Investigation of the potential for automatic ageing using image analysis: A pilot study

A. K. Morison and S. G. Robertson

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Final Report

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NON-TECHNICAL SUMMARY

96/136 Investigation of the potential for automatic ageing using image analysis: a pilot study

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Objectives:

1. Develop new methods for semi-automatic/automatic ageing of sectioned otoliths using image analysis software.
2. To validate automatic ageing using known age samples from species with clear otoliths.
3. To evaluate the potential of artificial neural networks for the process of objective age determination of fish.

Non Technical Summary

Knowledge of the age structure of exploited fish populations is crucial to understanding their dynamics and the impacts of exploitation. Currently most fish species are aged by counting growth increments on otoliths which, in most cases, require sectioning before all increments can be seen. Otoliths also need to be examined by an experienced reader to obtain accurate age estimates and fish ageing has often been referred to as 'art rather than science'. The process of ageing is laborious, time consuming and hence, relatively expensive. The development and application of a method for automatically estimating age would make the process more objective, reduce human error in the pattern recognition process, increase confidence in the stock assessment process, reduce sample processing time, and hence reduce the cost of age estimation.

The increasing processing power offered by modern computers allows for new approaches to this problem. Images of otoliths can be stored and processed digitally, and sophisticated numerical analyses can be used in the pattern recognition process.

In this study, age estimates were made using two approaches: trends in brightness levels, and artificial neural networks (ANNs).

1. Trends in brightness level.

Growth increments were identified by changes in brightness levels (luminance values) along a transect across an image of an otolith section. Within this approach, the capabilities of the existing image analysis software (Bioscan Optimate) used at the

CAF for automatic ageing were explored first. Then, new software was developed which detected and counted growth increments as changes in the trend in brightness level.

2. ANNs.

ANNs were used to examine information from images of sectioned otoliths, and develop criteria for classification of the images into age classes. ANNs are relatively new computer models which are currently used in a broad range of applications, from fingerprint analysis to stock market predictions. They are particularly suited to problems that involve detecting patterns in data, and the task of age estimation is essentially one of pattern recognition.

Three species were selected for testing for which there is an ongoing requirement for age estimation: black bream, snapper, and blue grenadier. Black bream otoliths have unambiguous otolith increments, snapper otoliths are also clear but more difficult, and blue grenadier otoliths have increments which require considerable experience to interpret.

The Optimate image analysis system has a facility for detecting opaque zone which uses the relative brightness level. However, trials showed that a single threshold level could not be found which provided accurate age estimates for a range of specimens of any species.

The regression methods applied proved inadequate at estimating age with a sufficient degree of accuracy. For all three species the estimation of age using the regression method on single transects yielded low accuracy and a high degree of variability.

The use of ANNs was more successful. Three different network structures were trialed and each was trained to be able to reproduce the observed ages on a training set of data. After training, at least one of the three networks correctly classified the age of fish from unseen transects for black bream and snapper at a rate comparable to an expert reader. For blue grenadier, a species with more complex otolith structure, error rates were unacceptably high for all networks. The trials indicate that ANNs have the potential to fully automate the production ageing process with the additional benefits of increased speed in processing, objectivity and repeatability of estimates, and of providing a measure of confidence in the estimate for each sample examined.

There is both the scope and the need to further refine the ANNs before they are employed in the production age estimation process. Improvements to the form of data inputs in particular offer the possibility of increased accuracy in network performance.

KEYWORDS: Otoliths, age determination, artificial neural networks

FINAL REPORT

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Project Background and Justification

Knowledge of the age structure of exploited fish populations is crucial to understanding their dynamics and the impacts of exploitation (Smith 1992). The age structure of the catch is the key input to the stock assessment process. Age composition data also provide fundamental insights into the fish biology and stock productivity and allow the estimation of basic parameters for describing growth, mortality rates and recruitment.

"The ageing of fishes, and consequently the determination of their growth and mortality rates, is an integral component of modern fisheries science. It is not an easy task: a wide variety of techniques are employed and continue to be developed, and discrepancies between different workers are common" (Paul, 1992). These discrepancies result from inconsistent pattern recognition by readers on the structures used for ageing. Currently most fish species are aged by using otoliths which usually require further treatment such as sectioning or 'breaking and burning'. Otoliths are formed by the deposition of calcium through metabolic processes. The rate of deposition and the lattice structure of the calcium is cyclic throughout the year creating alternating opaque and translucent zones from the primordium (otolith centre) to the edge of the otolith. Otoliths need to be examined by an experienced reader to obtain accurate age estimates. This is a time consuming process, and readers need to frequently re-examine previously aged material to check consistency in their interpretation (Morison *et al.,* in press). Williams and Bedford (1974) remarked "that otolith reading remains ...as much an art as a science" and the subjective nature of the interpretation of the increments has driven the search for a more objective method (eg Bagenal, 1978; Boehlert, 1985). The development and application of a method for automatically determining ageing would make the process of age determination more objective by reducing human error in the pattern recognition process.

Bioscan Optimas™, the image analysis software used at the CAF, includes the facility for automatic detection of opaque zones along a transect drawn across an image. This facility identifies zones as areas below a threshold brightness level (luminance value). However, the reliable discrimination of growth zones using this facility requires development before it could be routinely applied. Issues such as correction for variation in background illumination, selection of the optimal areas for counting, and choice of threshold luminance values require further development.

The increasing processing power offered by modern computers allows for new approaches to this problem. Images of otoliths can be stored and processed digitally, and sophisticated numerical analyses can be used in the pattern recognition process. Some approaches have used computers simply to assist in the process of data collection

(McGowan *et al.,* 1987; Small and Hirschhorn, 1987) or image enhancement (Estep *et al.*, 1995). Other attempts at automating the process have included the use of multiple regressions based on measurements of otolith and fish size (Boehlert, 1985), or by determining minima and maxima in the grey-level signal (Troadec, 1991; Welleman and Storbeck, 1995). Other recent approaches are not fully automatic in that they still require or allow interaction with the user to assist in the identification of increments (Macy, 1995; Cailliet *et al.,* 1996).

Two approaches are proposed:

1. A transect method based on peaks and troughs in brightness levels (luminance values) along a transect across a captured image.

The pattern of increments can be described by examining the luminance values along a transect from the centre to the edge of the otolith. The resultant pattern is similar to a sine wave, and by counting the troughs or peaks along this wave, above a threshold level, the age of the fish can be determined. This method is aimed at replicating methods others have developed to allow evaluation of their success relative to entirely manual methods, and to other methods developed by this study.

2. The application of artificial neural networks (ANN) for the processing of information from the whole image, to iteratively develop criteria for classification of images into separate selected categories (age classes).

This is essentially a new approach. The ANN techniques are more developmental but are currently used in a broad range of pattern recognition applications from fingerprint analysis to stock market predictions. The concept of neural networking is ideally suited to the problem of pattern recognition which is the fundamental process of age determination.

It was proposed that both the simpler transect method and the more sophisticated ANN be explored and developed. The Central Ageing Facility (CAF) provided staff who are experienced in the use and development of image analysis software and provided a repository of previously prepared and aged material suitable for this study. Their experience as a dedicated production fish ageing service also provided them with the experience to recognise and evaluate the utility of such new developments.

Need

Current age determination methods, even when enhanced by image analysis software still depend on interpretation by an experienced 'reader'. The process of ageing is also laborious, time consuming and hence, relatively expensive. At present, when readers change, there is a substantial training and verification period needed to ensure that the new reader is interpreting otolith structure in a consistent and correct manner (Morison *et al.*, in press). Automatic and semi automatic ageing would have the primary advantage of being a far more objective method than is possible with even the best training, reducing discrepancies both between readers and organisations. This factor will increase the precision of estimates and therefore provide greater confidence for the stock assessment process. Benefits associated with the development of this technique also include the reduced sample processing time which would increase the number of samples able to be processed in a given time and hence reduce the cost.

Objectives

1. Develop new methods for semi-automatic/automatic ageing of sectioned otoliths using image analysis software.

2. To validate automatic ageing using known age samples from species with clear otoliths.

3. To evaluate the potential of artificial neural networks for the process of objective age determination of fish.

Methods

Tests were conducted using the otoliths of three species: black bream *Acanthopagrus butcheri* (Sparidae) an estuarine species, snapper *Pagrus auratus* (Sparidae) a coastal marine species, and blue grenadier *Macruronus novaezelandiae* (Merlucciidae) a marine species inhabiting the continental shelf slope at 300-500 m depths. The species are all aged routinely at the CAF from sectioned otoliths and the methods of age estimation have been validated for each species (Morison *et al.*, in press, Francis *et al.*, 1992, and Kalish *et al.*, 1995 for the three species respectively). The increments on the otoliths of these species vary in clarity from unambiguous in *A. butcheri*, through quite clear in *P. auratus*, to moderately difficult in *M. novaezelandiae*. It was decided to include samples from a species with a range of readabilities, rather than just those with clear otoliths as stated in the initial objectives, to allow the limitations of the techniques to be more fully explored. Samples for the trials had been previously aged and 40 prepared sections of each species were selected from the Central Ageing Facility's collection to include between eight and eleven of the most frequently encountered age classes (Table 1).

The age of fish was estimated by experienced readers from otolith sections by manually counting the number of opaque zones, following the published and validated methods. These age estimates from zone counts were compared with estimates from objectively identified opaque zones. Agreement between methods was measured with the index of average percent error (APE) (Beamish and Fournier, 1981). Experience with using this index for comparing repeat readings for a large range of species at the CAF has shown that an APE should be less than 5% unless material is particularly difficult to age (Morison *et al.*, in press).

Table 1. Age composition of samples used for trial of neural networks for the three test species.

Age class	*P. auratus*	*A. butcheri*	*M. novaezelandiae*
1	1	5	0
2	6	5	0
3	5	0	0
4	5	5	3
5	5	5	5
6	3	5	4
7	0	5	3
8	3	5	4
9	2	5	3
10	6	0	4
11	0	0	3
12	4	0	5
13	0	0	5
14	0	0	1
Total	40	40	40

The proposal was for a pilot study with two broad components. The first component was to use existing image analysis capabilities to trial automatic ageing of species which have clear and unambiguous otoliths. This was to include daily ageing of larval fish. However, the approach was modified so as to focus exclusively on annual increments and to include species of a range of readabilities. This change was implemented to make the developments more relevant to the issue of production age estimation.

The second component looked beyond the capacities of the existing system and examined the potential for applying pattern recognition software to the problem automatic age determination. Artificial neural networks were investigated as they offered a new approach which was particularly suited to pattern recognition problems and which had not previously been applied to automatic age estimation.

Data Collection

An image of each section was saved in 8 bit bitmap format (256 levels of brightness from 0 for black to 255 for white). Data was collected from transects drawn on each image from the primordium to the ventral otolith edge approximately parallel to the sulcus (Figure 1), in the area of the section which would normally be used in ageing.

Results

Objective 1. Develop new methods for semi-automatic/automatic ageing of sectioned otoliths using image analysis software.

Attempts to reliably detect opaque zones and estimate ages from luminance values using the inbuilt Optimate™ facility were unsuccessful; a single threshold level could not be found which provided accurate age estimates for a range of specimens of any species. A threshold could be determined by trial and error which provided a correct marking of opaque zones on an individual transect. However, because of the variation in the luminance profile of different transects within and between specimens, the appropriate threshold value determined for one transect could not be confidently applied to another. It became apparent that a new approach would be required if a system of automatic age estimation was to be developed.

A custom-written application was developed in Visual Basic Professional 4.0 (Custom Application A, Figure 2). The input screen of this application (Figure 3) allowed the opening of image files saved using Bioscan Optimate®. On each image the primordium was selected and a line drawn as a transect across the image to the edge near the dorsal margin of the sulcus. The luminance value of each pixel along this transect was recorded as an array and displayed in the output screen where options could be interactively selected for data analysis (Figure 4). After analysis, data were exported to an Excel file together with specimen details.

For each image five transects were drawn from the primordium to the otolith edge near the dorsal margin of the sulcus. To make successive transects independent of previously drawn ones, only one transect was drawn on each image at a time, and the then the next image selected. The second and subsequent transects were drawn on separate passes through the list of images. The data was used raw (termed the base case) and also after smoothing (to reduce noise) with 3, 5 and 7 point running averages. The application also allowed viewing of plots of the smoothed data superimposed on the graphs of the raw data.

Opaque zones are minima in the luminance profile of the transect and these were detected as points of change from negative to positive slope in this profile. The slope was determined from regression equations which were calculated for the luminance values over a moving interval of a specified number of pixels (the search width). The sensitivity of the detection process depends on the distance over which the slope is calculated (the search width) so search widths of 3, 5, 7, 9, 11, 13, 15, 17 and 19 pixels were trialed (Figure 5). The number of slope changes detected provides the estimate of the number of opaque zones and hence the estimated age.

A second custom-written application developed in Visual Basic Professional 4.0 was written to facilitate batch processing of the data extracted with Optimate (Custom Application B, Figure 6). This application imported the data array, and calculated predicted ages based on the range of data pre-processing and regression options. Thus for each transect, 36 predicted ages were calculated (4 data inputs X 9 regressions). This was repeated for each of the 5 transects for each image, and for each of the 40 specimens. A further option was the use of an *a priori* criterion for the position of the first increment as being outside the first 40 pixels. This resulted in a total of 43,200 predicted ages (3 species X 40 specimens X 5 transects X 4 data inputs X 9 regression widths X 2 *a priori* options).

The next phase involved combining data from a series of transects radiating from the primordium of the sectioned otolith. It was expected that this approach would have reduced the noise inherent in any single transect, by allowing the annual pattern which was common to all transects to be more clearly identified. The custom-written application which applied the regression methods to single transects, was also written to include the facility to simultaneously extract data on luminance profiles from multiple transects on the one image.

Objective 2. To validate automatic ageing using known age samples from species with clear otoliths.

The regression analysis methods applied proved inadequate at estimating age with a sufficient degree of accuracy. For all three species the estimation of age using the regression method on single transects yielded low accuracy and a high degree of variability as measured by the average percent error APE. APE's exceeded 20% in all cases when pooled across age groups (Figures 7-9). APE values of over 10% are considered unacceptable.

The accuracy of the technique varied with the age of fish (Figure 10). Both the search bandwidth and the smoothing of the data had a large effect on the APE values obtained. Applying an *a priori* value for the width of the first zone only slightly reduced the APE. However, no single value of either search width of data smoothing produced estimated ages which were consistently more accurate. The search width which produced the lowest APE (best agreement between observed and predicted ages) decreased with increasing age. This effect is the result of the increment width decreasing with increasing age. The optimal search width therefore is narrower for outer increments than for inner increments.

However, after analysing the results of the single transect method, it was clear that the limitations of that type of approach could not have been overcome by using a series of transects. Therefore, rather than proceed with the collection and analysis of data with this technique, it was decided to concentrate efforts on developing the third phase of the project with ANNs.

Objective 3. To evaluate the potential of artificial neural networks for the process of objective age determination of fish.

A manuscript on this component of the project has been prepared for submission to the journal of Marine and Freshwater Research.

Data collection

Data was extracted from images as described for the previous components of this study. However, the ANN component required data for both training and testing of the ANN. One transect was sampled at a time from each image then another image selected. This was repeated for each of the 40 images until data were obtained from 400 separate transects as a training set to establish the ANN, and from an additional 200 transects for a test set, to test its performance.

A maximum transect length of 202 pixels was selected for the network input as being sufficient to allow the transect to cover the largest sections. Where the transect drawn included less than 202 pixels, the remaining array elements were assigned a value of 255. No data smoothing was used on the array.

Previously assigned ages were presented to the network as a binary array such that for a fish of age *j*, the *j*th array element was assigned a value of one, and all other array elements were zero. The array length was standardized to twelve elements for each of the three species trialed. Three custom-written applications were written to process data for the ANN (Figure 10): Custom Application A provided for interactive age estimation using ANNs, Custom Application C provided for age estimation in a batch mode, and Custom Application D provided for post-processing of ANN outputs.

Structure of the Artificial Neural Networks

All networks used in this study were written in Microsoft Visual Basic™ using the Ward Group NeuroWindows™ dynamic link library. The networks used in this study consisted of three layers of elements (or neurons): an input layer, a hidden layer and an output layer (Figure 11). The input layer contains the input data and has the same number of neurons as the input array of luminance values (202). The output layer contains the predicted ages and therefore must have at least the same number of neurons as the number of target age classes. The maximum number of age classes was 11 for *M. novaezelandiae* but for consistency at least one additional output neuron, with no observed ages in the input data set, was included in each network. The hidden layer (so called because it is not directly accessible as inputs or outputs) consists of variables used in intermediate calculations and the number of neurons in the hidden layer will vary depending on the nature of the problem. A geometric rule for estimating the optimal number of hidden layer neurons was applied as a starting point for the network design:

$$N = \text{int}\sqrt{m.n}$$

Where N = number of hidden layer neurons, m=number of input neurons and n= number of output neurons (Masters 1994). In this case, N = int√(12 X 202) = 49.

As the performance of the network can vary with the size of the hidden layer, networks with 10 more and 10 fewer hidden layer neurons were also trialed. Other features of the network structure do not vary, so the different network structures tested are referred to in the text by the number of neurons in the hidden layer and annotated as 39H, 49H or 59H. Therefore a total of 9 separate ANNs were tested (3 species X 3 structures).

All neurons in the three layers are fully inter-connected by a series of weighted coefficients so that values in each input neuron have some level of input to the values in each hidden layer neuron and each output layer neuron. The collections of weighting values between the input and hidden layers and between the hidden and output layers are termed the weighting matrices (*W1* and *W2*, Figure 11). It is these weights which are adjusted during network training, and the two final weight matrices define the trained network.

Network training

The networks used for this study are back-propagation feed-forward networks. The term back-propagation refers to way the model "trains" or "learns". Neural networks "learn" in much the same way that many statistical algorithms do estimation, and many neural network models are similar or identical to popular statistical techniques such as generalized linear models, polynomial regression and discriminant analysis (Sarle 1994). Weighting factors are adjusted iteratively during the training phase to reduce the network error as measured by the difference between the network output array (indicating the predicted ages) and the target array (representing the observed ages). The feed-forward term refers to the processing of data inputs by the network weights during the training and the test phases to produce the output values. When trained, a network can be viewed as having formed an internal representation of the data, thus generating the required output when given one of the training inputs (Newbury *et al.,* 1995). If the training is sufficient and the training data is representative, the network should also give correct classifications of new data.

The neural networks were trained in a six step process.

1) The input data array was standardised to the range of zero and one, then passed to the input layer neurons and multiplied by the weight matrix between the input and hidden layer (*W1*). The sum of the values at each hidden layer neuron was standardized within the range of zero and one using the logistic function to calculate the values for each hidden layer neuron.

$$f(x) = \frac{1}{1+e^{-x}}$$ where x = sum of input layer outputs

2) The hidden layer neuron values were then multiplied by the weight matrix between the hidden and output layers (*W2*), and again standardized using the logistic function. These results comprise the initial network outputs and their calculation completes the feedforward operation of the network.

3) The network error is calculated as the sum of the differences between the target output (a value of 1 in the appropriate element of the output arrray) and the output of the network.

4) Individual errors in each of the output neurons are used to change the connection weights between the hidden and output layers (matrix *W2*, Figure 11).

5) The individual error at each hidden neuron is used to adjust the weight matrix between the input neurons and the hidden neurons. (*W1*). The adjustment of the weight matrices *W1* and *W2* using the error is the backpropagation cycle.

6) One cycle of passing data forward and the backpropagation adjustments of the error is termed an epoch. Values for learning rate and momentum were used in the model to control the training time by specifying the amount of change in the weight matrices between the epochs. This process continues until network error was reduced to the arbitrary level of 0.1. After training ceased, the final weight matrices *W1* and *W2* were saved.

Network testing

The neural network was first tested using the training set to determine the level to which the network weight matrices correctly estimated the observed ages. The array element with the highest output value represents the model's predicted age (Figure 12). APE values for the differences between predicted and observed ages were calculated for each ANN trained.

The test data set for each species was then passed through each of the three trained networks for each species. Again, the predicted age was determined by the output neuron with the highest value. Overall performance of the networks on the test data was assessed by comparison of the predicted and observed age compositions and was quantified by the APE values. Bootstrap techniques (Efron and Tibshirani, 1993) were used to generate distributions (n = 2000) of potential APE values and so allow comparison of APE values. Network performance was also assessed using linear regression analysis of predicted age on observed age. Correctly estimated ages would produce a slope and an intercept not significantly different from one and zero respectively. Assessment of network performance by age class was obtained from age difference tables (which present the frequency of predicted and observed combinations), and from age bias plots (Campana *et al.*, 1995) which graphically display trends in these data.

Improved network performance was sought by applying two additional screening criteria to the network outputs: a minimum level for the highest output neuron, and a minimum distance between the two highest output values. Either a low maximum level in the output neurons, or a small difference between the two highest output values, would suggest that the inputs were not adequately described by the model, with a consequent failure to clearly predict age class membership. Two combinations of different levels for these screening criteria were applied: for type (i) screening, the maximum output value exceeded 0.8 and the next higest array element value was less than 80% of the highest array element value, and for the more stringent type (ii) screening,. the maximum output exceeded 0.9, and the next highest array element was less than 50% of the highest array element.

Results - Objective 3

Examples of the network outputs for the training and test sets for *P. auratus* (Tables 2 and 3, Figure 12) show two important features: although they are scaled from zero to one, the outputs do not constitute probabilities; and the second most likely age class predicted by the model (on Figure 12, age 6) is often not adjacent to the age with the highest network output value (age 2).

At the completion of training, APE values were below 0.4% for all networks for *P. auratus* and *A. butcheri*, but were at least an order of magnitude higher for all networks for *M. novaezelandiae*. For the first two species, all but a few samples were correctly classified by the networks (Tables 4-6). The APEs on the training sets for the 39H, 49H and 59H networks respectively were 0.35 %, 0.32% and 0.00% for *P. auratus,* 0.26%, 0.02% and 0.07% for *A. butcheri* and 4.92%, 7.17% and 7.78% for *M. novaezelandiae*.

The distributions of predicted ages generally approximate those of the observed ages for *P. auratus* and *A. butcheri* (Figure 13). The overall trends are well reproduced and although there are age classes whose abundance is matched exactly (age 1 for *A. butcheri*), some are overestimated by all networks (e.g. age 2 for *P. auratus*), and some are underestimated (e.g. age 8 for *A. butcheri*). However, for *M. novaezelandiae*, none of the three networks adequately predicted the age composition of the test set (Figure 13). For this species, the abundance of some age classes (e.g. ages 8, 9, and 13 for networks 39H and 59H) were highly exaggerated and others were ignored completely (e.g. age 11 for all networks).

The APEs from comparing predicted ages from test sets with the observed ages were substantially higher than those obtained on the training sets for each species. The values for the three networks were between 4% and 5% for *P. auratus* (Table 7), between 3% and 4% for *A. butcheri* (Table 8), but over 18% for *M. novaezelandiae* (Table 9).

Although low APE values were obtained for *P. auratus* (Table 7) and *A butcheri* (Table 8), the slope or intercept of the regressions of predicted age on observed age indicated significantly differences from a line of agreement for two of the three models. They were also significantly different for all three models for *M. novaezelandiae* (Table 9). High R^2 values of over 0.78 for *P. auratus* and of 0.87 for *A. butcheri* reflect the good fit of the ANN model for these species.

The percentage of the entire test sets which were classified correctly (or within year) was over 71% (over 84%) for *P. auratus* and over 75% (over 86%) for *A. butcheri* for all networks (Tables 7 and 8 respectively). Comparable values for *M. novaezelandiae* (Table 9) were under 12% (and under 30%).

Age difference tables (presented for unscreened data only) show that for both *P. auratus* (Table 10) and *A. butcheri* (Table 11) the majority of predicted ages agree, but those that do not are often several years away from the correct age. The maximum absolute values of these differences are greater for *P. auratus* than for *A. butcheri*. The data for *M. novaezelandiae* (Table 12) show diagonal patterns in the cell frequencies that reflect the pattern of over and under-estimated age frequencies seen in the age compositions (Figure 13).

Age bias plots of the unscreened data of the best fitting model for each species show there is no significant bias for most age classes of *P. auratus* and *A. butcheri*, but that the predicted ages for *M. novaezelandiae* are biased upwards for younger fish and downwardly biased for older fish (Figure 14). For all three species, there is a tendency for a downward bias in the older age groups.

The distribution of bootstrapped APE values (Figure 15) show that there are not only greater differences between the models for *P. auratus* than for *A. butcheri* but also greater variability among samples within each network type. This reflects the larger range of absolute differences between observed and predicted ages for *P. auratus*. The network outputs for *M. novaezelandiae* were not bootstrapped as the model failed to predict age class membership.

For *P. auratus* (Table 7) and *A. butcheri* (Table 8), screening of model outputs decreased the APE values, increased the percentage of samples correctly classified, and in a third of cases made the regression coefficients not significantly different. The screening excludes samples whose ages were poorly predicted by the networks; so there is a trade-off between model accuracy and the number of samples classified. The percentage of correct age classifications increased by up to 10% for *P. auratus* (Table 7) by excluding up to 40% of the samples. For *A. butcheri* screening improved correct classifications to over 96% but with a reduction in samples classified of 34%.

For *M. novaezelandiae* type (i) output screening produced little improvement in network performance and type (ii) screening reduced the percentage of correct classifications (Table 9). Even though over 95% of samples were screened out APE values remained over 16% for all networks.

Table 2. Example of output neuron values for four otoliths on the training set for *P. auratus*, and the predicted (P) and observed (O) ages. Non-zero values less than 0.001 shown as 0.001.

Output neuron												Age (P)	Age (O)
1	2	3	4	5	6	7	8	9	10	11	12		
0	0.923	0	0.007	0.038	0	0.041	0.014	0	0.001	0	0	2	2
0.001	0.989	0.001	0.003	0	0	0	0.006	0	0	0.001	0	2	2
0.003	0	0.001	0	0	0	0.985	0.028	0.060	0	0.001	0	7	7
0.001	0	0.001	0.001	1	0	0.001	0	0	0	0.001 0	0	5	5

Table 3. Example of output neuron values for four otoliths on the test set, and the predicted (P) and observed (O) ages. Non-zero values less than 0.001 shown as 0.001.

Output neuron												Age (P)	Age (O)
1	2	3	4	5	6	7	8	9	10	11	12		
0.001	0.430	0	0	0	0.398	0	0.672	0.029	0	0	0	8	6
0	0.014	0	0	0	1	0	0	0	0.001	0.001	0.001	6	6
0.003	01	0	0	0	0.024	0	0.009	0.002	0.001	0	0	2	2
0	0.062	0	0	0	1	0	0.013	0	0	0	0	6	6

Table 4. Observed and predicted ages for *P. auratus* after training has completed on the 49H network (APE= 0.33)

	Predicted												
Observed	1	2	3	4	5	6	7	8	9	10	11	12	All
1	10												10
2		60											60
3		6	44										50
4				50									50
5					49	1							50
6						30							30
7													
8								30					30
9									20				20
10										60			60
11													
12												40	40
All	10	66	44	50	49	31		30	20	60		40	400

Table 5. Observed and predicted ages for *A. butcheri* after training has completed on the 49H network. (APE = 0.02).

	Predicted									
Observed	1	2	3	4	5	6	7	8	9	All
1	50									50
2		50								50
3			0							
4				50						50
5					50					50
6						50				50
7						1	49			50
8								50		50
9									50	50
All	50	50		50	50	51	49	50	50	400

Table 6. Observed and predicted ages for *M. novaezelandiae* after training has completed on the 49H network (APE = 7.17).

	Predicted											
Observed	4	5	6	7	8	9	10	11	12	13	14	All
4	30											30
5	8	19			1	12	8			2		50
6	12		19				7			2		40
7				30								30
8	11				27					2		40
9	1					29						30
10	3	1		3			33					40
11						1		29				30
12	7	1			2	9			31			50
13										50		50
14		7									3	10
All	72	28	19	33	30	51	48	29	31	56	3	400

Table 7. Comparison of observed and predicted ages for networks on samples of *P. auratus* for complete and screened output data, including average percent error (APE), regression coefficients and 95% confidence limits. P = */ns if either slope or intercept are/not significantly different from 1 or 0 respectively.

Network	APE	R^2	Regression statistics						P	N	% Classified	% Correct	% Within
(Sreen type)			Slope	L 95%	U 95%	Intercept	L. 95%	U 95%					One Year
39	5.08	0.82	0.91	0.85	0.97	0.42	0.02	0.81	*	200	100	71.5	84.5
39 (i)	2.51	0.93	0.98	0.93	1.02	0.22	-0.08	0.53	ns	149	74.5	77.6	92.8
39 (ii)	2.53	0.93	0.98	0.93	1.02	0.23	-0.09	0.55	ns	125	62.5	78.5	92.6
49	5.57	0.78	0.93	0.86	1	0.19	-28	0.65	ns	200	100	72	87.5
49 (i)	2.89	0.88	0.96	-0.9	1.01	-0.17	-0.22	0.57	ns	149	74.5	81.9	92.6
49 (ii)	2.25	0.94	1	0.96	1.05	-0.002	-0.31	0.32	ns	120	60	60	83.3
59	4.15	0.81	0.87	0.81	0.93	0.53	0.12	0.94	*	200	100	75.5	89
59 (i)	2.57	0.91	0.96	0.91	1	0.21	-0.1	0.53	ns	165	85.5	81.8	91.5
59 (ii)	2.38	0.9	0.94	0.89	0.99	0.31	-0.03	0.65	*	138	69	81.2	92

Table 8. Comparison of observed and predicted ages for networks on samples of *A. butcheri* for complete and screened output data, including average percent error (APE), regression coefficients and 95% confidence limits. P = */ns if either slope or intercept are/not significantly different from 1 or 0 respectively.

Network	APE	R^2	Regression statistics						P	N	% Classified	% Correct	% Within
(Sreen type)			Slope	L 95%	U 95%	Intercept	L. 95%	U 95%					One Year
39	3.38	0.87	0.91	0.86	0.96	0.37	0.08	0.67	*	200	100	79	88
39 (i)	2.47	0.92	0.93	0.89	0.98	0.29	0.04	0.54	*	172	86	83.2	94.6
39 (ii)	1.76	0.95	0.97	0.94	1.007	0.17	-0.042	0.37	ns	148	74	87.2	94.6
49	3.8	0.87	0.93	0.88	0.98	0.2	-0.1	0.5	*	200	100	81	87.5
49 (i)	2.35	0.91	0.95	0.9	0.99	0.15	-0.11	0.42	*	158	79	87.3	91.1
49 (ii)	0.9	0.96	0.97	0.93	0.99	0.12	-0.06	0.31	*	132	66	96.2	97
59	4.02	0.87	0.98	0.91	1.02	0.08	-0.23	0.34	ns	200	100	75	86.5
59 (i)	3.46	0.92	1	0.95	1.04	-0.07	-0.33	0.19	ns	171	85.5	80.7	90.1
59 (ii)	2.89	0.93	0.99	0.94	1.04	-0.02	-0.27	0.23	ns	146	73	85.6	91.1

Table 9. Comparison of observed and predicted ages for networks on samples of *M. novaezelandiae* for complete and screened output data, including average percent error (APE), regression coefficients and 95% confidence limits. P = */ns if either slope or intercept are/not significantly different from 1 or 0 respectively.

Network	APE	R^2	Regression statistics						P	N	% Classified	% Correct	% Within
(Sreen type)			Slope	L 95%	U 95%	Intercept	L. 95%	U 95%					One Year
39	18.68	0.02	0.11	-0.004	0.23	8.03	6.95	9.11	*	200	100	11.5	29.5
39 (i)	16.13	0.23	0.34	0.16	0.52	6.89	5.26	8.52	*	52	26	11.5	25
39 (ii)	16.44	0.53	0.51	0.17	0.84	5.85	2.97	8.73	*	12	6	8.3	25
49	24.91	0.04	-0.21	-0.35	-0.07	10.8	9.51	12.1	*	200	100	8.5	19.5
49 (i)	20.1	0.002	-0.05	-0.22	0.22	8.97	6.41	11.53	*	66	33	10.6	22.7
49 (ii)	25.37	0.02	-0.15	-0.61	0.3	10.68	6.36	15	*	24	12	4.2	16.7
59	18.64	0.08	0.26	0.13	0.39	6.24	5.07	7.42	*	200	100	8	29.5
59 (i)	19.7	0.05	0.21	-0.01	0.43	7.15	5.14	9.16	*	66	33	9.1	25.8
59 (ii)	17.22	0.16	0.62	-0.02	1.26	3.72	-2.62	10.62	ns	22	11	4.5	31.8

Table 10. Age difference table for the three networks trialed for *P. auratus*. Values are percentages of each observed age class. Difference is observed age - predicted age.

Network	Difference	Observed age									
		2	3	4	5	6	8	9	10	12	All
39H	-4					7					1
	-3							20			2
	-2	9					7		15		4
	-1			36	12			30			9
	0	**91**	**92**	**32**	**76**	**87**	**87**	**45**	**70**	**60**	**72**
	1		8	20		7					4
	2			12	8				10	10	5
	3				4						1
	4						7			15	2
	5							5			1
	6								5	10	2
	7									5	1
49H	-5				4						1
	-3							20			2
	-2						13		20		3
	-1			8	12			35			6
	0	**91**	**76**	**56**	**76**	**80**	**73**	**40**	**80**	**65**	**72**
	1	9	24	36				5			10
	2				4		13				2
	3				4	7					1
	4					13				20	3
	10									15	2
59H	-3							20			2
	-2								10		1
	-1			20	24		7	30			9
	0	**94**	**96**	**68**	**72**	**80**	**87**	**45**	**70**	**55**	**76**
	1	6	4	12		13		5			5
	2				4				10		2
	3						7				1
	4					7			10	30	5
	7									5	1
	8									5	1
	10									5	1
N		35	25	25	25	15	15	20	20	20	200

Table 11. Age difference table for the three networks trialed for *A. butcheri*. Values are percentages of each observed age class. Difference is observed age - predicted age.

Network	Difference	Observed age								
		1	2	4	5	6	7	8	9	All
39H	-3		12							2
	-2		8				4			2
	-1			4		33		20		8
	0	**100**	**80**	**84**	**100**	**63**	**69**	**63**	**75**	**79**
	1						12			2
	2			12			12	10	10	6
	3						4		15	2
	4					4		7		2
49H	-3		4			8				2
	-1			4		13		20		5
	0	**100**	**92**	**80**	**96**	**71**	**65**	**63**	**85**	**81**
	1						12			2
	2			16		8	12	10	5	7
	3				4		12		10	3
	4							7		1
59H	-3					13				2
	-2		8				12			3
	-1					33		30		9
	0	**100**	**92**	**80**	**88**	**54**	**50**	**63**	**75**	**75**
	1						23			3
	2			20			8	3	10	5
	3				12		8		15	4
	4							3		1
N		25	25	25	25	24	26	30	20	200

Table 12. Age difference table for the three networks trialed for *M. novaezelandiae*. Values are percentages of each observed age class. Difference is observed age - predicted age.

Network	Difference	Observed age											
		4	5	6	7	8	9	10	11	12	13	14	All
39H	-6	20											2
	-5		12										2
	-4			20									2
	-3	13											1
	-2	27	8			10							4
	-1	33	16	10			13						7
	0	**7**	**52**		**13**			**35**					**12**
	1			70		5			40				11
	2		8		47	50			27	40			17
	3				7		33	10		4	24		8
	4		4			25	27	25	7				8
	5				7		13	10	13				4
	6				27	10			7	28			7
	7						7	5		4	36		6
	8						7	10		12	16		5
	9							5	7		8	40	3
	10									12			2
	11										12		2
	12										4		1
	13											60	2
49H	-6	13											1
	-5		24										3
	-4			5									1
	-3	47			53								8
	-2	13	64			35							13
	-1			70			20						9
	0	**7**			**20**	**5**		**25**					**5**
	1	13				10			27				4
	2	7		15		5				44			8
	3			10	13		27	25			32		11
	4		12		13	30		25					8
	5					15							2
	6						13			8			2
	7							25	40		16		8
	8						40			16			5
	9								7		8		2
	10								27		24		5
	11									32	4		5
	12										16	20	3
	13											80	2

Table 12 (Cont'd). Age difference table for the three networks trialed for *M. novaezelandiae*. Values are percentages of each observed age class. Difference is observed age - predicted age.

Network	Difference	Observed age											
		4	5	6	7	8	9	10	11	12	13	14	All
59H	-6	7											1
	-5		4										1
	-3	7			20								2
	-2	20				15							3
	-1	53					33						7
	0		**64**		**20**			**40**					**14**
	1			85	7	20			40				14
	2					35	7			28			8
	3	13			7		20	10			20		7
	4		32			15	20	20	7	4		20	11
	5			15		5			27	8			5
	6				47		7	15	7	44			12
	7					10					36		6
	8						13			4	16	20	4
	9							15		4	8		3
	10								20		4		2
	11									8			1
	12										16		2
	13											60	2
N		15	25	20	15	20	15	20	15	25	25	5	200

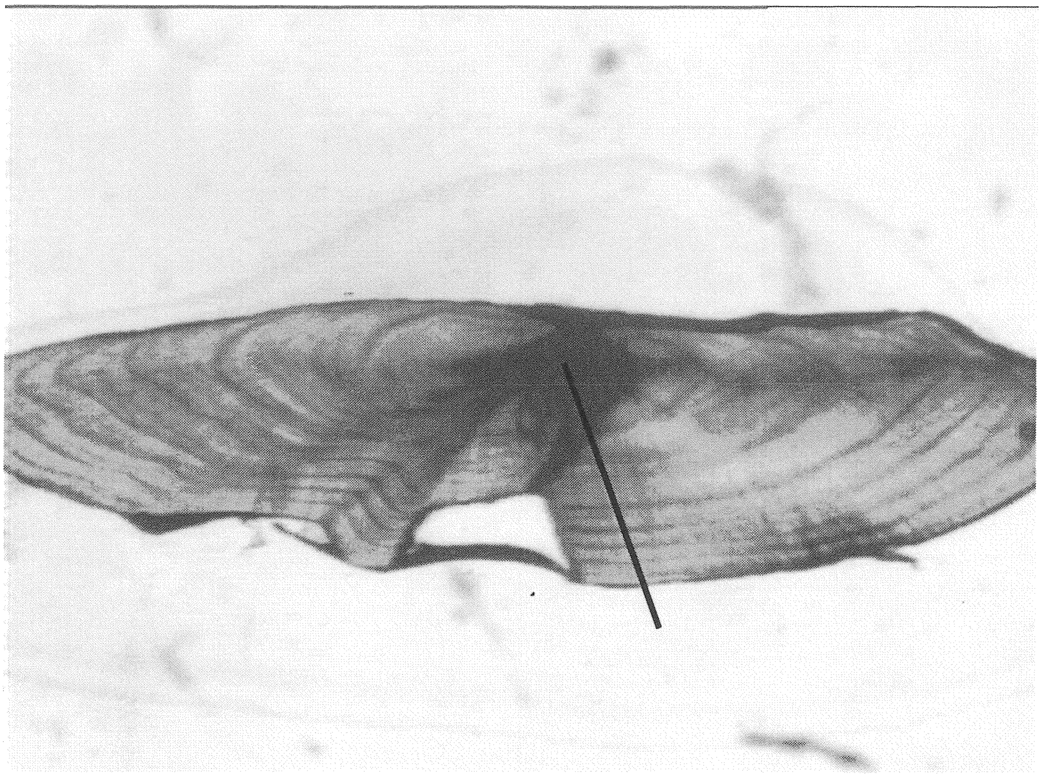


Figure 1. Transverse section of otolith of *A. butcheri* viewed with transmitted light showing transect used for age estimation and extraction of pixel luminance values.

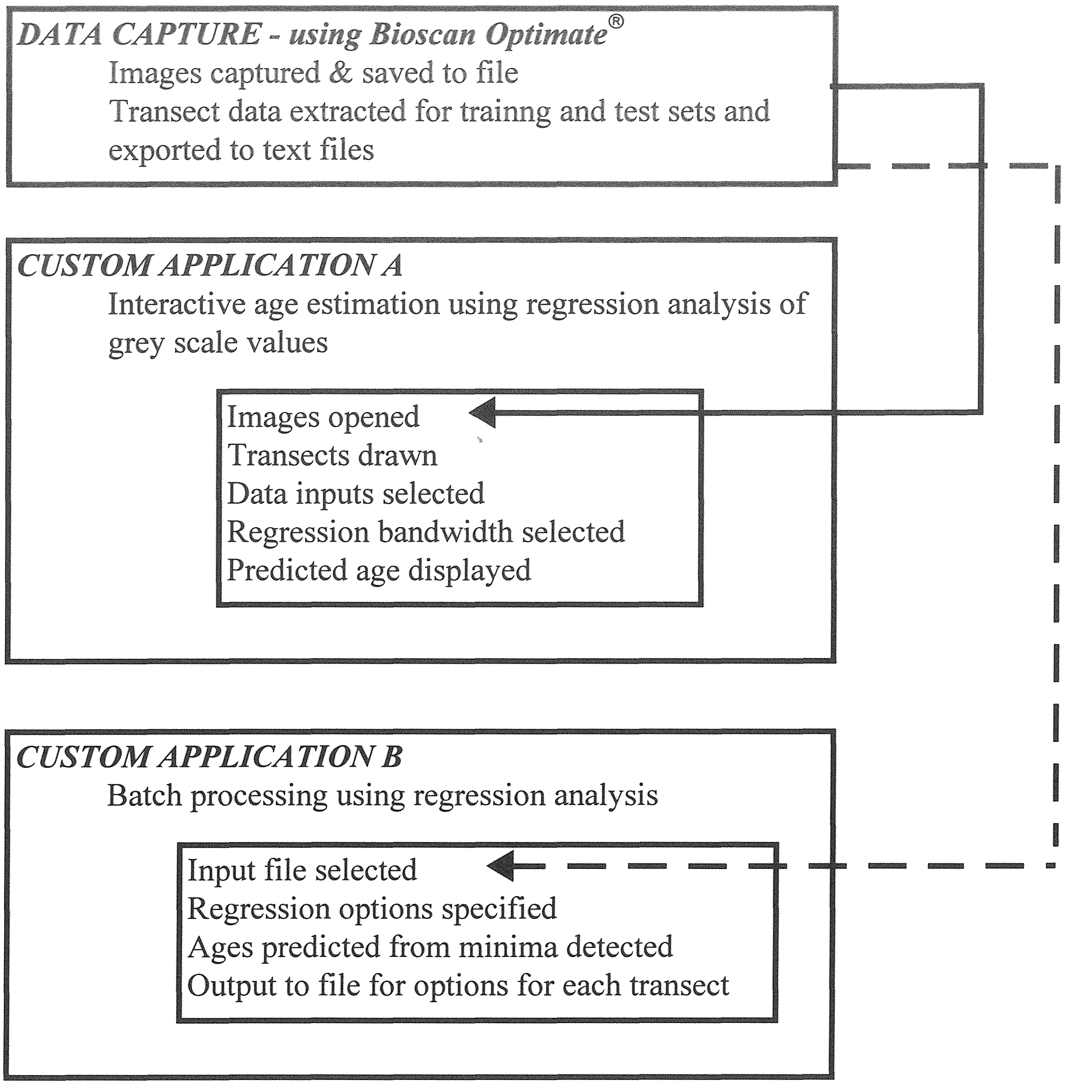


Figure 2. Data flow between components of automatic ageing system using regression analysis of transects. Solid lines represent transfer of images, dashed lines represent transfer of data files.

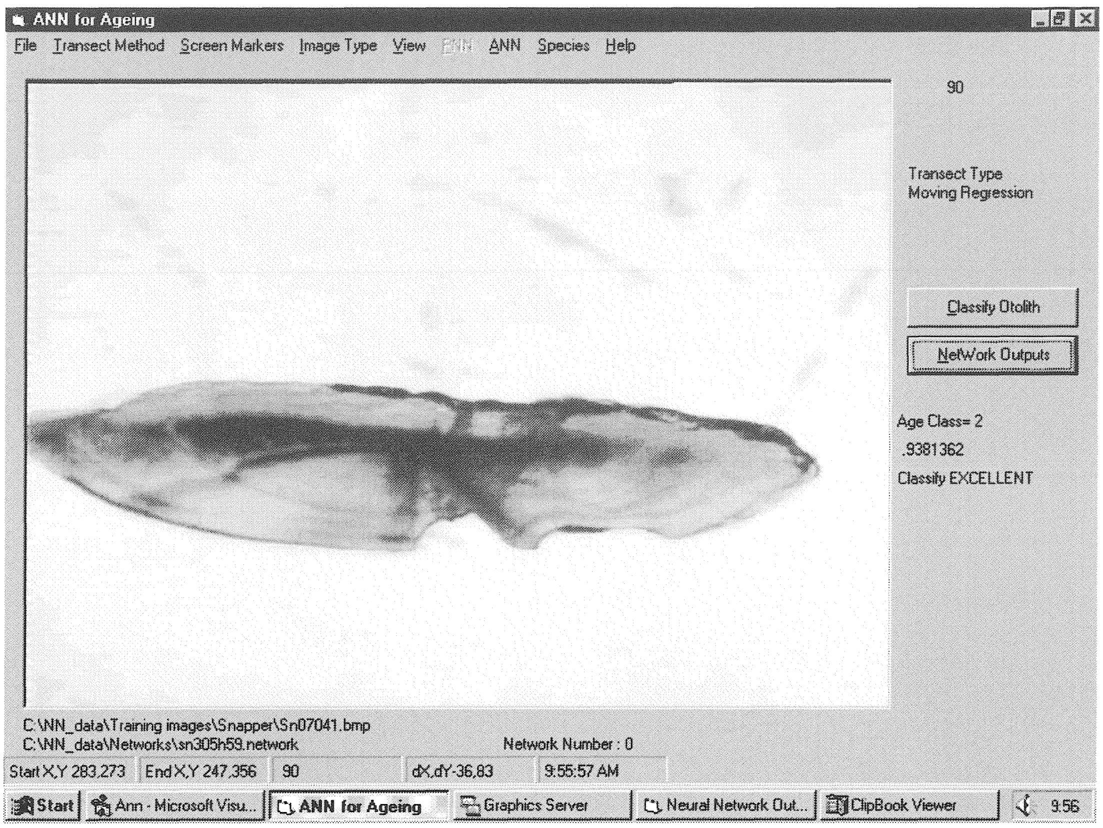


Figure 3. Input screen from Custom Application A (see Figure 2) which allows images to be opened, transects drawn, and luminence values extracted.

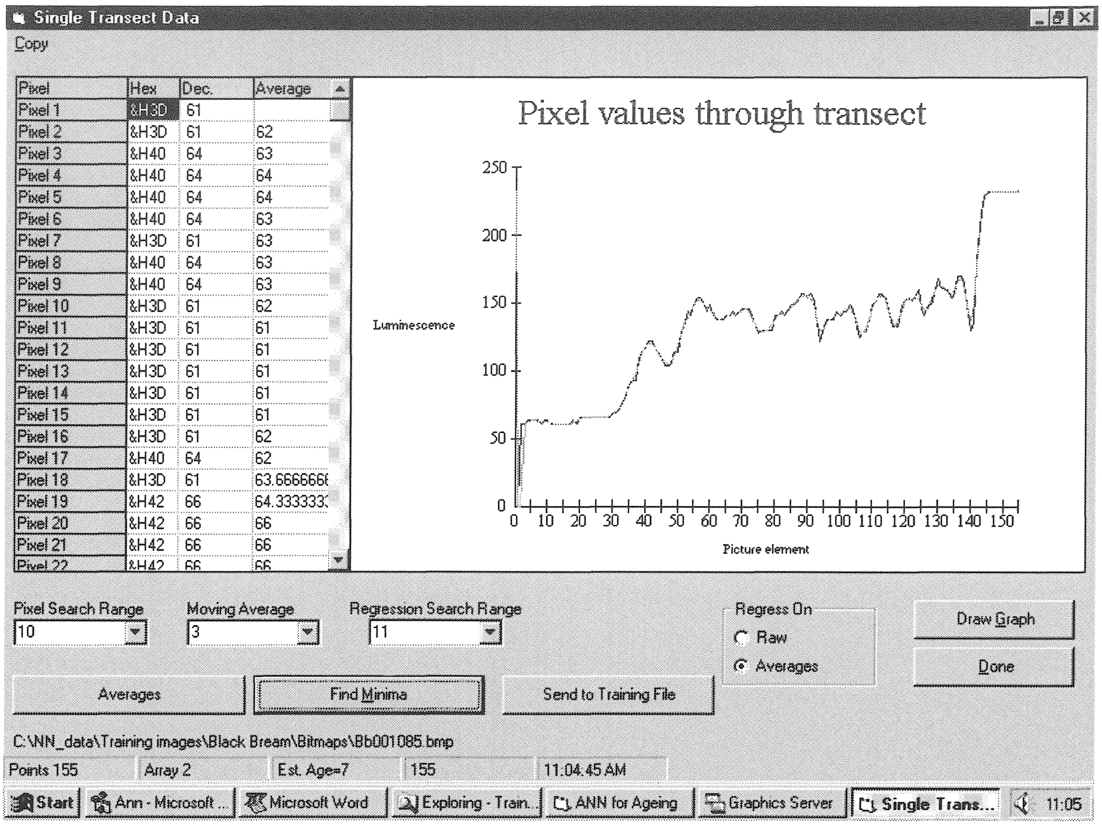


Figure 4. Output screen of Custom Application A (see Figure 2). Raw luminance values across transect are plotted together with a smoothed data set. Drop-down boxes allow selection of options for a pixel search range, data smoothing (Moving Average), and regression search width. Option buttons allow selection of regression on the raw or smoothed (Averages) data. The Find Minima button then causes execution of the program which determines the number of opaque zones according to the selected criteria. The estimated age is displayed in a box at the bottom of the screen.

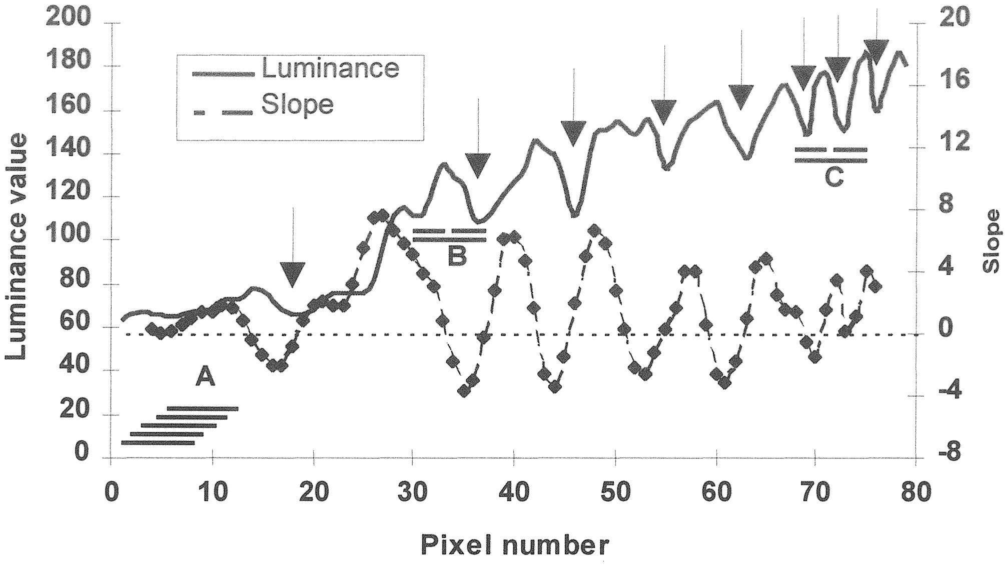


Figure 5. Example of transect of luminance values (solid line) showing the location of each annual increment (arrowed) and the calculated slope over successive widths of 7 pixels (dashed line). A: the first 5 intervals over which slope is calculated for a 7 pixel moving regression, B: an area where a wider search width will correctly overlook a false increment but a narrow search width will not, C: an area where a wide search width will incorrectly detect a single increment but a narrow search width will correctly detect two increments.

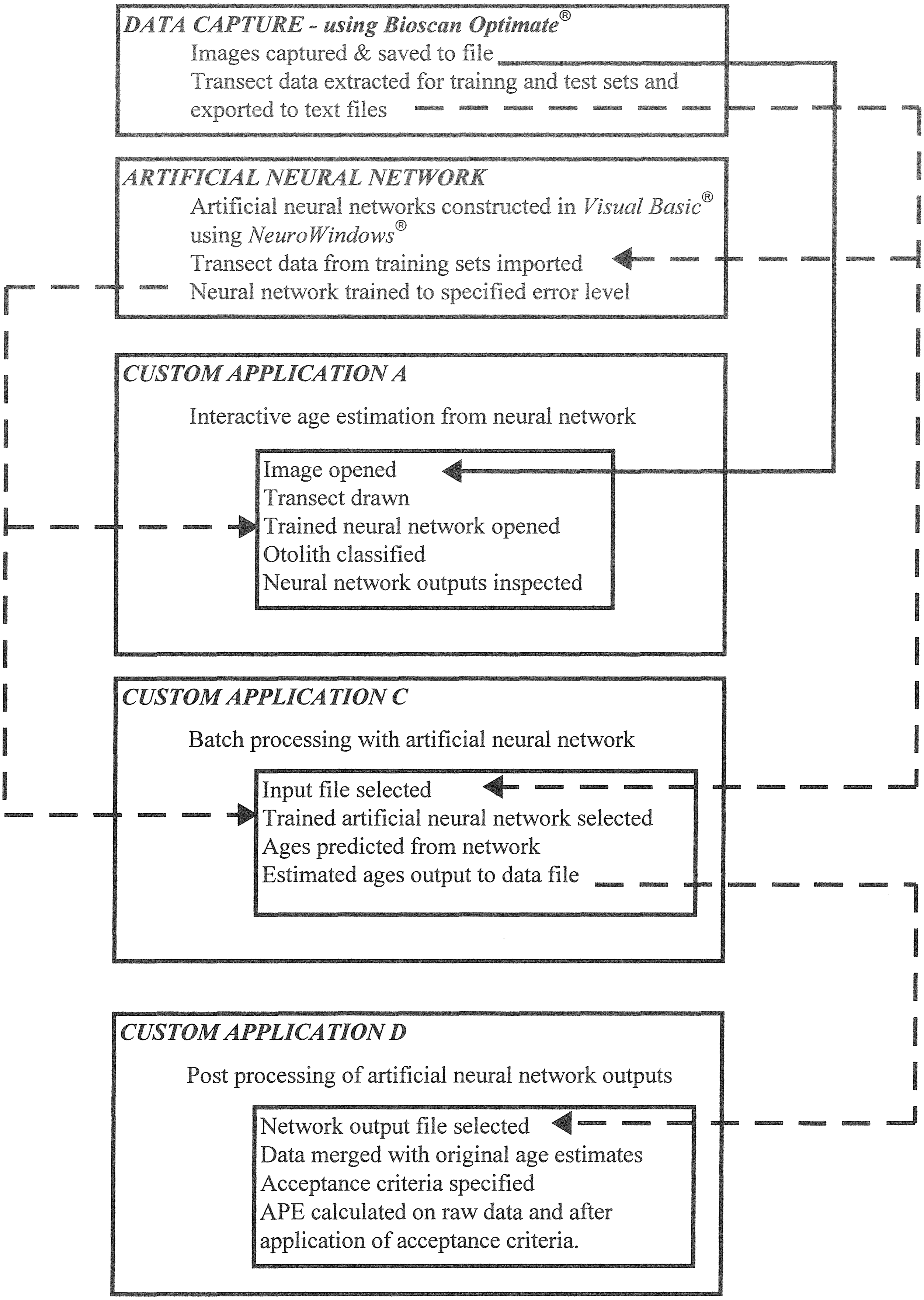


Figure 6. Data flow between components of automatic ageing system using Artificial Neural Networks. Solid lines represent transfer of images, dashed lines represent transfer of data or neural network.

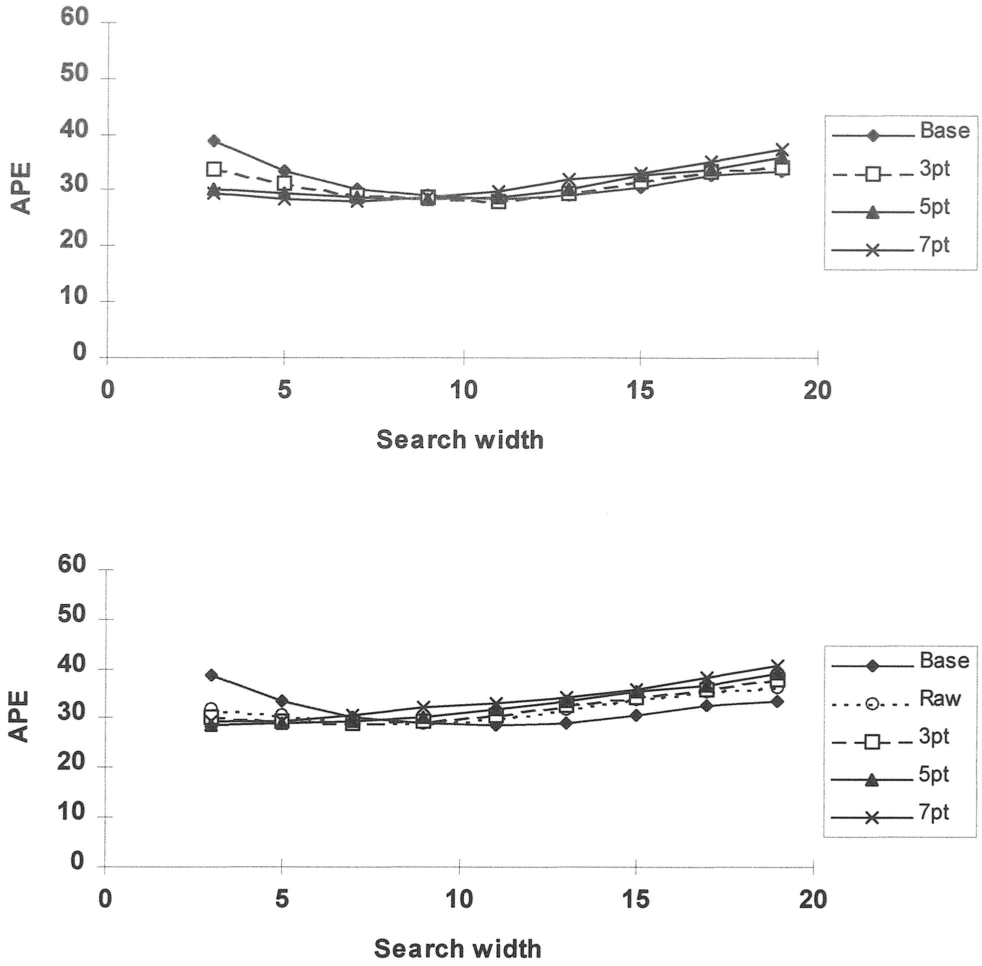
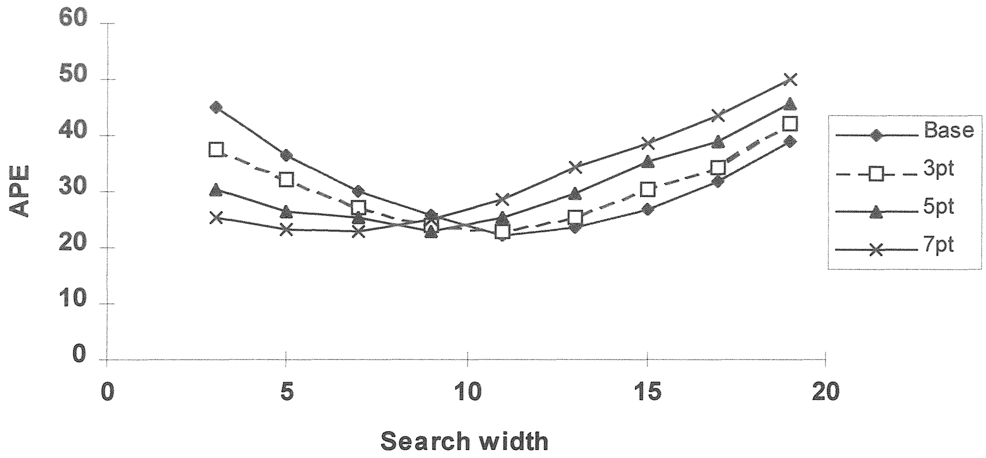


Figure 7. APE for ages of *P. auratus* predicted from transects versus original ages, by regression searchwidth and data smoothing, for no a priori width of first zone (top) and for an a priori width of 40 pixels (bottom). Base case is for no *a priori* width and no data smoothing.



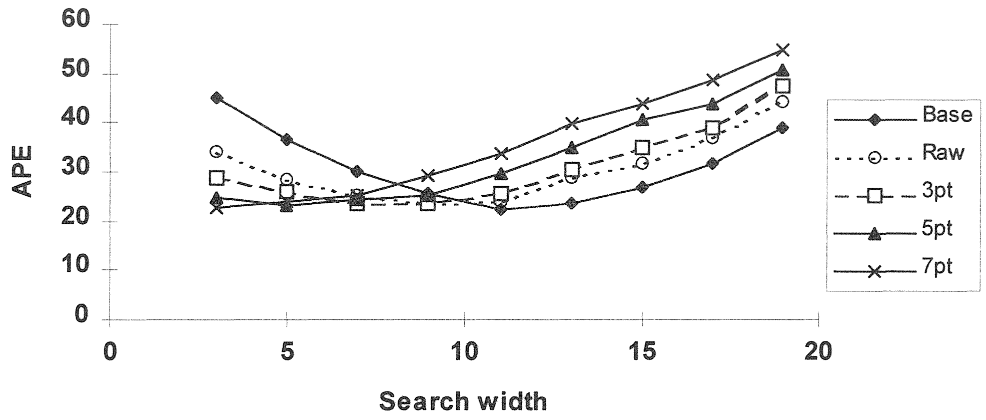


Figure 8. APE for ages of *A. butcheri* predicted from transects versus original ages, by regression searchwidth and data smoothing, for no a priori width of first zone (top) and for an a priori width of 40 pixels (bottom). Base case is for no *a priori* width and no data smoothing.

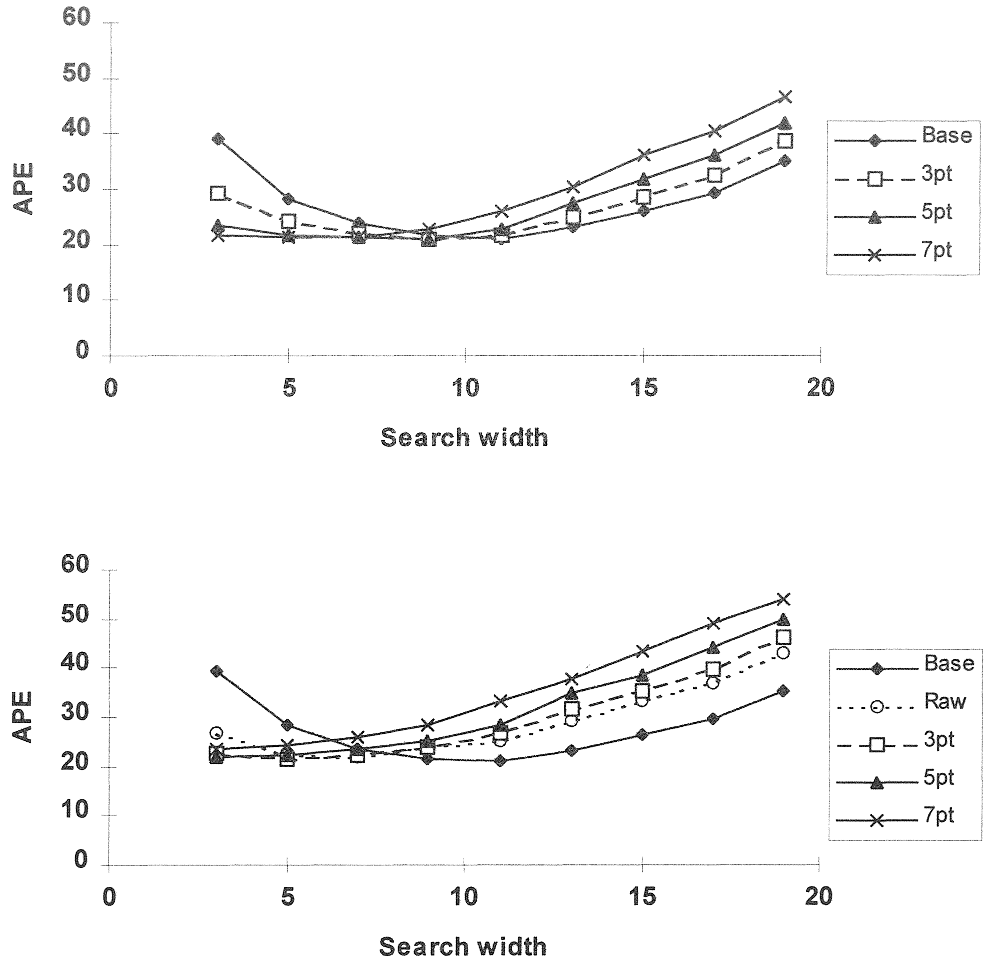


Figure 9. APE for ages of *M. novaezelandiae* predicted from transects versus original ages, by regression searchwidth and data smoothing, for no a priori width of first zone (top) and for an a priori width of 40 pixels (bottom). Base case is for no *a priori* width and no data smoothing.

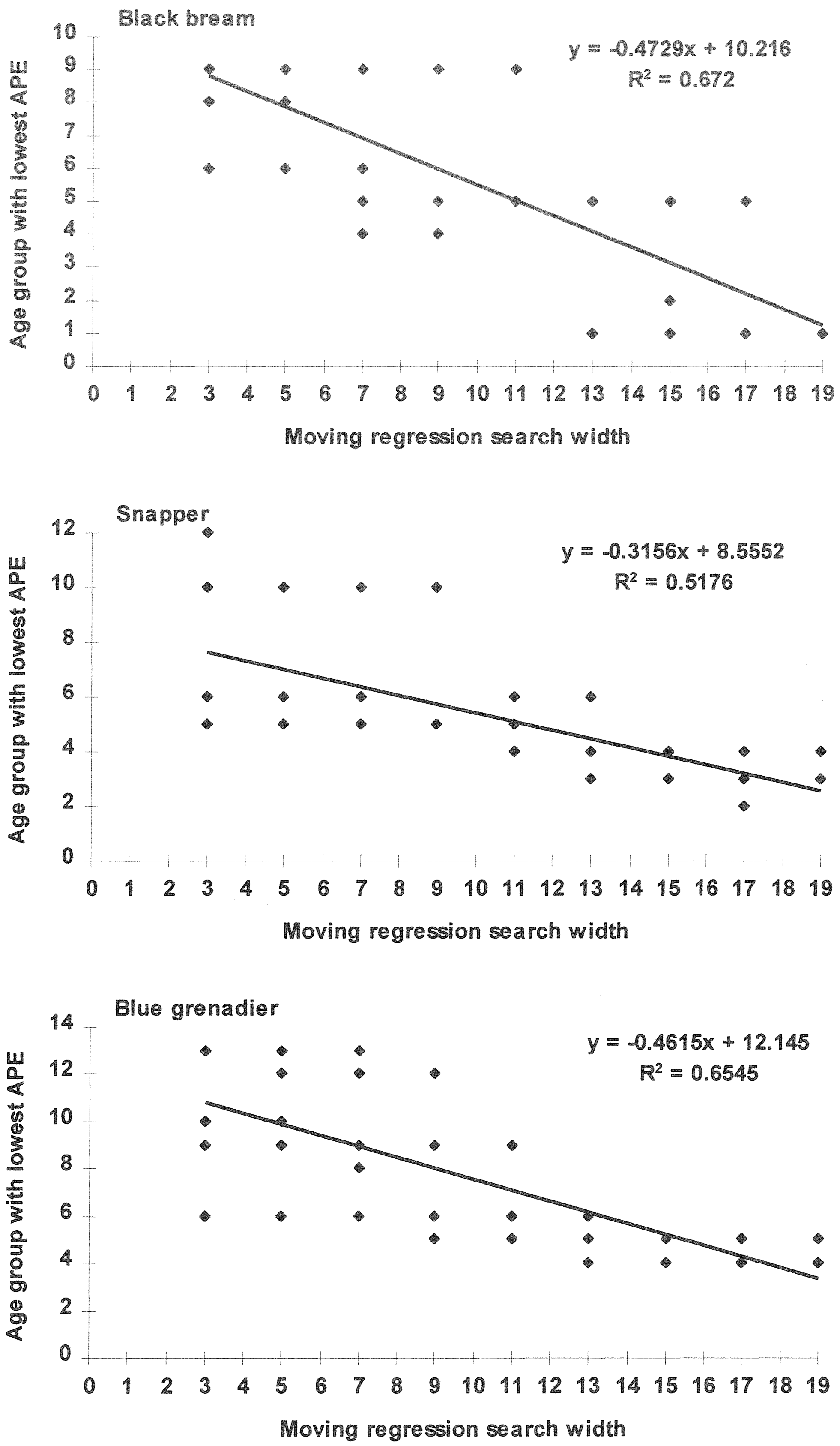


Figure 10. Age group with lowest APE against search width (in pixels) of moving regression for all possible combinations of a priori first increment width and data smoothing.

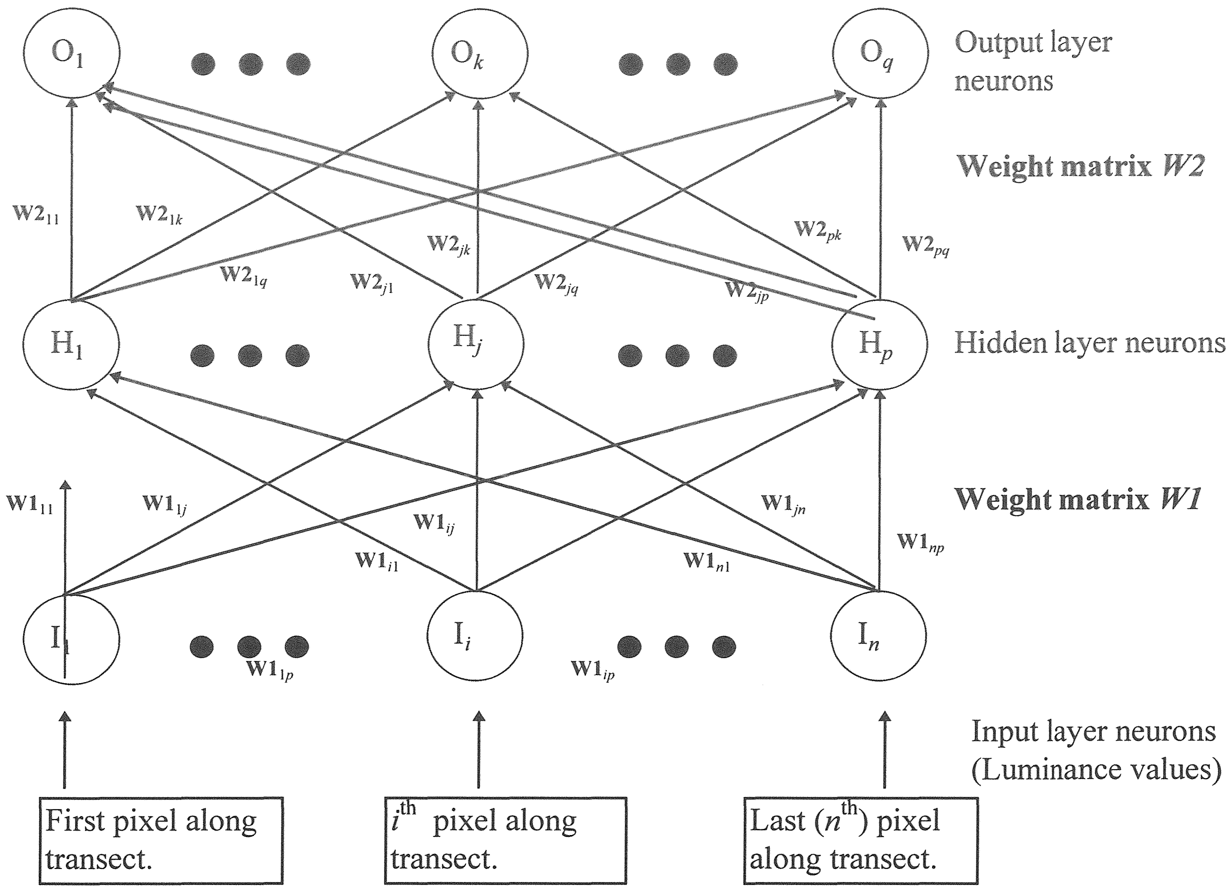


Figure 11. Structure of a three layer feed-forward back-propagation neural network as used in the present study (after Blum 1992). Note that a bias processor on both the input and hidden layers are not shown.

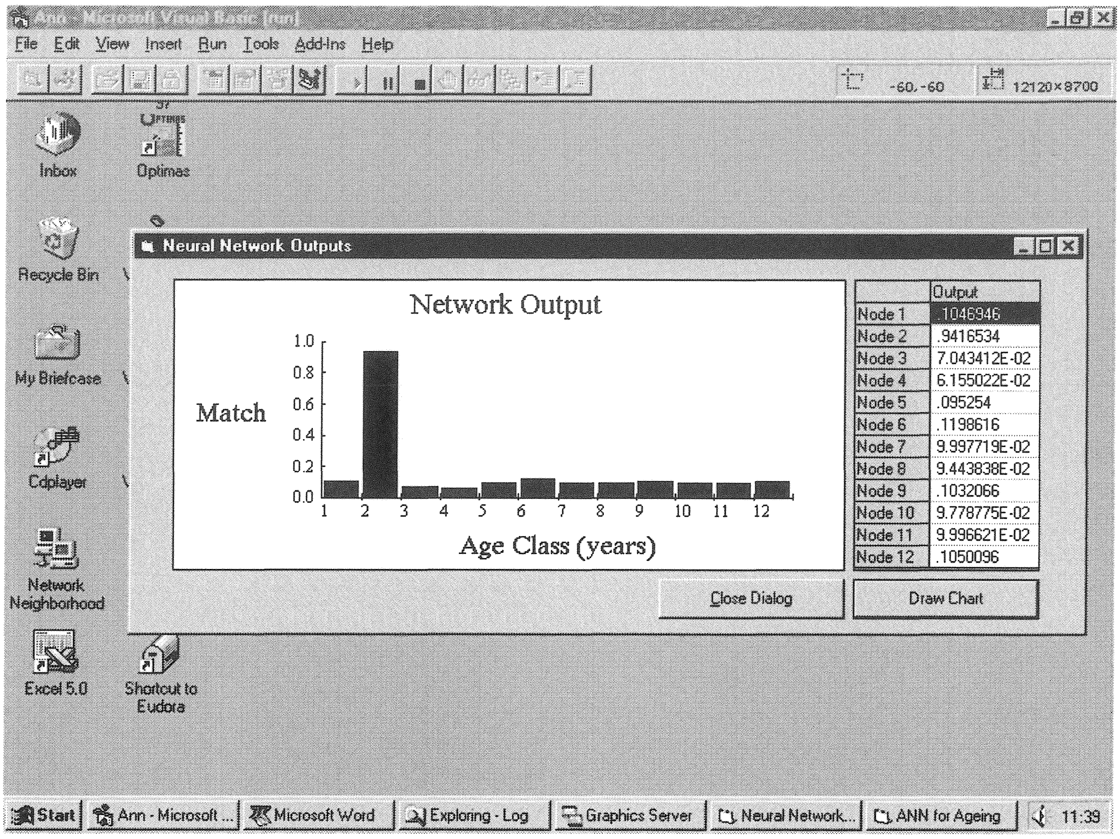


Figure 12. Example of output screen from ANN showing a network output array with a clear classification of age 2. Note that second most favoured classification is age 6.

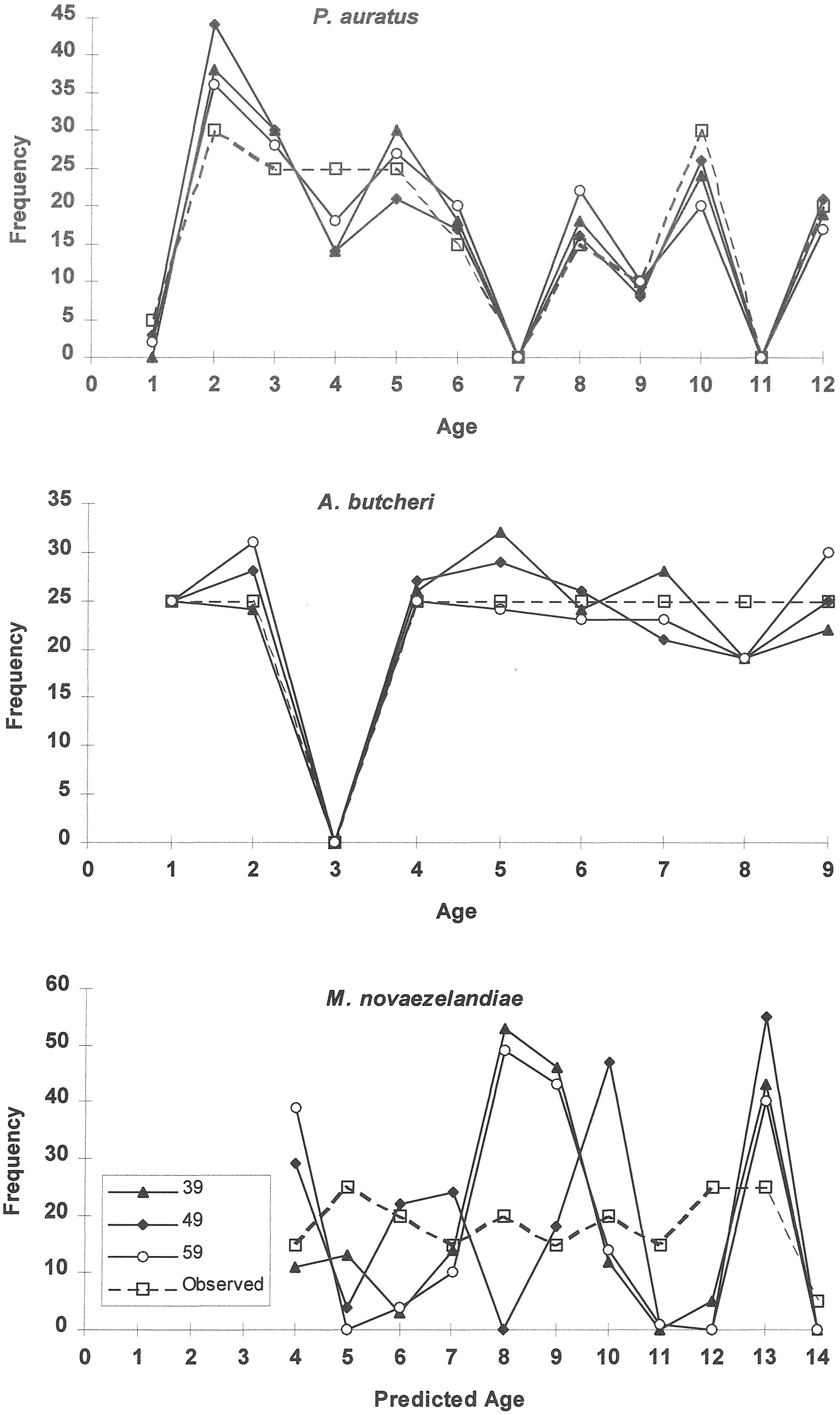


Figure 13. Age composition from observed and predicted ages for each network type for each species.

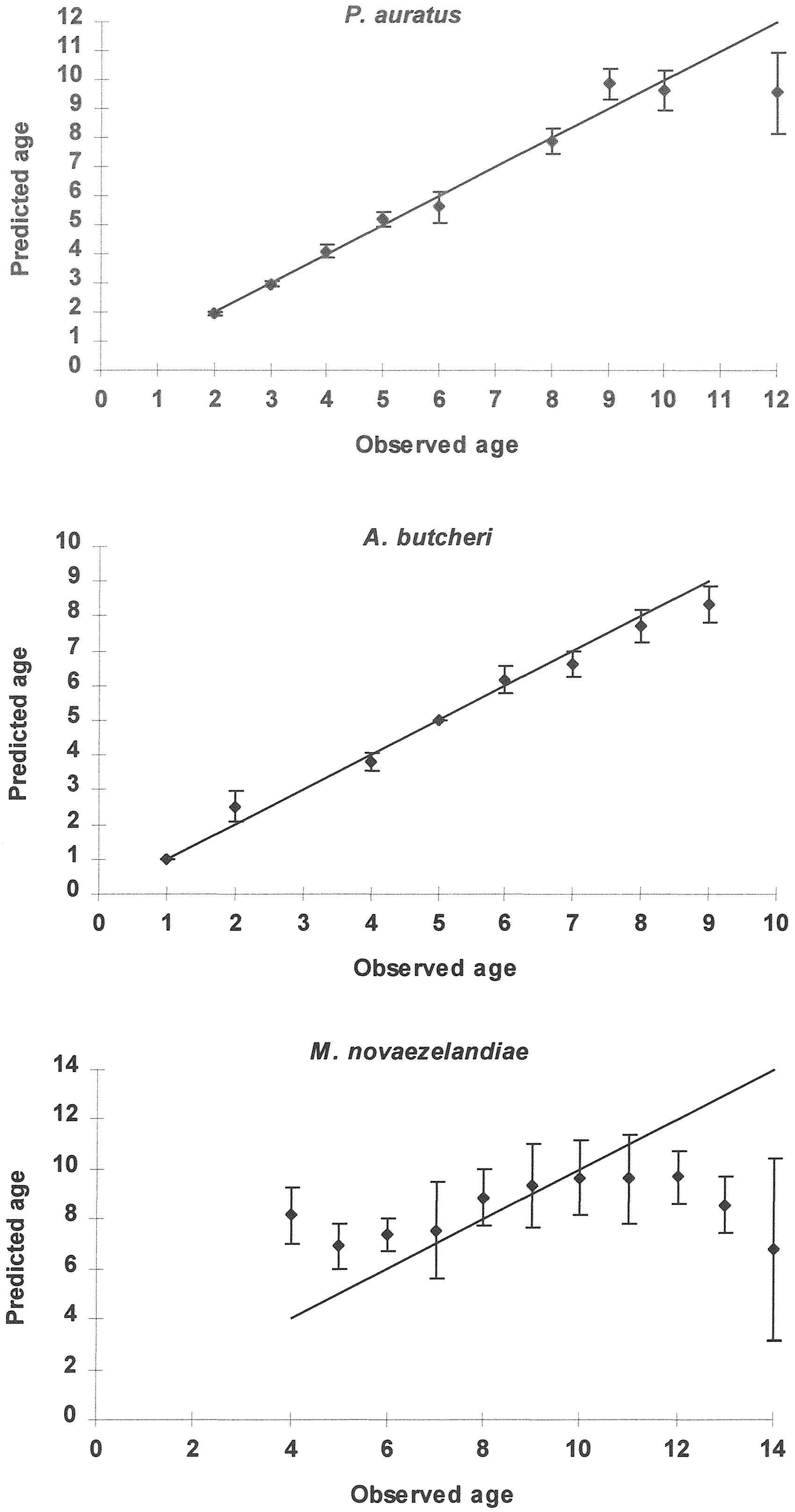


Figure 14. Age bias plots for the best fitting model for each species: 59H for *P. auratus*, 39H for *A. butcheri*, and 59H for *M. novaezelandiae*. Points are means +/- 2 standard errors. Line represents equal age estimates.

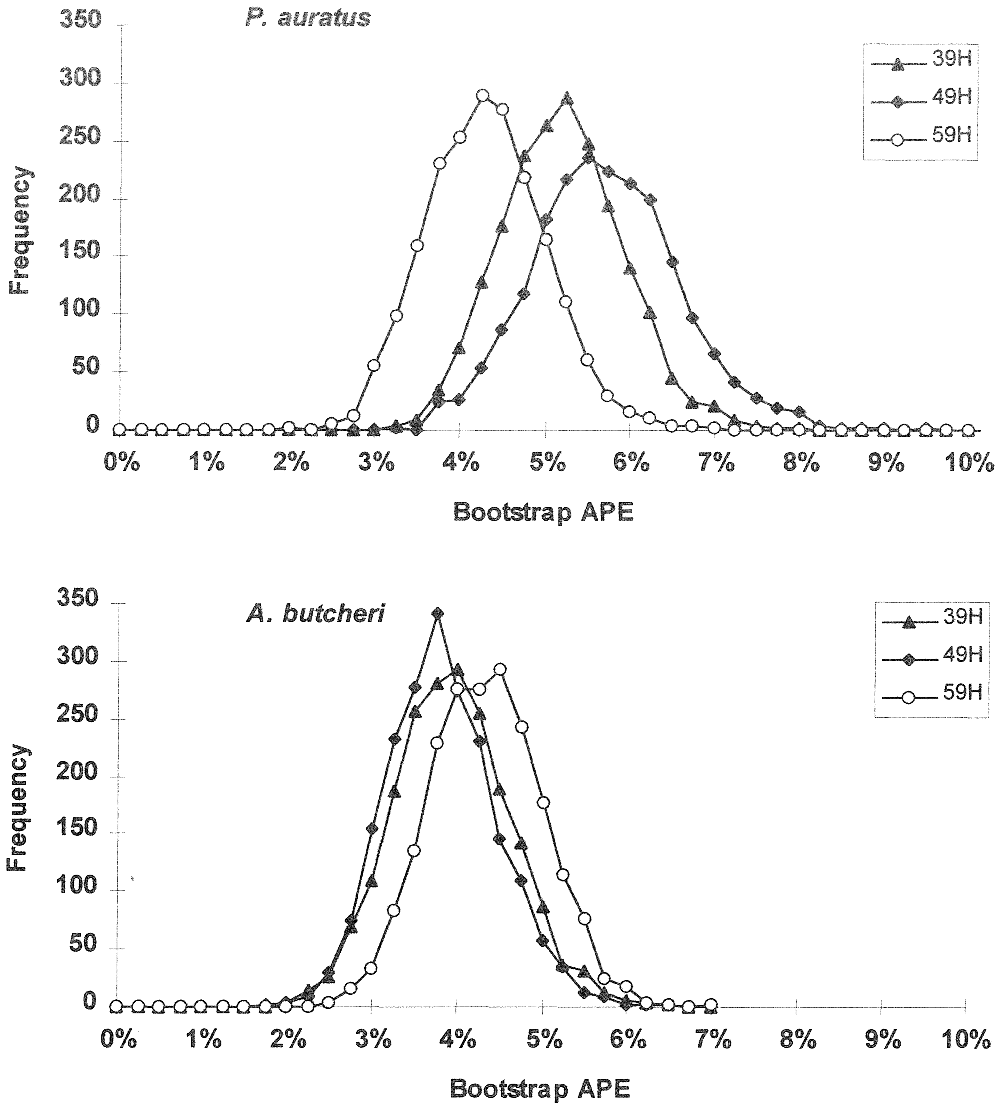


Figure 15. Distribution of bootstrapped APE values from each network type for each species.

Discussion

Of the methods tested in this project, the ANN offers the most obvious potential to fully automate the process of age estimation as used for production ageing. It adopts a fundamentally different approach to the problem than those used in previously published methods. As implemented in this pilot study, the ANN does not seek to provide an age estimate by identifying and counting individual increments on each captured image. Instead, it seeks to reproduce the age estimate through a numerical model which allows it to classify each type (age class) of input. In classifying each otolith the ANN is trained to match the decisions made by an expert reader - as presented to the ANN as the array of observed ages in the training set. This approach is particularly suited to ongoing production ageing, where a key concern is the maintenance of accuracy.

The transect methods were unsuccessful in providing age estimates with an acceptable level of accuracy. The variable width of the increments provided a major source of the error. Troadec (1991) used a combination of composite radial transects, a frequency demodulation technique, and spectral analysis, to overcome this problem on clear daily increments. However, on estimates of age in years we calculated the APE on his results for saithe otoliths (*Pollachius virens*) to be 6.2% and 16.3% for the two different data processing methods employed. The lower APE values obtained by the ANNs for *P. auratus* and *A. butcheri* suggest superior predictive power. Although such a comparison depends on the relative clarity of the increments on each of the species, Troadec (1991) states that the saithe otolith has no peculiar reading difficulties.

The neural network classified fish from the training set with a high degree of accuracy, and to an acceptable level with unseen data for two out of the three species examined. The two species for which the method was successful (*P. auratus* and *A. butcheri*) are those with the clearest patterns of increments in their otoliths. *M. novaezelandiae* has otoliths which are more difficult to read even for an experienced person. This is reflected in the APE values which are obtained by readers on repeat readings of otoliths of these species (0.34%, 0.39% and 4.6% respectively, Morison *et al.,* in press). The best performed networks produced APEs which were higher than these, but were sufficiently low for two species to indicate the potential of the method.

The transects used in the test set for the ANN were sampled from the sections from which the training set was drawn. A requirement of the ANN model is that the variability of inputs between age classes is greater than the variability between samples within age classes. In order for the model to correctly assign an age class, samples used for the training must also be representative of the patterns in the sample of unseen data. Samples which do not have the pattern of growth that was represented in the training set will not be accurately predicted by the model. Samples not described by the model will have a low maximum array value in the model output and can therefore be rejected by screening. These samples can then be examined by an experienced reader to assign an age class. In future developments of ANNs such samples could be added to the training set to improve future performance of a network.

The failure of the ANN model to predict ages for *M. novaezelandiae* could also indicate that the variability between samples within each age class was higher than the variability of samples between age classes.

One of the valuable aspects of the application of the neural networks to fish ageing is the quantitative model output for each estimated age. This provides a measure of the confidence in individual estimates of age, whereas ageing error estimated from repeat readings only provide a measure of overall precision. These output values are also the basis for the screening process. The screening of outputs used in this ANN model reduced the APE in both *P. auratus* and *A. butcheri*. Of the two components of the screening criteria, the threshold level had the greater influence on model performance. The value of the next highest array value in the network output accounted for less than 6% of the complete test set for *P. auratus* and less than 16.5% for *A. butcheri* using the type (ii) screening. Tables 7 and 8 suggests that with an optimal number of hidden neurons, no significant difference between the observed and predicted age classes was evident. The effect of screening is a reduction in the APE through the elimination of samples not adequately described by the model. However, this increased agreement is obtained at the cost of a reduction in the number of samples classified.

As they were implemented in the present study, the ANNs treat each of the elements in the input array as independent data values and therefore assumes no periodicity or cyclical pattern. Future experiments with neural networks will endeavour to supplement this approach by examining the luminance array in the frequency domain. This approach has been used successfully in the classification of two co-occurring species of *Ceratium* spp. from the North Atlantic (Simpson *et al*., 1992).

One of the problems with the ANNs as used in this study is the difficulty in determining the optimal number of neurons to use in the hidden layer. If too many are used, the model will train well but may 'memorize' or overfit to the training data and give unreliable estimates on unseen data. If too few hidden neurons are used, the model will be unable to generalize. Three different models differing in numbers of hidden neurons were used in this study. The results show that model 49H (with 49 hidden neurons) performed best for *P. auratus* model 59H best suited the transect data from *A. butcheri*. This suggests that the optimal number of hidden neurons in an ANN is species dependent. A sub-optimal number of hidden neurons may have contributed to the failure of the model to classify *M. novaezelandiae* transects.

Differences among species and network types in the frequency distributions of the bootstrapped APEs for both the training and test sets emphasise the importance of finding the optimal network structure for each species. For *P. auratus* the 49H network had the lowest APE of the three network structures, but for *A. butcheri* network 59H was superior. For both these species, the model with the lowest APE after training, also performed best on the test data. This suggests that precision in training is a good predictor of performance on unseen data.

ANNs are a new approach to the problem of automating the estimation of fish age. Previous approaches (e.g. Troadec, 1991; Welleman and Storbeck, 1995; Cailliet *et al.,* 1996) have tried to replicate the process of identifying and counting increments and have had limited success or have required the process to be overseen by an experienced reader. The ANNs implemented in the present study were presented with arrays of independent numbers (the luminance values of the transects) and developed the network from these inputs during training to match the required outputs (age classes). Once trained, the network provides a completely reproducible and objective means of estimating the age of a sample which requires no involvement from an experienced reader. The success of the approach lies partly in the flexibility of the network in fitting itself to the input data.

This approach has other advantages over traditional age determination methods in two distinct ways, the model provides a quantified level of confidence for each individual age estimate and outputs can be screened to discriminate samples for which this confidence is low.

The transect methods trialed which identify and count opaque increments from luminance values are theoretically applicable to any otoliths of any species, whereas each ANN is specific to the species presented in the training set. However, in practice, the transect methods have other limitations which have hindered their performance and required manual intervention when applied. Such methods ar not automatic to the extent that they require a trained reader to check on the classification of each sample. While each individual ANN is species (or training set) specific, an ANN can be trained to work on inputs of a range of species. Also, the ANN offers the added benefit that it provides a measure of the degree of certainty of the classification of each sample. A reader is not required to check each sample. The ANN approach also offers flexibility in the form of data inputs, and can be developed to incorporate a range of other data in a similar manner to the multiple regression approach of Boehlert (1985).

Benefits

The fishing industry will directly benefit from this research through reduced cost and greater objectivity in sample age determination. The time and costs involved in age estimation from key species will be reduced. This technology will be translated into higher and more accurate throughput of samples. This will, in turn, lead to a more accurate stock assessment tool with the potential for increased numbers of commercially important species being examined.

Further Developments

The approach of using a neural network can be further developed and refined to increase network performance. In particular improvements in the form of data inputs to the models would most likely lead to greater accuracy in estimated ages.

Final Cost

Refer to separate statement of Receipts and Expenditure.

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Appendix 1

Intellectual Property and Valuable Information

No Intellectual Property of commercial importance has been developed from this project. However, the approach of using a neural network for the problem of objectively ageing fish, is novel and will generate considerable interest in the fisheries science community. The manuscript which has been developed from this project will, when published, ensure the work funded by the FRDC will be exposed to this community.

Appendix 2

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