Tasman Rise (inside) and the 2001 South Tasman Rise fishing periods) and Figure 4. (Randomised harmonic differences between 2001 South Tasman Rise 1998 (outside) fishing area / periods).

Table 3. Observed harmonic distance between each group.

	63 Remote Zone 1998	112 STR 2001
62 STR 1998	0.0614	0.0487
63 Remote Zone 1998		0.0187

Table 4. Proportion of randomisations which produced harmonic differences greater than or equal to the observed harmonic distance for each group comparison (i.e. probability of the two groups under test coming from the same group).

	63 Remote Zone 1998	112 STR 2001
62 STR 1998	0	0
63 Remote Zone 1998		0.0002

Morphometric Analysis

The distributions of the otolith morphometric data is shown in Appendix 1, Figure 5 (Otolith perimeter), Figure 6 (Otolith area) and Figure 7 (Otolith circularity) for 1998 STR (inside), 1998 STR (outside) and 2001 STR. The distributions for otolith area, perimeter and circularity were all highly significant when tested with ANOVAs, ($F_{2,950}$=727, p<0.001, $F_{2,950}$=864, p<0.001, and $F_{2,950}$=205, p<0.001). The distributions of the morphometric parameters are similar between the Remote Zone (1998) and the STR (2001), STR (inside) 1998 show lower values in otolith area, perimeter and circularity, indicating that the otoliths were smaller and less complex in shape.

The results of the SNK test are shown in Table 5.

Table 5. SNK test used to determine groupings for otolith morphometric data. Groupings are underlined. (α=0.05)

	Area		
Circularity	STR (inside) 1998	STR (outside) 1998	STR 2001
Perimeter	STR (inside) 1998	STR (outside) 1998	STR 2001
Area	STR (inside) 1998	STR (outside) 1998	STR 2001

No differences between the STR (outside) 1998 and the STR 2001 samples were shown by the SNK test for otolith circularity and otolith perimeter. Otolith area was significantly different between all areas and fishing periods. The STR (inside) 1998 samples were significantly different from both the STR (outside) 1998 and STR 2001 samples for otolith area, circularity and area.

Age estimation

Samples

A total of 1171 samples were examined but age estimates were only possible for 1091 samples, as 70 samples proved too difficult to read. The number of samples that were processed for each location/time period group is shown in Table 6.

Table 6. Batch number, group, and number of samples processed.

Batch	Location and fishing period	Samples
62	STR (inside) 1998	474
63	STR (outside) 1998	345
112	STR 2001	272
Total		1091

Age estimation

The age distributions for 1998 STR (inside) 1998 STR (outside) and STR 2001 are shown in Figure 8, Figure 9 and Figure 10 respectively. All distributions were binned to five year-classes. The modal age of the 1998 STR (inside) sample was between 15 and 20 years. This contrasts 1998 STR (outside) and the 2001 STR modal age-class of between 25 and 30 years. The maximum age for the 1998 STR (inside) sample was 93, while the maximum age for the 1998 STR (outside) sample and the 2001 STR sample was 176 and 164 respectively. The minimum ages for the 1998 STR (inside) sample was 5 years, while the minimum age for the 1998 STR (outside) sample and the 2001 STR was 17 and 24 respectively.

Comparison of age estimates

The re-ageing of samples was completed on 242 randomly selected samples from the combined batches. The original Beamish and Fournier APE was 4.568%. bootstrapped APE was 4.563%. The bias-corrected APE was 4.573%. The 95% confidence interval was ± 0.442%.

The readability of the samples show most samples (75.8%) were classified with a readability index of 3 (readable with an approximate error of 5%). No samples were classified as 'ideal' with a readability of 1, and 5.98% of the samples were unreadable. This was due to the morphology being too complex to allow a sectioning plane that encompassed the zones throughout the life history of the individual, or small chips and breakage of the otolith rendered the sectioned sample unusable for ageing. These results are shown in Table 7.

Table 7. Readability indices for the orange roughy samples from the combined fishing periods / areas within the STR.

Readability index	*n*	Percentage
1	0	0
2	16	1.366354
3	888	75.83262
4	197	16.82323
5	70	5.977797

Length Frequencies

The length frequencies of these samples are shown in Figure 10, (1998 STR (inside)), Figure 11 (1998 STR (outside)) and Figure 12. (STR 2001). The Length frequency of the 1998 STR (inside) sample shows a distribution of smaller individuals compared to the length frequencies of the 1998 STR (outside) and the 2001 STR samples.

Discussion

The results from the Fourier shape analysis indicate that the orange roughy samples from the 1998 STR (both inside and outside the EEZ) and the the 2001 STR roughy all have morphologically distinct otoliths. As otolith shape is a phenotypic character, these differences may reflect underlying genetic differences, environmental differences, or a combination of the two. Otolith shape may differ among ages, sexes and year-classes (Castonguay *et al*. 1991; Campana and Casselman 1993) and variation in growth rates may be the most significant cause of significant variation in otolith shape (Campana and Casselman 1993). The Fourier descriptors were standardized for otolith size before analysis, which will at least partially remove any age effect on the differences. However, the extent to which otolith shape reflects growth rate rather than age, may still contribute to the observed differences. The mechanisms which drive the differences in otolith morphology between stocks are still poorly understood (Smith *et al*. 1998).

Given the differences with length frequencies, otolith morphometrics, age distributions and Fourier analysis; stock structuring is evident between the two areas inside and outside the EEZ. A stock structuring hypothesis is strengthened as these samples were collected within the same fishing period (1998). The differences in the samples from the STR (outside the EEZ), and the STR 2001 samples is evident, but less pronounced.

All ageing data has been utilised in modelling of the orange roughy stocks within the STR during ORAG. These data were supplied before the commencement of the ORAG for use by the modellers.

The objectives of the proposal have been fully accomplished and the ageing and Fourier analysis data have been utilised in quota management through the Orange Roughy Stock Assessment Group (ORAG).

Data Statement

All data generated from this study are stored on network drives which are backed up on a weekly basis. Further, all images and data have been recorded to Kodak CD-ROMs.

References

Anderson, J.R., Morison, A.K., and 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. In 'Age Determination and Growth of Fish and Other Aquatic Animals'. (Ed. D.C. Smith.) *Australian Journal of Marine and Freshwater Research* 43: 983-1013.

Beamish, R., and Fournier, D.A. (1981). A method for comparing the precision of a set of age determinations. *Journal of the Fisheries Research Board of Canada* 36: 1395-400.

Campana, S., E. and Casselman, J., M. (1993). Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*. **50**: 1062-1083

Castonguay, M., Simard, P. and Gagnon P. (1991). Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (Scomber scombrus) stock discrimination. *Canadian Journal of Fisheries and Aquatic Sciences*. **48**: 296-302

Efron, B., and Tibshirani, R.J. (1993). An Introduction to the Bootstrap. *Chapman and Hall, New York.* 436 pp.

Morison, A.K., Robertson, S.G. and Smith, D.C. (1998). An integrated production fish ageing system: quality assurance and image analysis. *North American Journal of Fisheries Management* 18: 587-598.

Smith, P.J., Robertson, S.G., Horn, P.L., Bull, B., Anderson, O.F., Stanton, B.R. and Oke, C.S. (2001). Multiple techniques for determining stock relationships between orange roughy, *Hoplostethus atlanticus*, fisheries in the eastern Tasman Sea. *Fisheries Research* 1339: 1-22.

Smith, D., C., Robertson, S.G. and Morison A., K. (1998). Age composition in the Eastern and Southern Management Zones. *Final Report to FRDC*. Project **95/032**

Zar, J. H. (1974). Biostatistical Analysis. Prentice-Hall, New Jersey.

Appendix 1.

Figure 2. Randomised harmonic differences between 1998 STR (inside) and the 1998 STR (outside) fishing area / periods. Observed harmonic is 0.0614.

Figure 3. Randomised harmonic differences between 1998 STR and the 2001 STR fishing periods. Observed harmonic is 0.0487.

Figure 4. Randomised harmonic differences between 2001 STR, 1998 STR (outide) area / periods. Observed harmonic is 0.0187.

Figure 5. Otolith perimeter for 1998 STR (inside), 1998 STR (outside) and 2001 STR.

Figure 6. Otolith area for 1998 STR, 1998 STR (outside) and 2001 STR.

Figure 7. Otolith circularity for 1998 STR (inside), 1998 STR (outside) and 2001 STR.

Figure 8. Age distribution of the STR (inside) 1998 fishing period, (n=474).

Figure 9. Age distribution of the STR (outside) 1998 fishing period, (n=345).

Figure 10. Age distribution of the STR 2001 fishing period, (n=272).

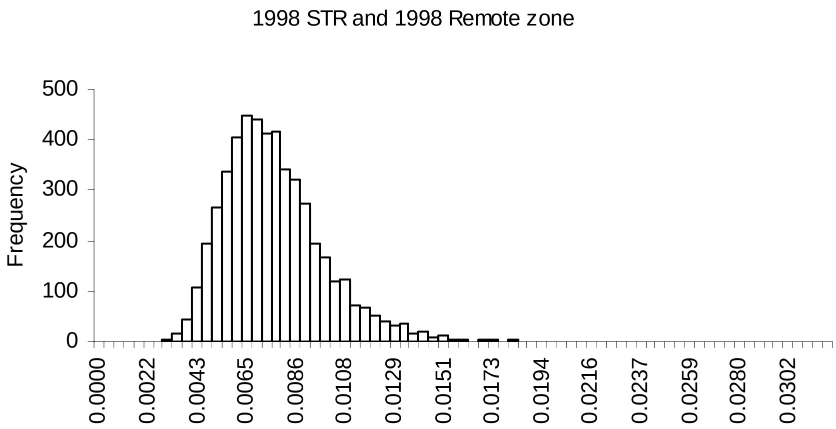

Figure 2. Randomised harmonic differences between 1998 STR (inside) and the 1998 STR (outside) fishing area / periods. Observed harmonic is 0.0614.

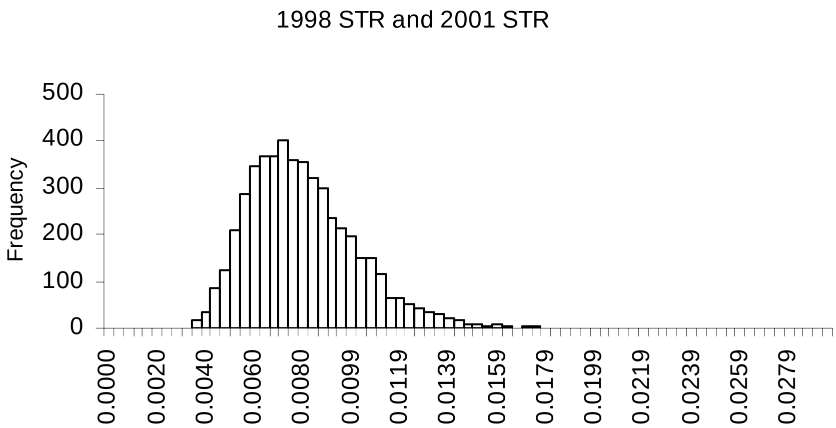

Figure 3. Randomised harmonic differences between 1998 STR and the 2001 STR fishing periods. Observed harmonic is 0.0487.

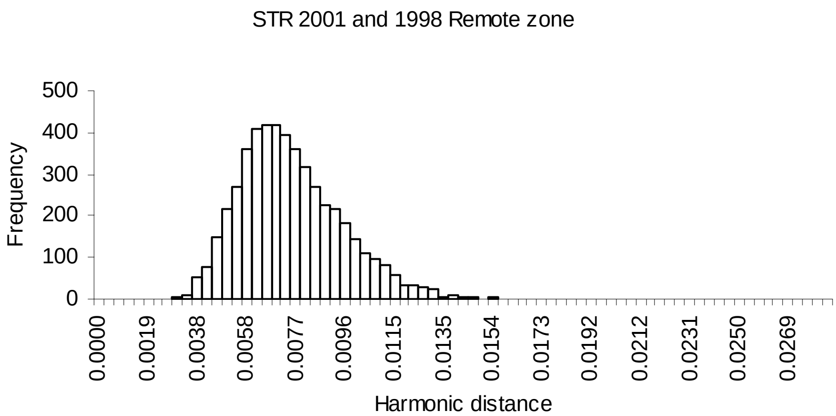

Figure 4. Randomised harmonic differences between 2001 STR, 1998 STR (outide) area / periods. Observed harmonic is 0.0187.

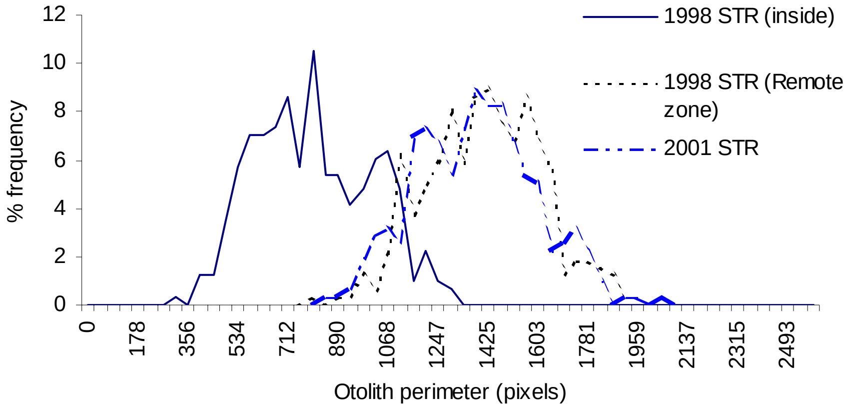

Figure 5. Otolith perimeter for 1998 STR (inside), 1998 STR (outside) and 2001 STR.

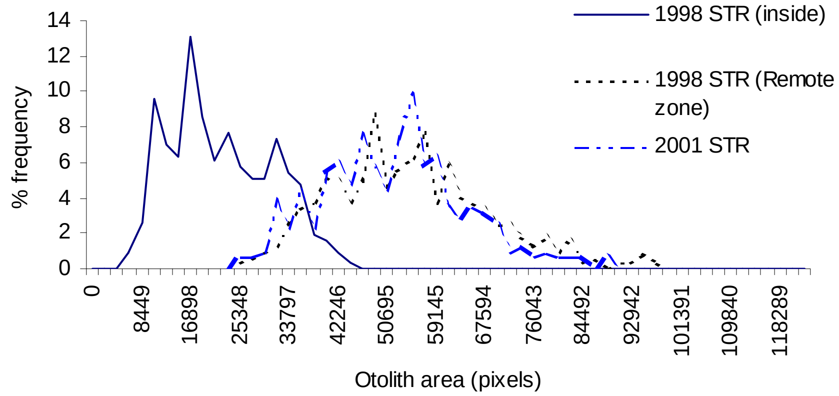

Figure 6. Otolith area for 1998 STR, 1998 STR (outside) and 2001 STR.

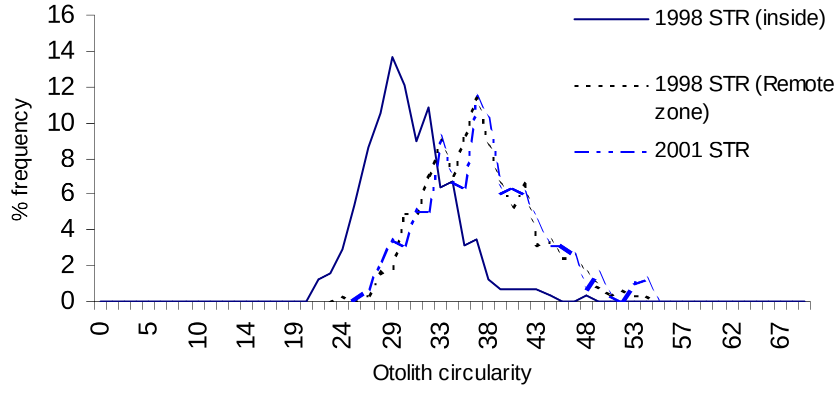

Figure 7. Otolith circularity for 1998 STR (inside), 1998 STR (outside) and 2001 STR.

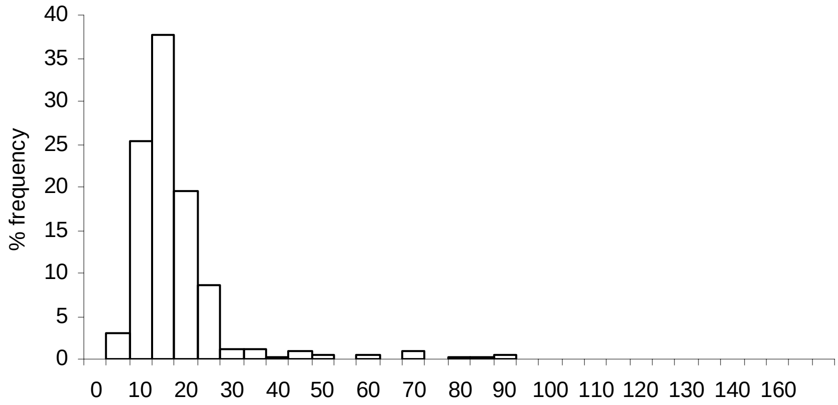

Figure 8. Age distribution of the STR (inside) 1998 fishing period, (n=474).

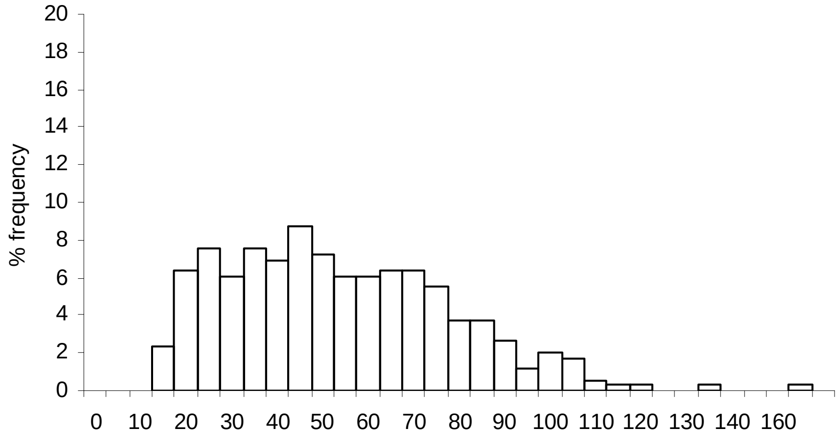

Figure 9. Age distribution of the STR (outside) 1998 fishing period, (n=345).

Figure 10. Age distribution of the STR 2001 fishing period, (n=272).

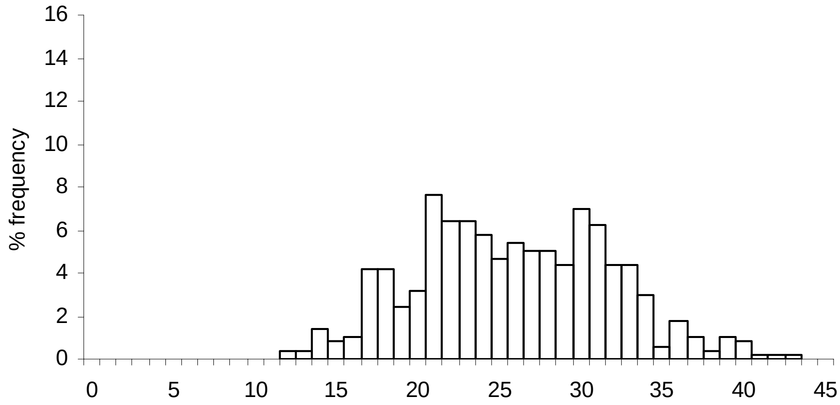

Figure 11. Length frequency of the 1998 STR (inside), n=499.

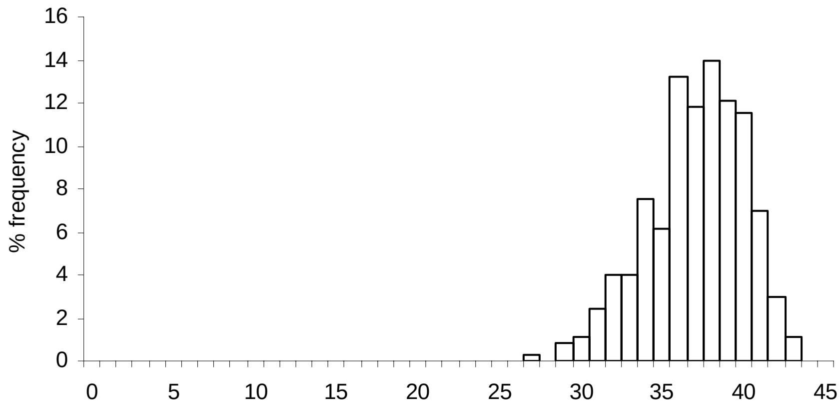

Figure 12. Length frequency of the 1998 STR (outside), n=372.

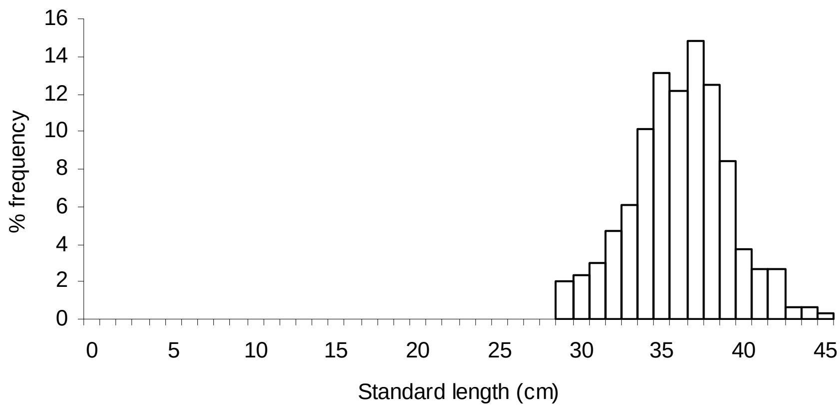

Figure 13. Length frequency of the 2001 STR, n=297.