Length-frequency Data for Tuna Baitfish
(Encrasicholina heterolobus and Spratelloides delicatulus) and the Cardinal Fish (Apogon rueppellii):
guidelines for employing computer packages to use length-frequency data to analyse age composition and growth of fish populations

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The management of fisheries resources is dependent on an understanding of the population dynamics of the species comprising those resources (Pauly 1987, Smith 1993). Furthermore, knowledge of the population dynamics of any given species cannot be acquired without first obtaining reliable information on the age and growth of that species (Casselman 1987, Summerfelt and Hall 1987).

The age of fish is traditionally determined by counting the number of annually-formed growth zones (annuli) on hard structures, such as scales, otoliths or spines (Casselman 1987, Beamish and McFarlane 1983, Hyndes et al. 1992). However, annuli are sometimes not formed regularly on such structures or are difficult to discern. Furthermore, under certain circumstances, it may not be feasible to extract, prepare and examine these hard structures. In these cases, the age composition of populations are often obtained by analysing the modes in length-frequency distributions determined for samples collected at regular intervals (Pauly 1987, Pauly and Morgan 1987).

During recent years, microcomputers have increasingly been used to analyse fisheries data. This has led to the development of a number of computer packages aimed specifically at analysing length-frequency data for fish populations in order to determine the age composition and pattern of growth in those populations. These computer packages include MIX, which analyses each individual set of length-frequency data independently from those calculated for the preceding and following samples, and ELEFAN and MULTIFAN which analyse the trends shown by modes in sequential sets of length-frequency distributions (Gayanilo et al. 1989; Fournier and Sibert 1990, Macdonald and Green 1990).

ELEFAN has been widely used for determining the age and growth of tuna baitfish populations (e.g. Dalzell and Wankowski 1980, Dalzell 1984, Dalzell et al. 1987, Tiroba et al. 1990). However it is now apparent that the growth parameters, and particularly $K$, calculated using ELEFAN for data on populations of the tuna baitfish Encrasicholina heterolobus and Spratelloides delicatulus in the Solomon Islands, differ from those calculated using the growth zones that are formed daily on otoliths (Milton et al. 1990, Milton et al. 1991, Milton and Blaber 1991).

The present paper demonstrates that length-frequency data collected for E. heterolobus and S. delicatulus are inappropriate for analysis by computer packages. The reasons for this inappropriateness are discussed. The length-frequency data collected for Apogon rueppellii, which show conspicuous and consistent modes in sequential monthly samples (Chrystal et al. 1985), have been analysed using MIX, ELEFAN and MULTIFAN to illustrate the type of data that is required to use these packages. Apogon

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rueppellii was also chosen because, like the above tuna baitfish, it occurs in the Indo-Pacific region (Allen and Cross 1989) and has a relatively short life cycle (c.f. Chrystal et al. 1985, Milton and Blaber 1991). The results obtained with A. rueppellii highlight the essential requirements of length-frequency data for use in age and growth studies, and emphasise the importance of selecting an appropriate sampling regime and considering the possibility that growth may be seasonal and vary with sex. Finally, guidelines are given for the requirements that should be met to produce and analyse length-frequency data by computer packages.

Materials and Methods

Tuna baitfish

Fish species used as tuna bait were collected at the Munda baitground in the Roviana Lagoon, and in the southern part of the similar Vona Vona Lagoon, both of which are located in the Western Province of Solomon Islands (for location of sampling sites see Fig. 1 in Rawlinson 1990). Munda is exposed to heavy commercial fishing pressure for baitfish, whereas Vona Vona is not fished for bait.

An underwater light was used to attract the fish, which were then sampled using a large 4 mm mesh net and then subsampled using a <1 mm mesh net as the fish in the buckets were being transferred into the baitwells (Rawlinson 1990). Fish were placed in 10% formalin and later sorted into separate species. It should be noted, however, that the smaller representatives frequently could not be identified at the species level. Identification of small fish was a particular problem with Encrasicholina heterolobus which, at fork lengths less than 25 mm, generally could not be distinguished from the co-occurring and also abundant E. devisi. The fork length of each identifiable representative of E. heterolobus and Spratelloides delicatulus was measured to the nearest 1 mm.

Apogon rueppellii.

This species was collected using seine nets during the day and otter trawls during the day and occasionally during the night at sites located throughout the Swan Estuary, Western Australia. Seine netting was carried out in near-shore shallow waters in each month between January 1979 and February 1982, while otter trawling was undertaken in offshore deeper water in each month between January 1979 and December 1982. The location of sampling sites and the depths of the trawl sites are given in Chrystal et al. (1985). The seine net was 133 m in length and comprised mesh of 25.4 mm in the wings and 9.5 mm in the pocket. The net swept an area of 2815 m². The otter trawl, which was 5 m long and consisted of 51 mm mesh in the wings and pocket, respectively, was towed for 5 min at 3-4 km/hour at each site on each sampling occasion. The cod end of the otter trawl consisted of 25 mm mesh in all but the months between July and December 1982, when it was replaced with 9 mm mesh.

The total length of each fish caught was recorded to the nearest 1 mm, except in the case of very large samples, when the measurements were restricted to a random subsample of at least 100 fish. Individuals in random subsamples of each sex were measured between May 1981 and December 1982 to allow the growth of males and females during this period to be plotted separately.

Analysis of length-frequency data

Details of the reproductive biology which provide information on the spawning times of the tuna baitfish E. heterolobus and S. delicatulus and of A. rueppellii, and thus the time when recruitment would be expected, are given in Milton and Blaber (1991) and Chrystal et al. (1985), respectively.

The lengths of the tuna baitfish species and A. rueppellii caught in each month were grouped into 2 mm size classes. Visual inspection was used in an attempt to elucidate the number of size cohorts in each of the length-frequency histograms and whether (if present) they followed a consistent pattern through each of the sequential monthly data sets.

Since the length-frequency data for the two baitfish species were unsuitable for analysis by computer packages (see later), the following account is restricted to the analysis of length-frequency data for A. rueppellii. MIX was used to determine the distributions of the size cohorts and the mean lengths of the normal curves fitted to those distributions in each of the sequential monthly histograms (Macdonald and Pitcher 1979, Macdonald and Green 1990). Growth curves were then constructed using a modification of the von Bertalanffy growth function, which was fitted to the mean lengths of the size classes in the sequential sets of length-frequency data using ELEFAN (Pauly 1987, Gayanilo et al. 1989) and MULTIFAN (Fournier et al. 1990, Fournier and Sibert 1990) and also another nonlinear curve fitting technique (Hall 1992) that employed the results of MIX. They each utilised a sinusoidal oscillation that simulated the seasonal trends exhibited by growth (Pauly and Gaschütz 1979).

The equation is:

\[ L_t = L_\infty \{1 - \exp(-K(t-t_0)) - C[K/(2\pi)] \sin(2\pi(t-t_0))\} \]

where \( L_t \) is the length at age \( t \) (in years), \( L_\infty \) is the mean asymptotic length predicted from the equation, \( K \) is the growth coefficient, \( t_0 \) is the hypothetical 'age' at which a fish would have zero length if growth followed that predicted by the equation, and \( C \) is a factor which expresses the amplitude of the oscillation that is imposed on the growth oscillation at age \( t = 0 \) (Pauly 1987).
A birth date of 1 January was estimated for the population of *A. rueppellii* in the Swan Estuary, on the basis of a combination of the trends shown by gonadosomatic indices and backwaters extrapolation of the lengths of the 0+ age class in the months when they are first recruited (Chryystal et al. 1985). This birth date was utilised by MULTIFAN to set the first month for each age class. The nonlinear curve fitting technique described by Hall (1992) was adapted to fit a curve to the means of the modes produced by MIX. The value of $L_\infty$ was obtained from MIX. The ELEFAN procedure involved first obtaining a seed value of $L_\infty$, using ELEFAN II. This value was then used in ELEFAN I to obtain estimates from $L_\infty$, $K$, $C$ and $t$. Further details of the above computer packages are found in Macdonald and Pitcher (1979), Pauly (1987), Gayanilo et al. (1989), Fournier and Sibert (1990), Fournier et al. (1990) and Macdonald and Green (1990).

**Results**

**Tuna baitfish**

Extensive sampling at Munda yielded large numbers of *Encrasicholina heterolobus* in most months between March 1987 and May 1989 (Fig. 1). Small fish less than 25 mm, could only occasionally be identified and fish greater than 60 mm were abundant only in a few months (Fig. 1). Although modes were present in some months, these did not follow any consistent trend with time.

The restricted size range and absence of conspicuous trends in modal lengths exhibited by the length-frequency data for *E. heterolobus* at the Swan Estuary was paralleled by the data collected at Vona Vona (Fig. 2).

In contrast to the situation with *E. heterolobus*, small representatives of *Spratelloides deliciatus*, i.e. <25 mm, were collected and could be identified, particularly from Vona Vona (Figs 3, 4). Thus, for example, a well defined mode of 15-17 mm was present in September, October and November of 1987 (Fig. 4). However, the corresponding fish were not nearly as well represented in the following three months and it is thus not clear from the frequency data how growth progressed. There was some indication from the length-frequency data that a single cohort might have been present in March, April and May of 1989, with the mode increasing from 25 mm to 39 mm during these three months (Fig. 4). However, there was no conspicuous and consistent progression exhibited by modes in the length-frequency data for either of the two species over a series of months.

**Apogon rueppellii**

The monthly catches of *A. rueppellii* taken by seine net in the shallows varied markedly (Fig. 5). However, catches tended to be greatest in summer or autumn and were often low in winter. Although the monthly catches in otter trawls also showed considerable variability, they tended to be greatest in winter (Fig. 5). The increase in otter trawl catches in winter represents an offshore movement at that time (see Chryystal et al. 1985).

Two well defined size cohorts were present in many of the individual monthly length-frequency histograms for *A. rueppellii* in each of the four years (1979-1982) that this species was sampled in the Swan Estuary (Figs 6, 7). The cohorts of the smallest fish, which represent the new 0+ recruits, first appeared in January in the Swan Estuary in 1979 and 1980 and in February in 1981 and 1982. However, the marked similarity in the modes of this cohort in February of each year, when they ranged only from 33 to 36 mm, indicates that spawning occurred at a similar time in each of those years.

The mode for the 0+ age class in 1980, i.e. the 1980 year class, increased progressively from 36 mm in January to 49 mm in May, to 57 mm in October and 60 mm in December (Fig. 6). The corresponding mode in 1981, i.e. when the fish had become one year old, increased from 67 mm in January to 77 mm in April, and remained at about this latter length until October (Fig. 7). By December 1981, the length distribution of the 0+ age class had merged with that of the 1+ age class (Fig. 7).

The trends exhibited by the modal lengths of the 1979, 1981 and 1982 year classes were similar to those described above for the 1980 year class (Figs 6, 7). However, the modal length for the 0+ age class did decline during the winter of 1981 and 1982 and the 1+ age class was not well represented in the second half of 1982. It should also be recognised that the 1+ age class was not always well represented in some of the monthly samples. The reasons for the above declines in length in winter and the occasional poor representation of the 1+ age class are discussed later.

The growth curves produced by the non-linear technique of Hall (1992), using the mean lengths of the 1980 year class in each of the monthly samples derived by MIX, show a pronounced seasonal pattern (Fig. 8). Thus, growth of this age class was rapid during the first summer and early autumn and slowed during winter, before increasing slightly in the following summer. This seasonal pattern was even more pronounced with the 1979 and 1981 year classes, to the extent that conspicuous ‘negative growth’ took place, particularly in the case of the latter year class. The ‘negative winter growth’ is reflected by values greater than 1 for $C$ in the growth equations for these two years (Table 1).

The value for $K$ in the growth equations was 0.66 for both the 1980 and 1981 year classes, which was
Figure 1. Length-frequency histograms for *Encrasicholina heterolobus* caught at Munda in the Solomon Islands.
Figure 2. Length-frequency histograms for *Encrasicolina heterolobus* caught at Vona Vona in Solomon Islands.
Figure 3. Length-frequency histograms for *Spratelloides delicatulus* caught at Munda in Solomon Islands.
Figure 4. Length-frequency histograms for *Spratelloides delicatus* caught at Vona Vona in Solomon Islands.
Figure 5. Mean monthly numbers of *Apogon rueppellii* caught by seine nets and otter trawls in the Swan Estuary, Western Australia, between January 1979 and November 1982. The horizontal black rectangles represent the summer and winter months and the open rectangles the spring and autumn months.
Figure 6. Length-frequency histograms for *Apogon rueppellii* caught throughout the Swan Estuary, Western Australia, between January 1979 and December 1980. The line separates the distributions corresponding to the 0+ and 1+ age classes.
Figure 7. Length-frequency histograms for *Apogon rueppellii* caught throughout the Swan Estuary, Western Australia, between January 1981 and December 1982. The line separates the distributions corresponding to the 0+ and 1+ age classes.
Tuna baitfish

Examination of the length-frequency data for the tuna baitfish *Encrasicholina heterolobus* and *Spratelloides delicatulus*, demonstrates that there were no conspicuous and consistent size cohorts that could be traced through the length-frequency histograms for these species in sequential months. However MULTIFAN and ELEFAN, which use length data obtained from sequential samples to derive growth curves, will both produce a curve or curves, even though clear-cut modal length progressions were not present. Furthermore MIX, which analyses the distributions in length-frequency data for individual samples, separated each of those distributions into cohorts which generally bore no resemblance to those produced for the preceding and following distributions. The lack of continuity in modes in sequential samples suggests that the sampling regime was not collecting comparable representatives of the populations in each month. Certainly, a lack of consistent representation could easily occur with baitfish, where their schooling and mobile behaviour could slightly higher than the 0.53 recorded for the 1979 year class. Although \( L_{\infty} \) for the three year classes ranged from 96 to 121 mm, these values were associated with a relatively high standard error. Despite this high standard error, the presence of correlation coefficients of 0.97 to 0.99 for the three year classes demonstrates that the curves fitted the data very well.

When the length-frequency data for the 1979 and 1981 year classes were subjected to MULTIFAN and ELEFAN, the values for \( L_{\infty} \) in the growth equations were always greater than 173 mm and on one occasion was 196 mm. These values are far higher than the maximum length recorded (105 mm), a feature attributable to the fact that the above two computer packages cannot effectively analyse length data which show ‘negative growth’ at some stage of the year, i.e. when the value for \( C \) is greater than 1.

The values for the parameters in the growth curve equations produced by MULTIFAN for the 1980 year class, which did not show ‘negative growth’, were virtually identical to those produced using the mean length calculated by MIX (Table 1). The corresponding values using ELEFAN did not correspond so closely (Table 1).

The growth of males and females started diverging after they had reached 48 mm (Fig. 9), which is reflected by differences in the values for \( K \) for the two sexes (Table 1). A likelihood ratio test (Kimura 1980) showed that the growth curves of males and females were significantly different \((P<0.001)\). ‘Negative growth’, reflected by a \( C \) value conspicuously above 1, was observed with males (Table 1).

**Discussion**

**Tuna baitfish**

Examination of the length-frequency data for the tuna baitfish *Encrasicholina heterolobus* and *Spratelloides delicatulus*, demonstrates that there were no conspicuous and consistent size cohorts that could be traced through the length-frequency histograms for these species in sequential months. However MULTIFAN and ELEFAN, which use length data obtained from sequential samples to derive growth curves, will both produce a curve or curves, even though clear-cut modal length progressions were not present. Furthermore MIX, which analyses the distributions in length-frequency data for individual samples, separated each of those distributions into cohorts which generally bore no resemblance to those produced for the preceding and following distributions. The lack of continuity in modes in sequential samples suggests that the sampling regime was not collecting comparable representatives of the populations in each month. Certainly, a lack of consistent representation could easily occur with baitfish, where their schooling and mobile behaviour could
Table 1. The parameters and their standard errors (in brackets) for the von Bertalanffy growth curves of *Apogon rueppellii*, constructed using the mean lengths produced by MIX, MULTIFAN and ELEFAN. $L_\infty$ is given in mm. See Materials and Methods for definitions of these parameters. NA, not applicable.

<table>
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<th></th>
<th>$L_\infty$</th>
<th>$K$</th>
<th>$t_0$</th>
<th>$C$</th>
<th>$t_z$</th>
<th>$r^2$</th>
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<td>MIX 1979 year class</td>
<td>121</td>
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<td>1.20</td>
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<td>(17)</td>
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<td>(0.16)</td>
<td>(0.10)</td>
<td>(0.10)</td>
<td>(0.01)</td>
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<td>-0.50</td>
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<td>(9)</td>
<td></td>
<td>(0.15)</td>
<td>(0.10)</td>
<td>(0.15)</td>
<td>(0.04)</td>
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<tr>
<td>1981 year class</td>
<td>96</td>
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<td>-0.61</td>
<td>1.74</td>
<td>0.10</td>
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<td>-0.46</td>
<td>1.02</td>
<td>0.16</td>
<td>0.99</td>
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<tr>
<td>(9)</td>
<td></td>
<td>(0.10)</td>
<td>(0.07)</td>
<td>(0.10)</td>
<td>(0.02)</td>
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<tr>
<td>Male</td>
<td>117</td>
<td>0.46</td>
<td>-0.58</td>
<td>1.22</td>
<td>0.16</td>
<td>0.99</td>
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<tr>
<td>(11)</td>
<td></td>
<td>(0.09)</td>
<td>(0.58)</td>
<td>(0.11)</td>
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<td>(0.6)</td>
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<td>ELEFAN 1980 year class</td>
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<td>0.96</td>
<td>0.10</td>
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result in the collection of different size cohorts in different months.

It is essential to recognise that the data for the two baitfish species have other inherent deficiencies which would essentially invalidate any attempt to construct growth curves. For example, since these species spawn throughout the year and with highly variable intensity (Milton and Blaber 1991), it is not possible to assign a precise birth date to any cohort. While this problem could be overcome by estimating a birth date using the trends shown by the growth of the smallest fish, small fish were not well represented in the data. This was particularly the case with *E. heterolobus*, where the small members of this species could not be separated from those of *E. devis* with which they co-occurred. However, even in the case of *S. delicatulus*, where small fish (<25 mm FL) were present and could be identified in a few months, there were no corresponding cohorts in the following months that would be consistent with the type of modal progression that would represent growth.

The ability to use MULTIFAN and ELEFAN to analyse length-frequency data also depends on recruitment occurring at regular intervals. However, since tuna baitfish spawn throughout the year and the precise timing and intensity is highly variable at Munda and Vona Vona (Milton and Blaber 1991), recruitment must be highly uneven. This feature by itself precludes the use of MULTIFAN and ELEFAN.

Although *E. heterolobus* and *S. delicatulus* apparently spawn throughout the year but with irregular intensity (Milton and Blaber 1991), the spawning of another Indo-Pacific species, *Stolephorus nelsoni*, occurs in the spring (Hoedt 1990). As a result, recruitment of this latter species is restricted to one time of the year and the mode corresponding to the 0+ age class can be clearly traced through length-frequency data for sequential months (Hoedt 1990).

*A. rueppellii*

As with the length-frequency data for *S. nelsoni*, those for *A. rueppellii* show clearly defined cohorts, whose modes could be traced through sequential monthly length-frequency histograms. The well defined modes reflect a relatively narrow spawning season. The presence of a single annual spawning season means that a new 0+ age class is recruited only once in each year. An ability to define a realistic birth date, both from reproductive data and from the trends shown by the modes corresponding to the smallest size class, combined with the presence of well defined cohorts, make the length-frequency data for *A. rueppellii* ideal for analysis using either MIX, ELEFAN or MULTIFAN.

It is noteworthy that the growth curve parameters calculated using MULTIFAN and employing the mean lengths generated by MIX were almost identical in the case of the 1980 year class. However, the presence of apparent 'negative growth' for the 1979 and 1981 year classes in the winter resulted in the production of completely unrealistic values for $L_\infty$ in those year classes when using MULTIFAN and ELEFAN. This is because these two computer packages cannot accommodate 'negative growth' since $C$ is constrained between 0 and 1 (Fournier and...
With the 1981 year class, the 'negative growth' was reflected a relative reduction in the proportion of the resultant retention of a higher proportion of smaller between the catches of fish in seine nets and otter being taken in shallow waters and the larger fish of fish. The absence of 'negative growth' with the 1981 first time, the growth of males and females started to of the sexes will lead to greater definition of the age composition of fish in catches can be influenced by diverge. While this did not produce a very marked

The ability to use a computer package successfully is related to a shift from the use of 25 mm to 9 mm mesh in the cod end of the otter trawl in July and the resultant retention of a higher proportion of smaller fish. The absence of 'negative growth' with the 1981 year class can be attributed to the better balance between the catches of fish in seine nets and otter trawls, with the smaller fish of both the 0+ cohort being taken in shallow waters and the larger fish of this cohort being collected in deeper waters. Furthermore, the catches included good numbers of the 0+ and 1+ cohorts (see also Chrystal et al. 1985).

The above examples demonstrate that the size composition of fish in catches can be influenced by such factors as the mesh size of the net and the seasonal movements of fish. Our data also demonstrate that, as A. rueppellii approaches maturity for the first time, the growth of males and females started to diverge. While this did not produce a very marked change in $K$ in the growth equation, it is important to recognise that, in those cases where differential growth between the sexes is conspicuous, separation of the sexes will lead to greater definition of the age classes in length-frequency data.

**Guidelines for Acquiring Data for Computer Analysis**

**Sampling regime**

The ability to use a computer package successfully is dependent on establishing the type of sampling regime that will yield appropriate length data. The sampling regime should thus be designed to obtain a full range of fish sizes. This may require more than one method in the case of fish that grow to a relatively large size.

It is also necessary to ensure that a fully representative sample is obtained by sampling widely in a given area to avoid any bias that might be incurred through schooling and seasonal or other movements, such as from inshore to offshore. It is also important to use a mesh size sufficiently small to catch the smaller representatives of the species. Furthermore in the case of small fish, it is important to measure the length accurately, usually to the nearest 1 mm.

For small, fast growing fish, e.g. tuna baitfish, it is advisable to sample at least fortnightly, whereas monthly sampling should be adequate for fish species that grow to a large size, such as snappers and emperors.

The gonads of representative fish should be collected and allocated to stages to determine whether spawning is continuous or occurs at regular intervals. If the latter is the case, the data can be used to calculate the spawning period and thereby assign a birth date for use in growth equations.

**Type of computer package**

Prior to analysing length-frequency data, it is important to examine the data visually to determine whether there are a series of well defined modes that can be traced through length-frequency data for sequential samples. Consequently, the way in which the data are plotted is crucial. Small class intervals or even individual lengths of fish should be used for small species.

The choice of computer package depends on the characteristics of the data set. MULTIFAN and ELEFAN analyse the lengths in preceding and following samples to elucidate the distribution of the size cohorts and then uses the mean lengths of those cohorts to produce a von Bertalanffy growth curve. The use of MULTIFAN and ELEFAN thus depends on growth conforming to the von Bertalanffy growth equation, i.e. growth becomes asymptotic with age. These two computer packages also assume that recruitment occurs at regular intervals, e.g. once yearly or once every six months, and they cannot accommodate "negative growth".

In contrast, MIX determines the distribution of the size cohorts in each sample separately. The means of the distributions of those cohorts in successive samples can then be used to construct a growth curve. These curves do not necessarily have to conform to the von Bertalanffy growth equation, or require recruitment to have occurred at regular intervals.

In summary, the successful use of each of the above computer packages requires the following:

1. The presence of conspicuous and consistent modes which can be traced through sequential length-frequency data. Unless this requirement is met, it is thus not appropriate to submit length-frequency data to MIX, MULTIFAN or ELEFAN.
2. Length measurements of a sufficiently large number of fish covering the complete range of fish lengths.
3. Samples should be collected at regular intervals, the precise interval between sampling depending on the lifespan of the species. Sampling should also attempt to collect representatives from throughout the range of distribution of the population.
4. Measurements should be made in units appropriate for the length range of the species (i.e. 1 mm for small and short-lived species) and the data plotted in appropriate size intervals.
5. Ideally, the data should include details on the reproductive cycle so that a birth date can be assigned.
References


Hall, N. 1992. Fitting a seasonal growth curve with SAS. Takestock No. 3, 4-5.


