Estimation of length and weight growth parameters in populations with a discrete reproduction characteristic

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CONTENT

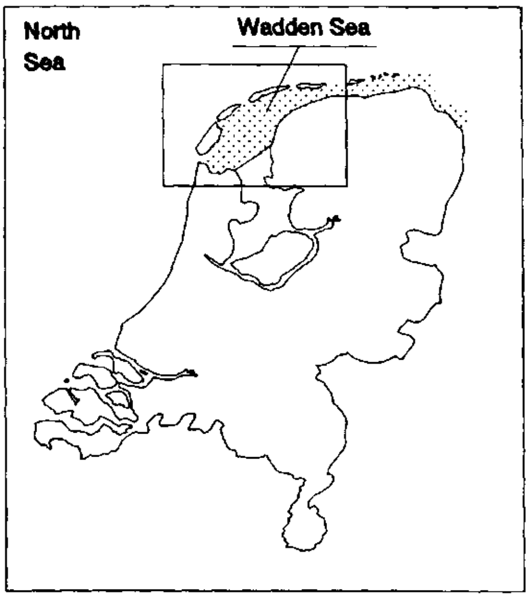
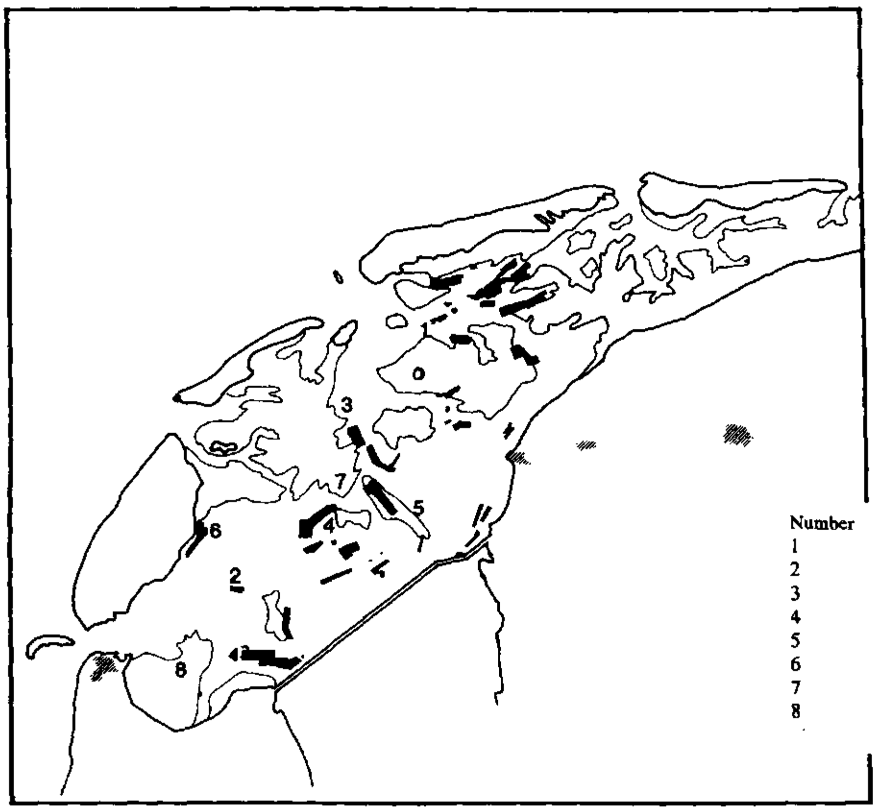


Figure 1. Location of research area.



Number	Codes	Name
1	K,L,M	Meep
2	A,B	Scheer
3	I	Inschot
4	S	Scheurrak
5	Z	Zuidoostrak
6	U,T	Texel
7	V	Oude Vlie
8	X,Y	Mosselgaatje
·	R	IBN-mesocosms

Figure 2. Sample locations in the Dutch Western Wadden Sea.

1. INTRODUCTION

1.1. Background

At the Institute of Forestry and Nature Research on Texel, during a number of years a large number of data are gathered on length frequency distributions and length-weight relationships on mussels (Mytilus edulis). The mussels were sampled from mussel beds and mussel culture plots in the Dutch Wadden Sea in order to get better information on mussel development from year to year, and during the year, on several characteristic locations.

To describe, e.g., growth of mussels during a year, the rate of change of the average length has to be known; to describe animal condition and the effect of feeding, length-weight relationships are needed. At the end of the sampling activities, over 600 data-files were available.

1.2. Objectives

To derive the characteristic parameters from the data, the software program MUSSEL was developed. Length-frequency data are divided into maximal three size classes by the program, and average lengths of each size class, and numbers of animals that belong to each class are computed. Length-weight relationships are computed using an allometric equation. Finally, the rate of change of average lengths and the course of the animal's weight is results from subsequent samples.

The existence of a discrete reproduction characteristic is essential, otherwise random sampling from a population never would yield information on growth. Mussels reproduce once a year in spring, and the method of analysis is very well applicable.

In this report, the underlying computation method is explained and documented. Emphasized is the proper use of a non-linear parameter estimation technique and the computation of the confidence of the derived parameter values.

The resulting parameters describe each sample and contain information on the sample location. Further data analysis is needed to relate the results to environmental conditions; such an analysis is *not* discussed here.

Some results are presented. However, a complete data set analysis will be reported separately.

The developed method, implemented in the MUSSEL program, is applicable to all other datasets where distinct length classes are present.

In appendix A the use of this package is explained.

1.3. Mussel reproduction and growth characteristics

Mussels feed on algae, bacteria, microfauna and detritus present in the pelagic part of the system. Their growth occurs mainly in summer and early autumn, while spawning takes place in spring. During the growing season, both length and weight will increase. In the last part of autumn and during winter, food is less available and mostly a decrease in body weight is observed. In spring, weight usually increases until spawning takes place. As a result of the spawning, the average body weight decreases drastically.

1.4. Area description

The sampling has been carried in the Western part of the Dutch Wadden Sea (Figure 1), a tidal area ($14 \cdot 10^3$ km^2), with an average depth of 3.3 m. The area consists of tidal flats (that part of the area above mean low tide level (MLT)), subtidal parts (the area between MLT and MLT -5 m) and the deeper channels (up to about 30 m deep) for 32, 53 and 15 area-% and 6, 39 and 54 volume-%, respectively (derived from EON, 1988-1).

Mussels in the Wadden Sea occur on two types of locations. Firstly, they occur grow naturally on musselbeds on tidal flats or in subtidal parts, down to about -3 m below MLT. Secondly, mussels are cultured. After spawning in spring, mussel seed (10 to 15 mm length) is caught the next year (May-June) and brought to special growing areas, the culture plots. That year they grow to 35-45 mm and may be fished and relaid several times. At the end of the next growing season these mussels they have a length of 50-70 mm, and are fished for consumption (Dankers *et al.* 1989).

1.5. Available datasets

Both the naturally occurring musselbeds and the culture plots were sampled. On musselbeds all year classes are present; on culture plots usually just one or two year classes will be found.

Nine locations (Figure 2) were sampled five times a year (Figure 3). The sampling has been carried out by the Institute for Forestry and Nature Research and the fishery inspectors of the Ministry of Agriculture, Nature Management and Fisheries. The locations were chosen in such a way that different parts of the system were covered. Length and weight of individual mussels were measured and length-frequency distributions were determined.

In Figures 4 and 5, examples of the obtained data sets, a length-frequency distribution and a length-weight relationship, respectively, are presented. The sample notation is explained in Table I.

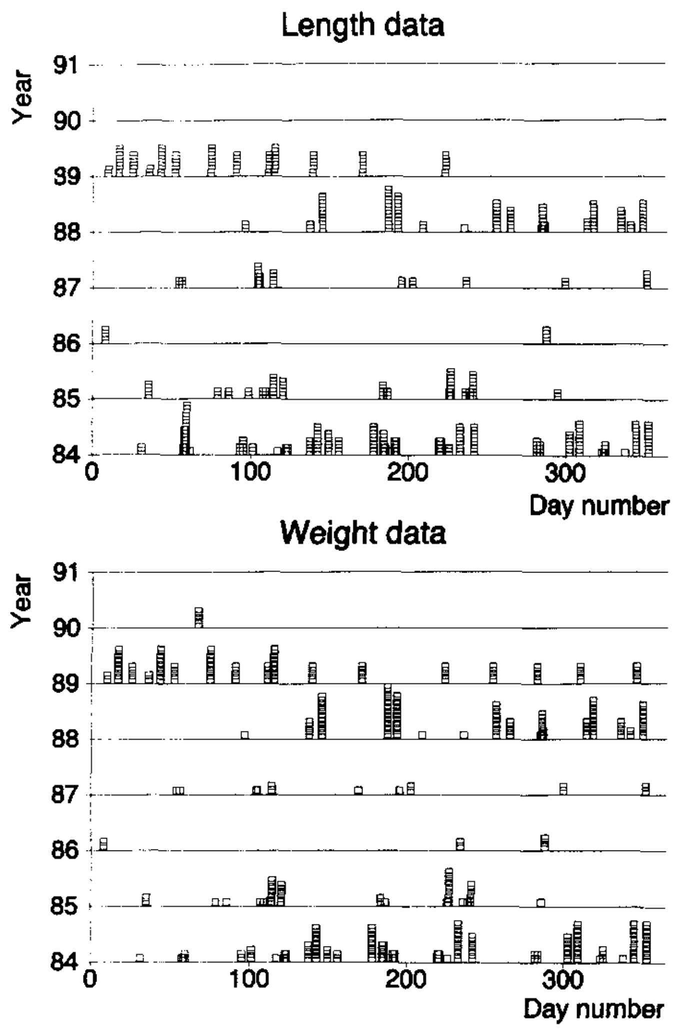


Figure 3. Sampling dates during the research period.

Table I. Notation of sample location and numbers.

Sample number notation : B1XYMMDD means

B	Sample site (see Figure 2). An R denotes samples from the IBN mesocosms at Texel
1	Sample location inside site. Most times this is a number. 'T' denotes: all the data from one site together
X	'A' denotes length-weight data; 'L' length-frequency distribution data
Y	Year number in the eighties. '0' means 1990
MM	Month
DD	Day

e.g., A1L80412 means : site A, position 1, length data, on April 12[th] 1988

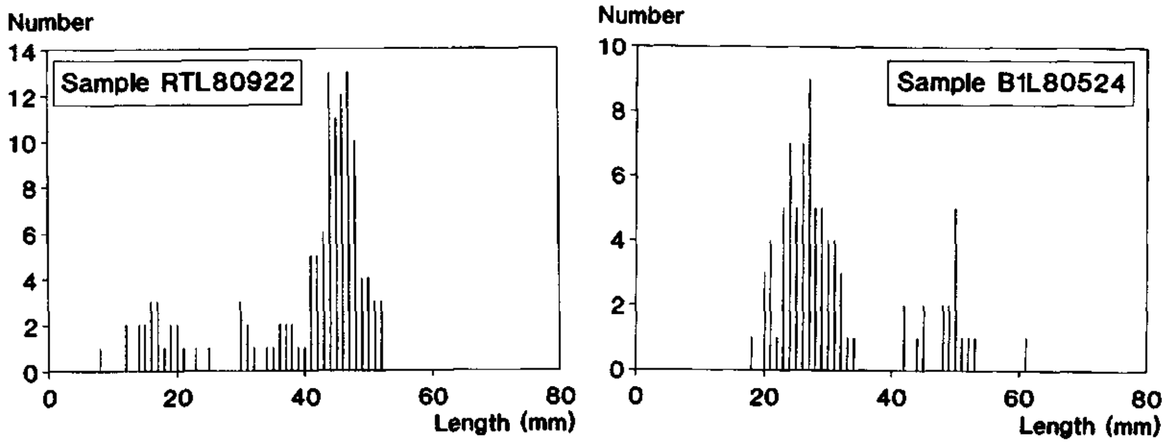


Figure 4[a-b] Examples of length-frequency distributions at two sample stations.

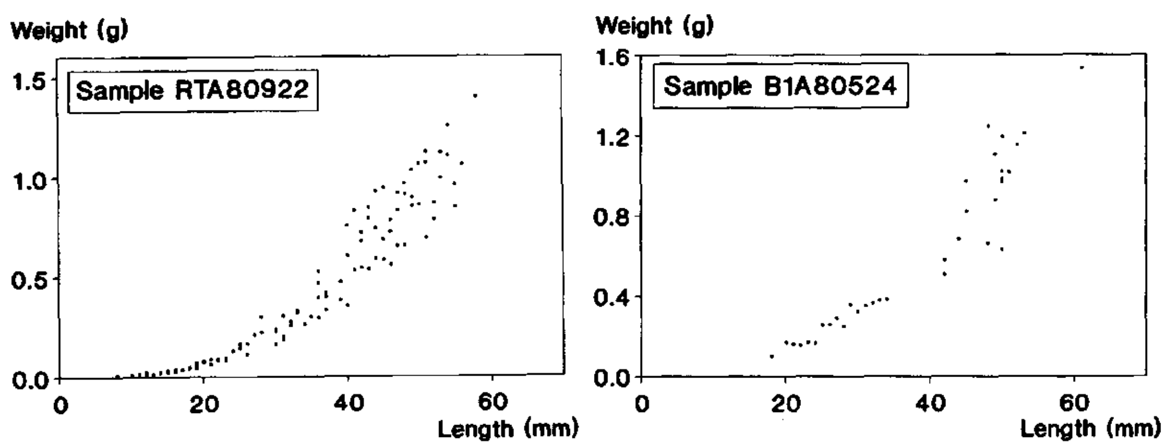


Figure 5[a-b] Examples of length-weight relation data at two sample stations.

2. MATHEMATICAL DESCRIPTION

2.1. Introduction

2.1.1. Length-frequency distribution

Generally spoken, length-frequency distributions can only be analyzed successfully when the number of size-classes present in the samples is limited. This implies that reproduction is a more or less discrete process: it has to take place in a certain period of the species' lifespan. It will be clear that the analysis will be more successful when the variation in size within one size-class is small. When size variations are large, classes will overlap and separation will be hard. Thus, the data should clearly show some distribution when inspected 'by eye'. Fournier *et al.* (1990) encountered the problem that length-frequency distribution data in Southern Bluefin Tuna (*Thunnus maccoyii*) populations contained many age-classes. They were forced to use an estimate for the value of the mean length of each age-class according to a Von Bertalanffy growth curve, and then they were able to compute age-distributions from their sample data. This length assumption is very crucial in their approach. Their data structure indicates quite clearly that the results of the calculations, e.g. numbers of fish of a certain age within the sampled population, will be very sensitive to variations in the assumed lengths; a statement already made by Fournier *et al.* themselves. Richardson *et al.* (1990) showed for example that for *Mytilus edulis* populations there is a large discrepancy between observed and theoretical lengths, the latter calculated according to a Von Bertalanffy length-age relationship. Thus, when the aim is to estimate growth characteristics, the method as outlined in this present study is restricted to samples with a limited number of size-classes (say three or four). Otherwise, other methods have to be applied. For shellfish, the use of internal growth bands, according to Richardson *et al.* (1990), probably is a suitable method to gather additional information on the population age structure.

Graphical methods, that use cumulative length-frequency distribution plots, are not discussed here. One of the goals is the compute the reliability of the resulting parameters; when using graphical methods uncertainty calculations are hardly possible.

The general numerical method is to assume the existence of a certain number of length classes (the use of the term 'year-class' is avoided here). Each length class is thought to have a normally distributed length-frequency characteristic. The measured population length-frequency distribution is then fitted using some least-square estimation procedure; the parameters of the several length classes are to be estimated.

In the present case, since mostly fishery cultures were sampled, just a very few length classes are present, and the difficulties mentioned above are hardly here.

2.1.2. Length-weight relationship

Usually, there are few difficulties in establishing the length-weight relationship in some population. Many investigators perform such analysis. However, two remarks are important.

Generally, the length-weight formula reads

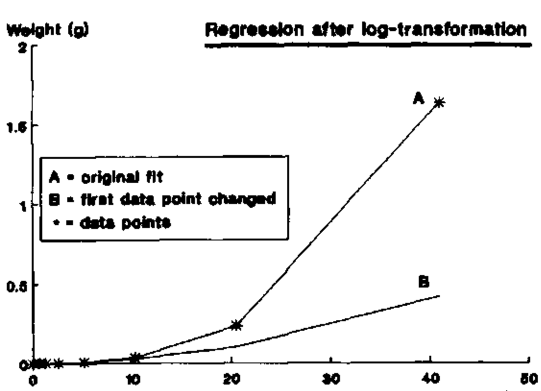
$$Weight = a \cdot Length^{b} \tag{1}$$

in which the dimension of **a** is determined by the value of **b**, too.

Firstly, when such an equation has to be used for comparing one population with another, one has to be sure that *the* **b**-*values are equal*. Otherwise, comparing **a** and **b** values for different samples is meaningless.

Secondly, software packages may have the option to estimate both parameters **a** and **b**. Unfortunately, sometimes this is done after a logarithmic transformation. This turns the non-linear equation into a linear one, and a simple linear regression will do for the calculation of log(**a**) and **b**. After back-transformation, **a** and **b** are returned to the user. Generally, this is wrong, since it assumes that the *relative* errors in measured weights and lengths are equal for all the data points. Thus, this procedure minimizes the low weight errors in the absolute way, permitting large absolute high weight errors to exist although these are small in a relative way. One has to be very careful when using such software package options. In Figure 6a an example is given elucidating the unreliability of the logarithmic transformation method. Both lines in Figure 6 represent eq. 1. Both lines are fitted to the datapoints showed, the only difference being the first datapoint: for line A the ordinate value of the first datapoint is 10^{-7}, for line B it equals 10^{-6}. In fig. 6b both datasets are fitted without the logarithmic transformation. This example is an extreme, but it may be useful in parameter estimation discussions.

In §2.3 results of the different estimation methods are discussed.



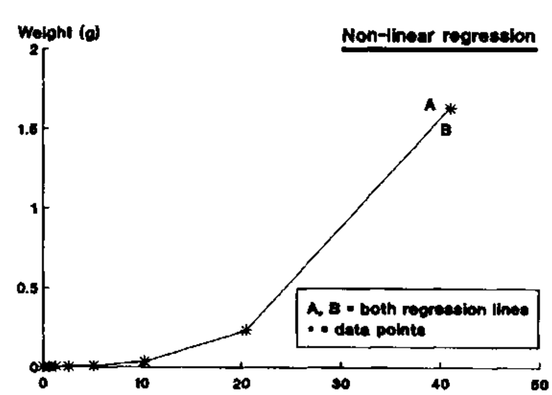


Figure 6ª. Regression analysis results after logarithmic transformation. Datapoints are given in table 1.

Figure 6ᵇ. Regression analysis results without any transformation. See table 1 for datapoints.

Table II. Datapoints used in Figures 6[a] and 6[b]. Only the first Y-value is different for the second Y-column.

Length (mm) X	Weight (g). Line A Figure 6	Weight (g). Line B Figure 6
1.00 E-2	1.26 E- 07	1.00 E-06
6.40 E-1	1.43 E-05	
1.28 E00	9.98 E-05	
2.56 E00	6.95 E-04	
5.12 E00	4.84 E-03	
1.02 E01	3.37 E-02	
2.05 E01	2.35 E-01	
4.1 E01	1.64 E00	

2.2. Description of the mathematical technique for calculation length classes and the use of uncertainty analysis

2.2.1. Estimation of the length class parameters

It is assumed that a normal length-frequency distribution exists. Although a slightly right skewed length-frequency distribution (Figure 7) may be more realistic, such adjustments have not been studied.

The length-frequency distribution of each length class i is given by the standard equation

$$N_i(l) = \frac{A_i}{\sigma_i \cdot \sqrt{(2 \cdot \pi)}} \cdot \exp(-0.5 \cdot \frac{(l - l_{0,i})^2}{\sigma_i^2}) \qquad (2)$$

with

$N_i(l)$= number of length class i individuals with length l
$l_{0,i}$ = average length of the class i
l = length of each individual
σ_i = standard deviation of the distribution of class i
A_i = total number of individuals in this length class i

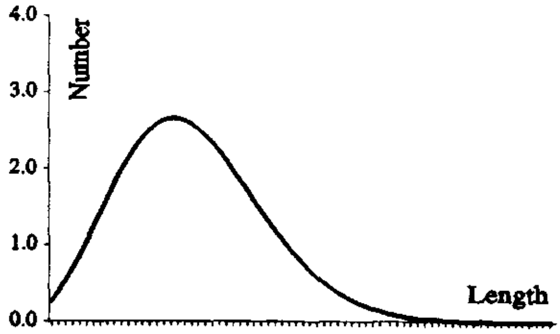


Figure 7. Right skewed frequency distribution.

Thus, the total number of individuals N_{tot} of a given length l is, assuming there are l_{max} length classes present:

$$N_{tot}(l) = \sum_{i=1}^{l_{max}} N_i(l) \tag{3}$$

Since length-frequency distribution data are available, the parameters of the l_{max} length classes may be estimated. The target of this parameter estimation is to minimize

$$S = \sum_{l=lmin}^{lmax} [N_{tot}(l) - N_m(l)]^2 \tag{4}$$

in which $N_m(l)$ is the measured number of individuals with length l, and $N_{tot}(l)$ the calculated number. lmin and lmax are the minimum and maximum length, respectively. The minimum value lmin equals 1 mm in the present case of mussel sampling. The parameters l, σ and A are stored in vector $\mathbf{x} = (x_1, x_2, .., x_p) = (l_{0,1}, \sigma_1, A_1, \ldots, l_{0,lmax}, \sigma_{lmax}, A_{lmax})$. The minimum of S is reached when $S(\mathbf{x}) = S(\hat{\mathbf{x}})$, with $\hat{\mathbf{x}}$ being the final estimate, and for all the p parameters:

$$\frac{\partial S}{\partial x_i} = 0 \qquad (i=1..p \ , \ p=3 \cdot l_{max}) \tag{5}$$

The set of p ($p=3.l_{max}$) equations (5) is non-linear in l and σ, and the minimization had to be carried out using a modified Marquardt technique (Marquardt, 1963). Disadvantage of such techniques is that they may get stuck in local minima. However, the use of different starting values for the l_{max} $l_{0,i}$ parameters turned out to be successful in all the cases.

2.2.2. Parameter significance

The significance of the estimate is important for the interpretation of the results.

There are three groups of methods to be used for the calculation of the parameter significance.

1- the calculation of individual confidence intervals for each parameter (Draper and Smith, 1981).
2- the calculation of combined confidence contours for two parameters (Draper and Smith, 1981).
3- the calculation of sets of parameters using a Monte Carlo-like simulation technique. Each parameter set meets certain error conditions (Keesman, 1990).

Methods 1 and 2 are used here. When p is the number of parameters and M is the number of individuals measured, the equation

$$S(x) = S(\hat{x})\cdot[1 + \frac{p}{M-p}\cdot F(p,M-p,1-\alpha)] \tag{6}$$

has to be solved, in which equation $F(p,M-p,1-\alpha)$ is Fisher's distribution value for the $(1-\alpha)\cdot 100\%$ confidence level.

Eq. 6 may be solved in several ways. First, the Taylor expansion

$$S(\bar{x}+\overline{\Delta x}) = S(\bar{x}) + \frac{\partial S}{\partial \bar{x}}\cdot\overline{\Delta x} + \frac{1}{2}\cdot\frac{\partial^2 S}{\partial \bar{x}^2}\cdot(\overline{\Delta x})^2 \tag{7}$$

may be used. Since the first derivatives equal zero (eq. 5), the Hessian matrix [pxp] containing the second order terms is needed for calculating the Δx values. So, validity of eq. 7 is assumed. The solution of this quadratic equation for the chosen parameters x_a and x_b results in elliptic confidence contours. Usually, eq. 7 is only valid when $\hat{x}$ only slightly differs from $\hat{x}$; the solution of (6) generally results in much larger deviations. So, this is not a very suitable way of estimating confidence contours.

Secondly, starting from $\hat{x} = \hat{x}$, the region of $\hat{x}$ may be scanned by subsequently changing one of the two parameters x_a and x_b for which the confidence contour has to be calculated and solving eq. 6 for the other parameter. Each solution of eq.6 serves as the starting point for the next computation. This method has been used in the present calculations.

2.2.3. Results

Length-frequency distribution

For the present study, the assumption of three length classes will do, since the mussels in the Wadden Sea are fished at the end of their third growing season. For natural musselbeds, the larger mussels were taken together. Previous investigation of the data sets showed this to be a realistic approach; the introduction of a fourth or even fifth length class would introduce extra parameters that could not possibly be estimated with some significance.

Parameter constraints were:

$$0 \le A_i \tag{8a}$$
$$0.5 \le \sigma_i \le 8 \tag{8b}$$
$$\begin{aligned} 0 &< l_{01} \le 25 \\ 20 &\le l_{02} \le 40 \\ 35 &\le l_{03} \le 70 \end{aligned} \tag{8c}$$

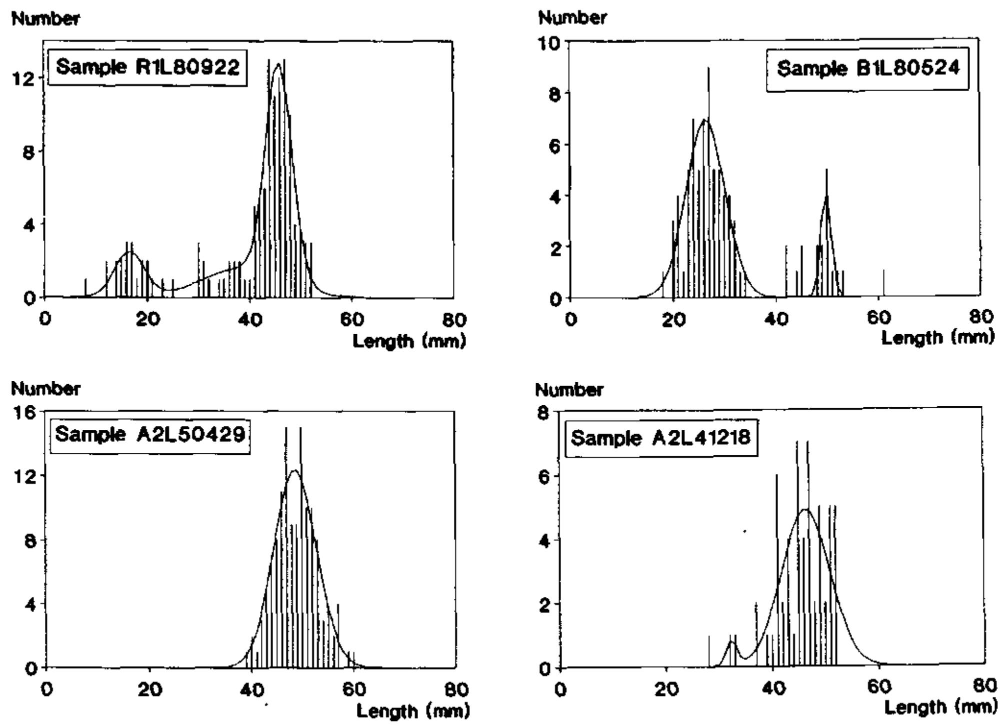


Figure 8. Measured (bars) and calculated (lines) length-frequency distributions for four samples. Sample codes refer to location and date as explained in section 1.4.

The latter constraints imply that the l_i results have to be checked constantly, since as a final estimate, in theory, $l_{01} > l_{02}$ or $l_{02} > l_{03}$ is a possibility. In Figure 8 some resulting estimated curves are shown. Location codes and sample dates refer to Figures 2 and 3, respectively. In Table III, estimated average lengths, distribution standard deviations (σ) and numbers (A) are listed.

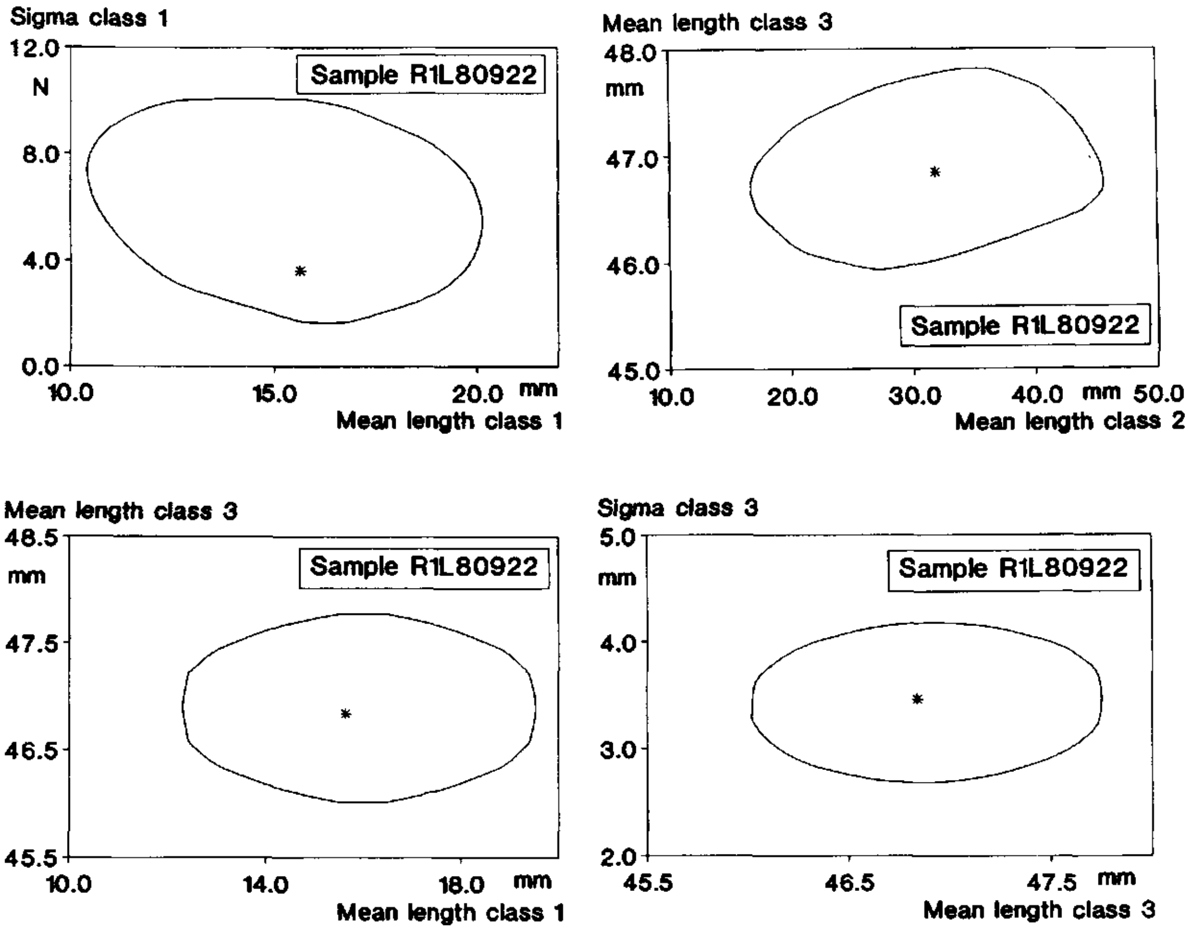


*Figure 9. Confidence contour examples for some parametercombinations from eq.2. * = calculated best fit. Sample notations refer to locations and dates as explained in section 1.4.*

Table III. Estimated average lenghts, σ and A-values for the samples shown in Figure 8.

Sample	R1L80922		B1L80524		A2L50429		A2L41218	
	avg	dev	avg	dev	avg	dev	avg	dev
l-1	14.43	2.127	--	--	--	--	--	--
l-2	33.1	5.789	25.61	0.7572	--	--	--	--
l-3	45.47	0.4067	49.36	0.4965	48.07	0.5663	44.81	1.546
σ-1	1.687	2.15	--	--	--	--	--	--
σ-2	5.341	6.095	3.221	0.6246	--	--	--	--
σ-3	2.263	0.3382	0.4562	0.7752	3.742	0.4608	3.542	1.235
A-1	9.284	9.02	0	0	0	0	0	0
A-2	15.44	15.04	56.57	9.33	0	0	0.168	6.666
A-3	67.76	8.5	4.728	4.069	116.9	12.43	42.34	13.38

Confidence contours

In Figure 9 four confidence contours are shown. As expected, the confidence intervals for the A- and l-parameters are more or less normally distributed; the one for σ is right-skewed, caused by the non-linearity of σ in eq.2.

It has to be stressed that the contour lines shown in Figure 9 actually are sums of squares to which some α-value is assigned according to eq. 7. The contours are exact, the error level is approximate.

Discussion

The length-frequency distribution computations never got stuck in local minima; so the used strategy satisfies: all the computations are carried out threefold, with different starting values for the three l_i-parameters. The parameters that yield the lowest S-value (eq.11) are stored. These calculations are the most reliable way of calculating average lengths for each year class. Any subjective decision is left out of these computations. Only the choice of the number of length classes and the parameter constraints are more or less subjective, but these choices are made in advance, and not during the calculations. This is different from the method Fournier *et al.* (1990) employed; their method allows some subjective decisions during the computations, and, of course, is not suited for year-class length estimations, since this is one of the parameters computed in advance according to a Von Bertalanffy equation.

The interpretation of the growth in terms of weight and length increase is statistically correct because the reliability of the parameters is computed. The 95% confidence contours shown in Figure 9 all concern sample R1L80922 (mussels sampled from the IBN mesocosms on September the twenty-second, 1988), the length-frequency distribution of this sample is shown in Figure 8. It is clear from Figure 8 that the first and the third length class are present in the sample, and the second length class is slightly visible on the third length class' left shoulder. The total number of animals in the first length-class is much smaller and the σ_1-value is larger than for the third length class. Mind that a large σ-value implies that the distribution is 'flat'; it does *not* necessarily mean that the *reliability* of the mean length of the same length class is bad. This reliability is given by the *individual confidence interval* of this mean length. In the combined confidence contour diagrams (Figure 9) the 95% boundaries for the y-value at the mean x-value are good indications of the 95% individual confidence interval of the y-mean. The interpretation of all the length-frequency results from the Western Wadden Sea mussel sampling will be reported separately.

2.3. Description of the mathematical technique for calculation of length-weight parameters and the use of uncertainty analysis

2.3.1. Estimation of length-weight parameters

According to section 2.1.3,

$$W_{c,j} = a \cdot L_j^{\,b} \tag{9}$$

is used in the present approach. By fitting the calculated weights W_c to W_m, the measured weights, the parameters **a** and **b** are estimated.

The target is, analogue to eqs. 4 and 5, to minimize

$$S(a,b) = \sum_{j=1}^{M} (W_{c,j} - W_{m,j})^2 \tag{10}$$

where M is the number of measurements. For the final estimate (,)

$$\frac{\partial S}{\partial a} = \frac{\partial S}{\partial b} = 0 \tag{11}$$

In section 2.1.3 it is explained that for comparison reasons **b** must have a constant value. $2 < b < 3.5$ usually is appropriate. **b** = 3 implies equal growth in all directions, **b** = 2 implies that, e.g., when doubling the length, the animal increase √2 in size in both other directions. **b** > 3 will be observed for animals that become thicker. When **b** is *not* estimated (kept constant), eq. 9 is linear in **a**, and a simple linear regression method will do.

There is, however, a second reason why estimating **a** as well as **b** is dangerous.

Close examination of the behaviour of eq. 9 shows that usually **a** and **b** are very strongly, negatively correlated : a decrease in **a** needs an increase in **b** to yield almost the same S-value. The more the individual animal length measurements are restricted to a relatively small interval, which is rather often the case, the worse the discrimination between **a** and **b** will be.

The developed computer program MUSSEL (section 2.4) allows both estimation of **a** with a fixed **b**-value and simultaneous estimation of **a** and b. When both **a** and **b** are to be estimated, the same non-linear modified Marquardt routine is used as is applied for the length-frequency distribution calculations.

When **b** is kept constant, eqs. 9, 10 and 11 yield

$$a = \frac{\sum_{j=1}^{M} W_{m,j} \cdot L_j^b}{\sum_{j=1}^{M} L_j^{2 \cdot b}} \tag{12}$$

which is a normal linear regression calculation with a zero intercept.

2.3.2. Parameter significance

Simultaneous non-linear estimation of a and b.

In order to calculate the significance of the **a** and **b** parameter, the same confidence contour calculation method as mentioned in section 2.2.2 is being employed: starting from the calculated best fit, the region of this best fit is scanned by solving eq.7 subsequently for each of the two parameters **a** and **b**.

Also, individual parameter confidence intervals are computed.

Estimation of a after linear estimation

When **b** is kept constant (according to eq. 12), the standard deviation of **a** is given by

$$\sigma_a(a) = \sqrt{\frac{\sum_{j=1}^{M} (W_{c,j} - W_{m,j})^2}{(M-1) \cdot \sum_{j=1}^{M} L_j^{2b}}} \tag{13}$$

The average population weight for mussels with given length l, and its error, is given by

$$W_c(L) = a \cdot L^b \pm t(M-1, 1-\alpha) \cdot \sqrt{\frac{\Sigma(W_c - W_m)^2}{(M-1)}} \cdot \sqrt{1.0/M + \frac{(L^b)^2}{\Sigma(L^b)^2}} \tag{14}$$

where t(M-1,1-α) is Students-t distribution value for M-1 degrees of freedom at the (1-α)$\cdot$100% confidence level (Snedecor and Cochran, 1980). Weights of individual mussels and their uncertainty are given by

$$W_c(L) = a \cdot L^b \pm t(M-1, 1-\alpha) \cdot \sqrt{\frac{\Sigma(W_c - W_m)^2}{(M-1)}} \cdot \sqrt{1.0/M + 1.0 + \frac{(L^b)^2}{\Sigma(L^b)^2}} \qquad (15)$$

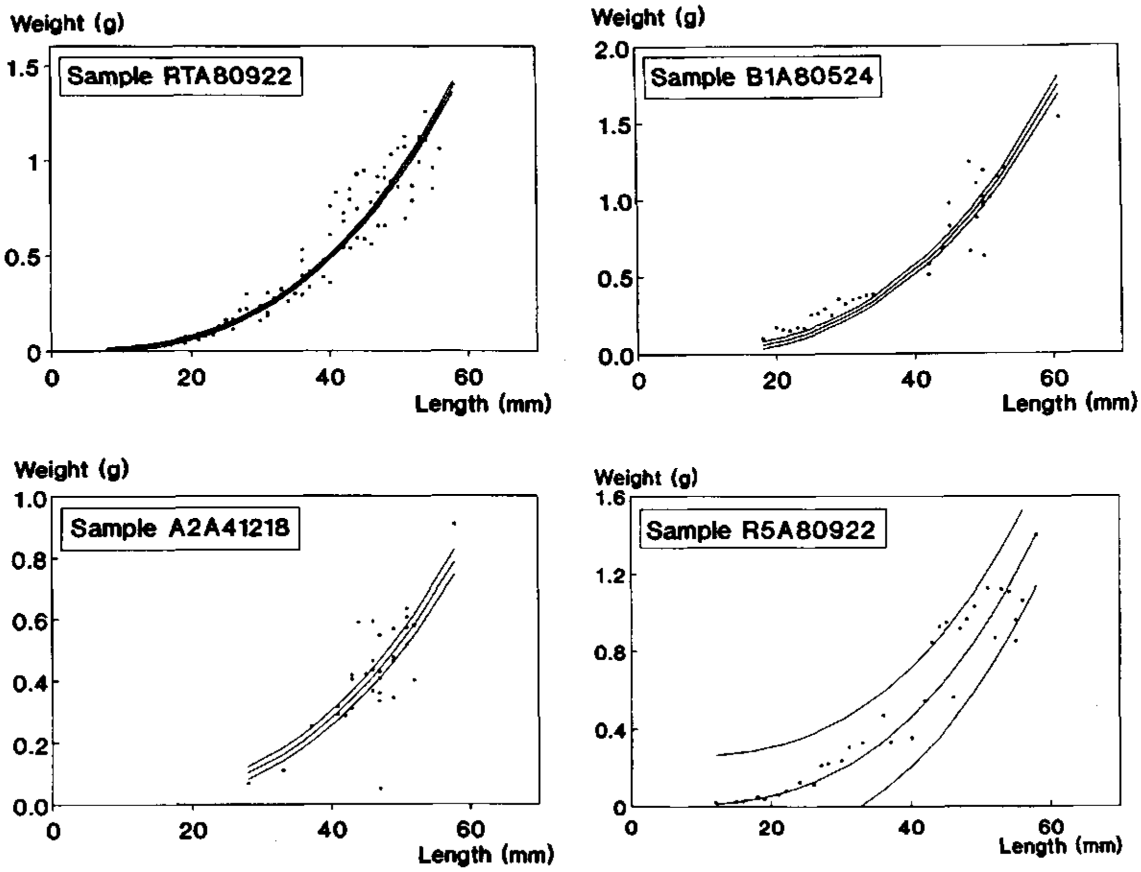


Figure 10. Best length-weight fits after estimation of **a**, **b**=*2.8 Fig. 10a-c: 95% confidence boundary for population is shown (eq.14); Fig. 10d: 95% confidence boundary for single animal (eq.15) is shown. Sample notation: see section 1.4.*

2.3.3. Results

Length-weight parameters

In Figure 10 computation result are given, together with the 95% confidence boundaries. It will be clear that these boundaries are relatively narrow when the number of measurements is large. Note that the boundaries denote the 5% probability that the calculated length-weight relationship is wrong. The chance on finding a single mussel with a certain length inside this weight interval is much smaller than 95%, see eq.15. In Figure 10^d an example of such a confidence region is shown.

Confidence contours

The combined 95% confidence contours of **a** and **b** are shown in Figure 11. It will be clear that estimating both **a** and **b** generally yields very unreliable parameter values. The minimization routine even stops at an arbitrary point because it is programmed in such a way that it not only stops searching when a minimum is reached, but it also stops when further progress (in terms of eq.10) is smaller than a certain minimum value.

The location of the minimum found by the routine often is not exactly in the middle of the banana-shaped curves shown in Figure 10. Also, the confidence contour calculating routine does not even detect the extremes of these curves, which illustrates the difficulty of the search for the 'true' values of the **a**- and **b**-parameter.

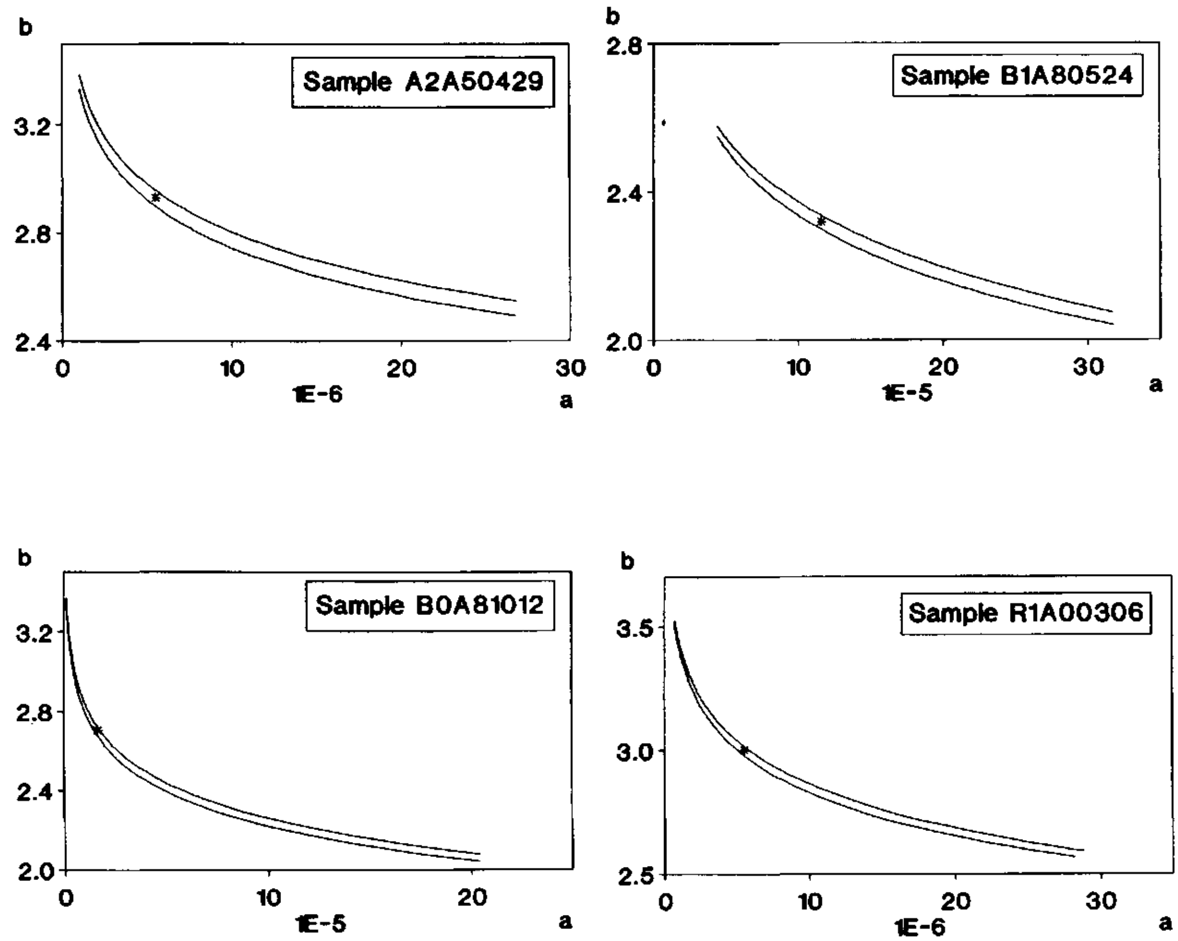


Figure 11. Combined 95% confidence contours for **a**- *and* **b**-*parameter values after simultaneous estimation of* **a** *and* **b**. *Sample notation: see section 1.4.*

Non-linear estimation techniques versus regression after logarithmic transformation

It was stated in section 2.1.3 that the use of a least-square method after logarithmic transformation of length and weight values may result in very bad estimates. In Figure 12 it is shown that in the present case the use of the logarithmic transformation method will result in minor errors when calculated weights are compared. The estimated parameter values also differ from the results obtained using the correct methods, these deviations may *not* be minor. As a result of the high interchangeability of the **a**- and the **b**-parameter (Figure 11) a wide range of combination of **a**- and **b**-values will more or less satisfy the eq.11 criterion. In Table IV the relevant parameter results are given.

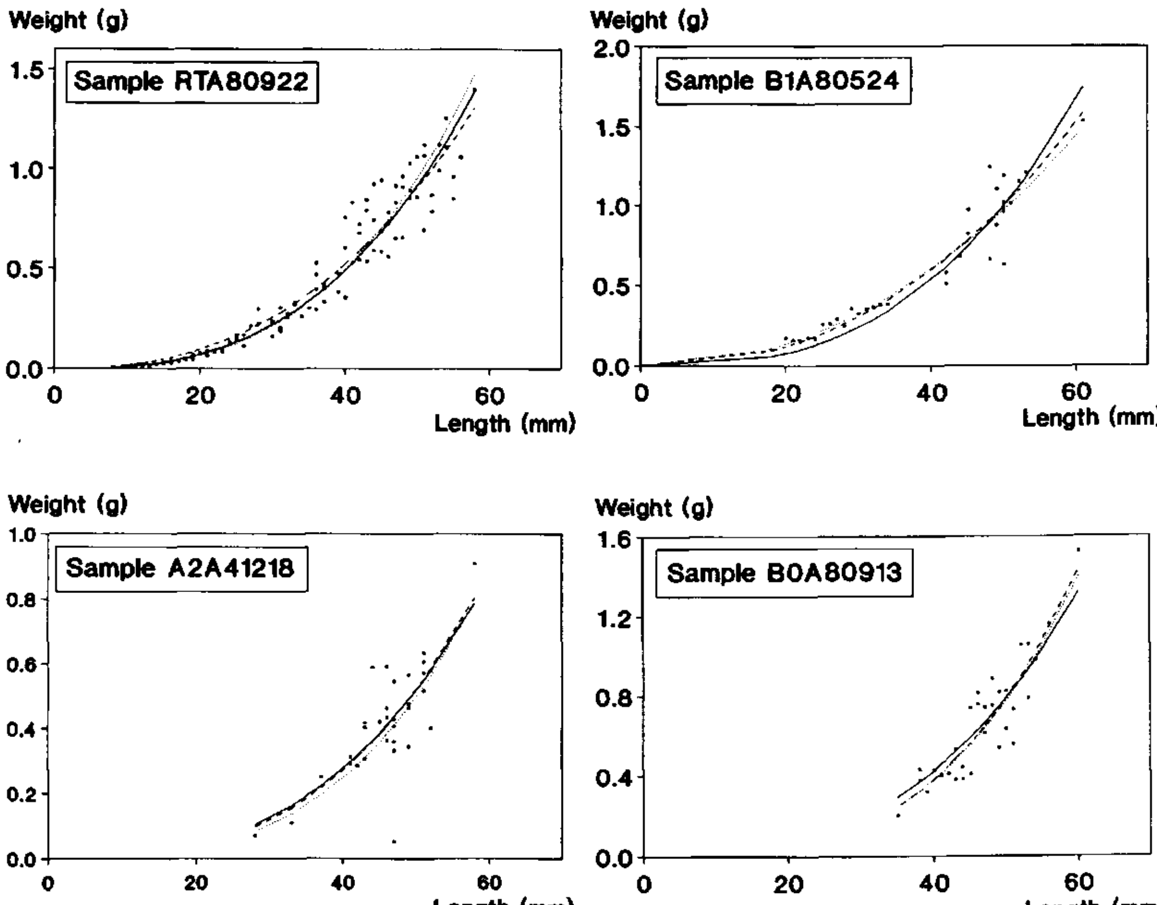


Figure 12. Best length-weight fits using three different estimation methods. Line: **a** *estimated,* **b** *=2.8 refers to eq.12; Dots:* **a,b** *estimated (log) refers to* **a** *and* **b** *calculation after logarithmic transformation; Broken line:* **a,b** *estimated refers to the non-linear estimations according to eqs. 9-11.*

Table IV. Length-weight parameters after three different estimation methods. Sample notations: see section 1.4.

Sample	a-parameter			b-parameter	
	a,b non-linear estimation	**a** estimated, b=2.8	**a,b** estimated after log transformation	**a,b** non-linear estimation	**a,b** estimated after log transformation
RTA80922	$5.69\ 10^{-5}$	$1.60\ 10^{-5}$	$9.57\ 10^{-6}$	2.473	2.942
B1A80524	$1.15\ 10^{-4}$	$1.75\ 10^{-5}$	$2.15\ 10^{-4}$	2.318	2.151
A2A41218	$5.47\ 10^{-6}$	$9.06\ 10^{-6}$	$2.47\ 10^{-6}$	2.930	3.121
B0A80913	$2.17\ 10^{-6}$	$1.39\ 10^{-5}$	$2.69\ 10^{-6}$	3.273	3.214
R1A80922	$1.61\ 10^{-5}$	$1.16\ 10^{-5}$	$7.07\ 10^{-5}$	2.410	2.891

Discussion

The reliability of the combination (**a**,**b**) is rather good (Figures 10 and 12), the reliability of each separate parameter is extremely small (Figure 11). This again supports the recommendation *not* to estimate **a** and **b** simultaneously when comparison of **a**-values from different samples is one of the goals of a research. However, when the calculated parameters only are to be used for the sample itself, estimation of **a** *and* **b** gives a better fit and is *not* to be rejected. Also, when the **b**-value is not known, one might start with estimation **a** and **b** simultaneously for all samples, average all the **b**-results, and restart all the computations using this averaged **b**-value as the fixed **b**.

2.4. Software program MUSSEL

In order to perform the mentioned calculations automatically, the computer program MUSSEL has been developed, applicable not only to mussel samples, but to all samples from populations having discrete reproduction behaviour. The program runs on any IBM-compatible personal computer running under MS-DOS equipped with a numerical co-processor. Also some additional calculations are performed, such as population biomass-frequency distributions:

$$\boldsymbol{Biomass_l} = \sum_{j=1}^{3} a \cdot L_{l,j}^{b} \cdot N_l \tag{16}$$

All the MUSSEL results are stored in such a way that further processing by spreadsheet programs such as LOTUS 1-2-3 or -Symphony is accounted for. The MUSSEL program is described in appendix A in more detail.

2.5. Discussion

The procedure described here allows the user to calculate a number of population characteristics, as well as the reliability of the results. This automated process is preferable since subjective estimations are avoided. The method used by Fournier *et al.* (1990) allows subjective decisions, which is a disadvantage, although the authors conclude otherwise. Also, their method uses the very important pre-assumption that the length of each year class lies on the Von Bertalanffy growth curve. First, this makes it impossible to derive growth characteristics from the results; secondly, since deviations from this growth curve are substantial in nature, the interpretation of the results in terms of year class abundances is not very reliable.

The MUSSEL program does not yield nice estimates when applied to length-frequency data with many length classes. For the gathered mussel data from the Dutch Western Wadden Sea this problem is avoided by taking the larger mussels (Length > 50 mm) together. For other populations other solutions may be necessary. The main criterion is how well size classes can be distinguished: the lesser the spread in size, the better size class parameters can be calculated.

The calculations also make clear that one has to be very careful when estimating length-weight parameters; especially the estimation of both the **a**- and **b**-parameters from eq. 9 is disputable. If both **a**- and **b**-parameters are estimated, the initial values used for the estimation procedure will determine, to a large extend, what the result will be.

The MUSSEL program has been used extensively for analyzing about 600 data sets from the Dutch Western Wadden Sea, mostly containing length-frequency distribution data as well as length-weight data. In Figure 13, length-distribution results from sample A1L4* (year 1984) are shown. In Figure 14, an example of calculated **a**-parameters (eq.9, **b** = 2.8) for five samples in 1984 is given. The results imply that during the year 1984 the average weight of mussels with a certain length decreased. This a-factor is often called the mussel condition factor. Such results may be combined with other environmental data such as food availability or temperature.

In Figure 15, the length of mussels from the same location is shown, derived from the analysis already given in Figure 13. In Figure 16, the weight of a single mussel during that year is drawn. This weight is derived from results for the length-frequency distribution and the length-weight relationship.

MUSSEL computes the biomass of the length-weight distribution samples and of the length-weight samples. These values can be compared to the measurements. Generally, as is expected when applying a correct parameter estimation procedure, measurements and computations do not differ much, as can be seen from Table VI.

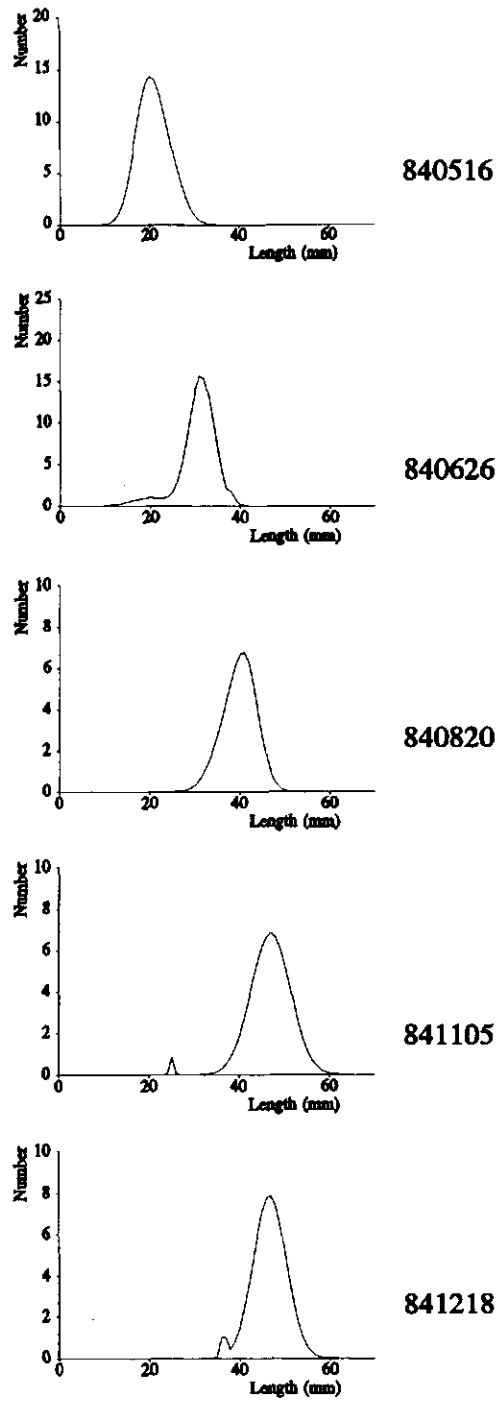


Figure 13. Computed length-frequency distribution for sample A1 for five dates in 1984.

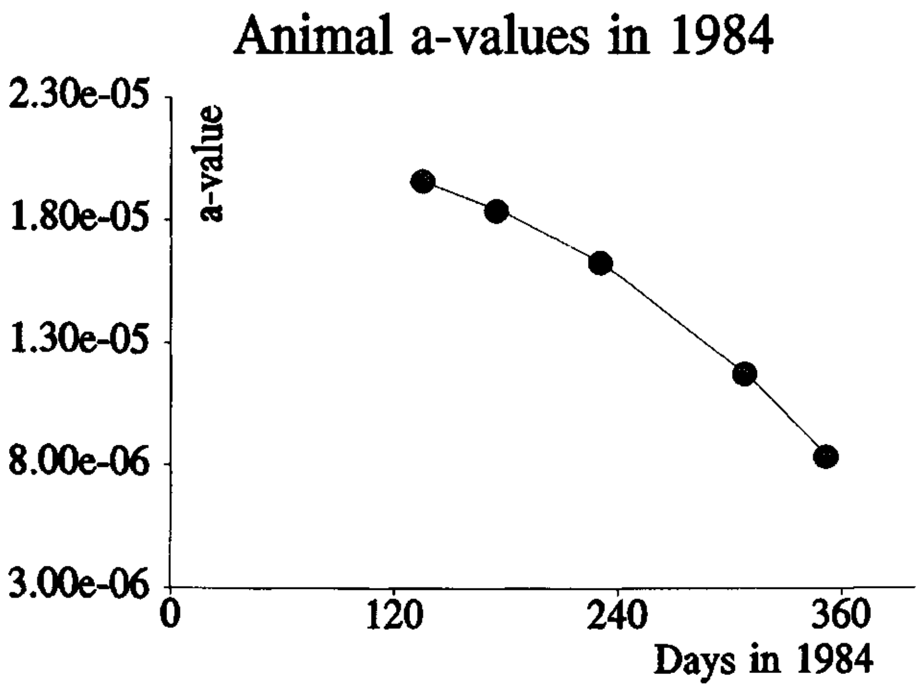


Figure 14. Estimated a-values for sample location A1 during 1984 b = 2.8.

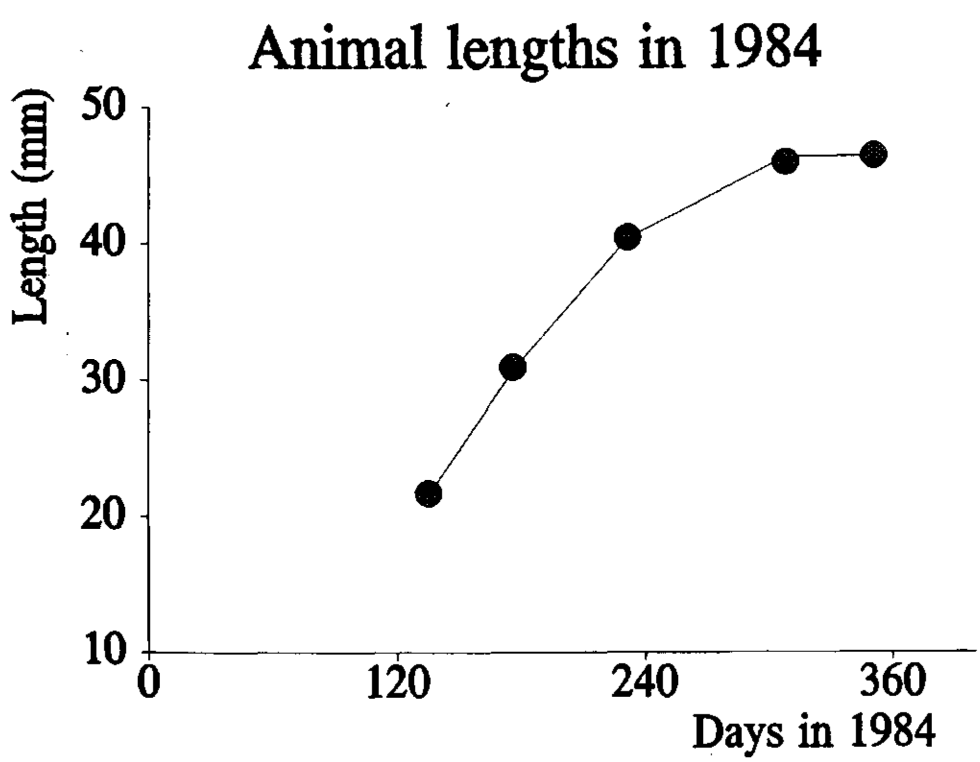


Figure 15. Estimated lengths for one mussel year-class; sample location A1 during 1984.

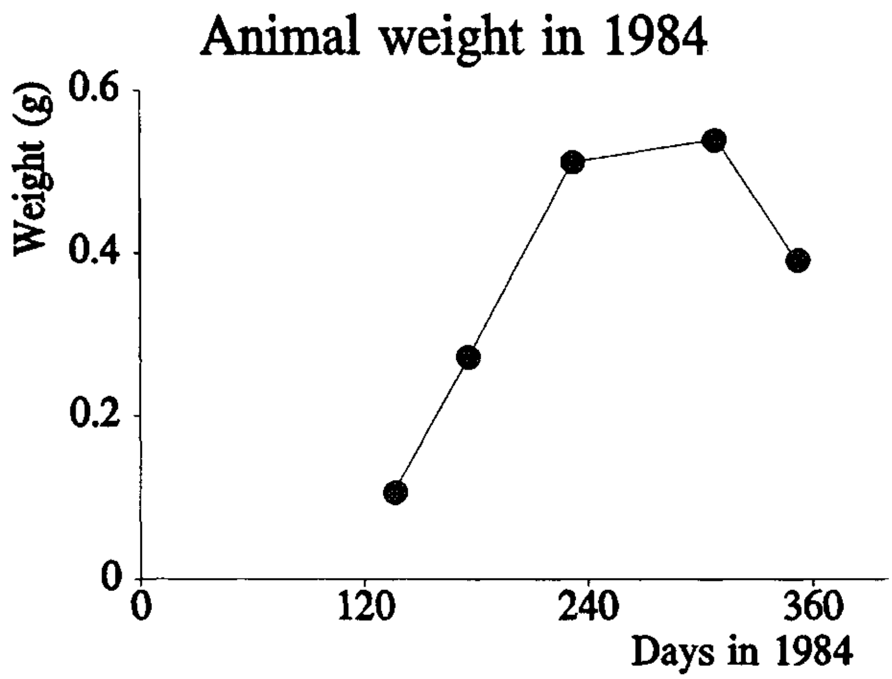


Figure 16. Computed animal weight for sample A1during 1984.

Table VI. Computed and measured total sample weight of some length-weight samples. Sample notation after section 1.4.

Sample	Measured weight (g)	Computed weight (g)
B1A41218	13.36	13.50
B1A80524	21.23	20.05
R1A80922	13.62	13.35

Complete results will be reported separately.

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A The use of the MUSSEL computer program

A The use of the MUSSEL computer program

A.1 Introduction

In the following sections, the use of the MUSSEL program will be explained briefly. This appendix is not a manual; however, the examples of input and output files (App. A.2) will give sufficient information to minimize, or even eliminate, user problems.
In appendix B, the MUSSEL software is given in detail. This appendix is not an integral part of this report, and is available on request. See also section A.5 on support and program supply.

MUSSEL treats two different data-sets, and computes
1- a length-frequency distribution
2- a length-weight relationship.
Then, outcomes of both computations are coupled: weights of all size-classes are calculated and summed. As an option, MUSSEL computes individual confidence regions and combined confidence contours for the parameters estimated for the length-frequency distribution and for the length-weight relationship. The applied methods are explained in chapter 2.

For the computations, three ASCII-files are needed by MUSSEL.
1- the measured length-frequency data have to be available;
2- the measured length-weight data are needed.
Both tables are independent, that is, they may concern different length ranges. The format of the files is treated in App.A.2.2 and A.2.3.
3- a configuration file is needed by MUSSEL, where the different options have to be chosen.
In the configuration file one has, among others, to define the min-max ranges for the length-classes and to set flags whether a confidence contour computation is wanted or not. This configuration file *has* to be named MUSSEL.CFG, and comes with the MUSSEL-program in a standard format, in ASCII. Using some standard text editor, options may be changed by the user. MUSSEL.CFG is treated more in detail in A.2.2.1.

The computation of confidence contours needs two table-files (BBSTUDEN.GEG and BBFISHER.GEG), that are part of the software, and do not need adaptation by the user.

MUSSEL produces a number of output-files. These files all have names that are derived from the names of the input-files. These output-files concern:

1- the results of all parameter estimations (extension: .PAR)

2- the results of the length-frequency distribution, including the weights of the animals when weight-data are available. Input-data are printed also (extension: .OUT).

3- the results of the length-weight relationship: parameters and computed plus measured weights (extension: .GEW).

4- the results of the confidence contours (extension: .UNC).
Usually this concerns two files: one on the length-frequency computation, and one on the length-weight data. The latter is only of interest when the user asks for the estimation of both the a- and the b-parameter. The names of the files are derived from the input-file names.

5- a log-file (extension: .LOG) is produced containing all intermediate results. This is done for a possible check afterwards; usually this can be omitted (log-switch in MUSSEL.CFG is 'off'), or the file can be deleted after a proper program run.

A.2 Running MUSSEL

A.2.1 Calling MUSSEL and window layout

MUSSEL is written in MODULA-2 (Wirth, 1985; Logitech, 1987; Verhulst, 1986). Before the starts of MUSSEL, Logitech's Window Machine driver (WM.EXE) must be installed; it is used by MUSSEL. Then, MUSSEL can be run. The program then reads MUSSEL.CFG (A.2.2), BBFISHER.GEG and BBSTUDEN.GEG, and asks for the file containing the length-frequency data and for the file containing the length-weight data. Is one of both files not available, a '0' should be typed instead. The computations regarding these data then are skipped.

The calculations then run. A summary of results is displayed during the program run. After finishing the computations, MUSSEL does not close it's screen until a key is pressed. On this screen final results are being displayed, so the user keeps an overview. In case of batch operation, this option can be skipped by changing the relevant switch in MUSSEL.CFG. Figures A.1[a,b] show examples of screens during and after finishing the calculations, respectively.

```
Datafile length-frequency data     :       spista.prn
Datafile length - weights          :       spistop.prn
File length-frequency results      :       spista.out
File length-weight results         :       spistop.gew
Log-file                           :       spista.log
File with starting values          :       mussel.cfg
Parameter-results                  :       spista.par
Uncertainty results Length-Freq    :       spista.unc
Uncertainty results Weight-Length :        spistop.unc

Startvalue number   1
Iteration-number   23     ;  sum-of-squares: 2.590E+001


Mean               Sigma            A
 1.7991E+001        1.4022E+000      4.3769E+000
 2.8599E+001        2.2919E+000      3.0620E+001
 2.9055E+001        1.3075E+000      2.2189E+000
```

```
Datafile length-frequency data     :       spista.prn
Datafile length - weights          :       spistop.prn
File length-frequency results      :       spista.out
File length-weight results         :       spistop.gew
Log-file                           :       spista.log
File with starting values          :       mussel.cfg
Parameter-results                  :       spista.par
Uncertainty results Length-Freq    :       spista.unc
Uncertainty results Weight-Length :        spistop.unc

Best (MSA)-set    2        ;  sum of squares   2.542E+001
Mean               Sigma            A
 6.8955E+000        9.6104E-001      6.8654E-007
 1.7992E+001        1.4052E+000      4.3857E+000
 2.8699E+001        2.2023E+000      3.2789E+001
Computed a voor G=a*L^b, b fixed          :  8.292E-006
Sumn-of-squares a,b-computation G=a.L^b  :  2.876E-002
a-value  :  7.4610E-006         b-value  : 3.0304E+000

        ESC= stop, elke andere toets= verder gaan
```

Fig. A.1a,b MUSSEL screen during and after finishing the computations

A.2.2 Data-files

As mentioned before, three basic input-files come with MUSSEL. The configuration file MUSSEL.CFG is treated below; both files BBFISHER.GEG and BBSTUDEN.GEG are given in Appendix B.

A.2.2.1 The configuration file MUSSEL.CFG

MUSSEL.CFG is read automatically.

In Table A.I this file is given. Most of the parameter meanings will be clear as a result of their explanation in section 2, and also as a result of the comments in the file. Please notice that the text 'data:' is a code: parameter reading does not start until that code is read by MUSSEL. The test preceding this code is regarded as comment; thus all kind of explanation can be added to this file. Hence, a short introduction will do here.

- Relative step-size: the non-linear iteration procedure computes the step-size of the parameter corrections, finally leading to the best estimation. It appeared to be useful not to use the complete correction, but just part of it. This step-size parameter tells the parameter estimation procedure which relative part of the computed correction should be realized ($0.0 < \text{stepsize} <= 1.0$). Usually this parameter needs no adaptation.
- Iprint: frequency of output to screen and to log-file. When Iprint=1, extra output to the log-file is produced. Generally, Iprint= 3 to 5 is most convenient. The computation time is only slightly influenced by Iprint.
- Relative error: stop criterion for iteration. Concerns the relative accuracy of the computed numbers. Or, the wanted sum of squares $\Sigma_i R_i^2$, with i=1..M. M is the total number of lengths (which is maximal 100, see A.2.2.2), and R_i is the difference between computed and measured number of animals of length i.

Table A.I The configuration file MUSSEL.CFG

```
MUSSEL configuration file.
Contains parameters for length-frequency iteration and for length-weight iteration. Also some flags/switches have to be set properly.
Iprint is the frequency of intermediate iteration result output to the screen and the LOG-file.
When Iprint=0 there is no output of intermediate results.
Beta is the power of the allometric length-weight equation for the case only a has to be estimated. It also serves as starting value when a and b have to estimated simultaneously.
At least 30 positions serve for comment ! Tabs are not allowed.
Note that 'data:', starting as the first word on a line, serves as marking code for the start of relevant input-data!
---------|---------|---------|30
data:
Relative stepsize                       0.4
Iprint                                  2
relative error criterion                1.0E-3
improvLimit                             1.0E-3
maximal number of iterations            300
Batch (1) or file-by-file (0)?          0
beta value (G=alfa*L^beta)              2.8

Boundaries for estimators.
Animal lengths
min                                     max
data:
0                                       20
20                                      40
35                                      70

Standarddeviations of the distributions
min                                     max
data:
0.5                                     5
1                                       8
1                                       12

Numbers. Only the minimum is of importance.
min
data:
0
0
0

Uncertainty parameters. First the number of contours is needed, and then what the alfa-values are.
Note: have 30 positions for comment on each line ! And: 'data:' denotes start of input.
---------|---------|---------|30
data:
uncertainty contours?                   1
number of alfa-values                   2
95% confidence contour                  0.05
90% confidence contour                  0.10

Then, the number of the parameter combinations and the parameter combinations for which the contours are wanted have to be given.
Numbering:
l-mean = 1 4 7                          For length-classes 1 , 2 resp. 3
sigma  = 2 5 8                          For length-classes 1 , 2 resp. 3
A      = 3 6 9                          For length-classes 1 , 2 resp. 3
In case the number of animals in a length-class is less than 10% of the total number, the uncertainty computation is not done.
---------|---------|---------|30
data:
number of combinations                  6
length class 1: l & sigma               1 2
length class 2: l & sigma               4 5
length class 3: l & sigma               7 8
length class 1 & 2 : l-mean             1 4
length class 1 & 3 : l-mean             1 7
length class 2 & 3 : l-mean             4 7

Then it is asked whether simultaneous a- and b- parameter estimation should be done for the allometric length-weight equation (G=a*l^b). Note that a, with b fixed, is always computed when length-weight data are available.
Combined confidence contours can be computed. Alfa-values are asked (0.30, 0.10, 0.05 en 0.01 are possible; 0.10 and 0.05 are recommended).
Again: 30 positions at least for comment, and 'data:' denotes start of data.
---------|---------|---------|30
data:
estimate a and b ? (1/0)                1
confidence contours? (1/0)              1
number alfa-values ?                    2
95% confidence contour                  0.05
90% confidence contour                  0.10
```

- improvLimit	dummy
- max number of iterations	when the number of iterations exceeds this number, the iteration stops unsuccessfully. In general, a value of 300 will do. A limit is needed to avoid extreme long sessions.
- Batch ? or file-by-file ?	when 0, MUSSEL does not end immediately but keeps showing the final parameter results until any key is pressed. When 1, MUSSEL does stop, and a next run (e.g. in a batch-file) may be performed.
- b - value	the length-weight relationship is always computed with a as the only estimator, and (this) b-value as the fixed parameter. When (see below) also an estimation of a *and* b is asked, this b value serves as starting value.
- min / max values for l	minimum and maximum values for the three size classes. Should be between 0 and 100. MUSSEL computes starting values from the lengths present in the data-set.
- min / max values for σ	minimum and maximum values for the standard deviation of the distribution. Maximum values should not be too large (related to the size class range), minimum values should be larger then 0.
- min value for A	MUSSEL computes starting values from the data.

The estimation is repeated three times using different values as starting values for the average lengths. First the mean value as computed from the data (for the relevant range) is used, then the average of this value and the minimum, and then the average of this value and the maximum value as given in this parameter file. It is useful to have the ranges overlap each other somewhat. The choice of the ranges mainly depends on the kind of animal and the size classes present. Using different scales (cm instead of mm, which is the unit of length with mussels) other animals may become subject of these computations. When animals have sizes varying from 0.2 to 12 mm, e.g., it is advisable to scale the lengths to integer values from 1 to 60, perform the computations, and then rescale later on. Of course, a length-frequency distribution does not allow real values as lengths.

- confidence contours ? 1 = YES, 0 = NO. Concerns only the length-frequency distribution.
- number of alfa's May be 1, 2 or 3.
- alfa-values $100 \cdot \alpha$ = uncertainty boundary (%). For as many as there are α's asked, an value for α must be given. Possibilities are: 0.30, 0.10, 0.05, 0.01.
- number of combinations is the number of confidence contours computed by MUSSEL
- parameter combinations MUSSEL reads the above mentioned (number of combinations) which parameter combinations should be subject of the confidence computations. The numbering is as follows: length, σ and A of size class 1 have numbers 1, 2 and 3. Those of class 2 have numbers 4, 5 and 6, and those of class 3 have 7, 8 and 9. When less than 10% of the number of animals belongs to a certain size class, then the parameters from this size class will be skipped from the uncertainty computations. This is because the sum of squares will be very little sensitive to changes in such parameters, and computation of the confidence contours will give very wide and unrealistic regions. Also, MUSSEL may not be sufficiently protected against extreme parameter values.

Also the computation of confidence contours for the a- and b-parameter from the length-weight relationship needs some parameters. Note that if only the a-parameter is wanted, none of the input data can be skipped!

- estimate a *and* b ? 1 = YES, 0 = NO. Note that a alone will *always* be computed when length-weight data are available.
- compute confidence contours ? 1 = YES, 0 = NO.
- number of α-values May be 1 , 2 or 3
- which α-values ? the above number times an α-value. Possible values are 0.30, 0.10, 0.05, 0.01

Now, MUSSEL.CFG is outlined. A user usually may change the value of Iprint, Batch (1/0), the desired b-value, both switches that indicate whether a confidence contour computation has to be computed or not, and the values of the l-range and the σ-range of the three size classes. Other parameters and flags should only be adapted when strictly necessary.

A.2.2.2 Length-frequency data

In Table A.II an example of a length-frequency data file is given. It consists of pairs of (lengths,number) data, both being integers. Lengths have to be integers ranging between 1 to 100. 0 is not allowed. Lengths may appear more than once, the numbers being summed. Not all lengths between 1 and 100 are needed: all lengths are initialized with zero numbers.
The file is closed with '-99' as final length; no number is needed.

A.2.2.3 Length-weight data

In Table A.III an example of a length-weight data-file is given.
It consists of pairs of (lengths,weight) data. Lengths are integers, weights are real numbers. Lengths may appear more than once. **The maximum number of data is 1400. If more, a program error occurs.** The file is closed with '-99' as final length; no weight is needed.

Table A.II File with length-frequency data

11	1
13	2
15	4
14	5
20	12
19	14
18	14
17	5
16	6
30	2
29	2
28	2
27	6
26	5
25	7
24	8
23	10
22	13
21	18
31	1
-99	

Table A.III File with length-weight data

30	0.1859
32	0.2002
36	0.2467
37	0.2936
37	0.44
38	0.2402
38	0.2986
39	0.3504
39	0.2805
40	0.3509
40	0.4798
41	0.4326
41	0.4766
42	0.4732
42	0.5752
42	0.3999
42	0.6381
43	0.5367
43	0.5503
43	0.5426
43	0.6122
-99	

A.2.3 Results of the computations

In A.1 it is mentioned that five different files are produced by MUSSEL. All are in ASCII-format, and suitable to be imported into a spreadsheet program such as LOTUS-123 or Quattro-Pro for further processing. Figures can be produced, or results from several computations may be combined.

A.2.3.1 Parameter results

Estimated parameter values are printed to file .PAR; in Table A.IV an example is presented. For each of the three length-classes the computed average length, standard deviation of the distribution and the A-value are given. Also, the individual confidence intervals (75%, α=0.25) are given for each parameter. For the length-weight relationship the computed a-value that comes with a fixed b-value and its standard deviation (σ_a(a)) are printed. When a and b are also estimated simultaneously, both are printed. Otherwise, their values will equal 0.

Table A.IV Results of the parameter estimation (.PAR file)

	min-value	estimated	max-value	avrg +/- range
"length"	8.596E-001	1.500E+001	2.617E+002	1.304E+002
"length"	3.712E+001	3.815E+001	3.919E+001	1.034E+000
"length"	4.292E+001	4.372E+001	4.457E+001	8.258E-001
"standard deviation"	1.719E-001	3.000E+000	5.235E+001	2.609E+001
"standard deviation"	1.294E+000	1.851E+000	3.323E+000	1.015E+000
"standard deviation"	2.028E+000	2.595E+000	3.399E+000	6.858E-001
"A-value"	8.042E-008	1.403E-006	2.449E-005	1.220E-005
"A-value"	1.986E+001	3.222E+001	4.476E+001	1.245E+001
"A-value"	5.072E+001	6.557E+001	8.022E+001	1.475E+001
"alfa"	6.474E-006			
"$\sigma(a)$"	2.340E-007			
"beta"	2.800E+000			
"alfaD"	6.472E-006			
"betaD"	2.800E+000			
"number of weight data"	32			
"sum weights l-G measured"	6.777E+000			
"sum weights l-G computed"	7.005E+000			
"sum weights l-N measured"	0.000E+000			
"sum weights l-N computed"	2.244E+001			

The printed values only are of interest when the relevant data-file was available. When one of the data files was absent, the initialization values as given in MUSSEL.CFG are printed.

The user has to check the relevance of the results. The possibility not to print non-relevant data is rejected in order to keep the output-files identical.

A check is performed: the sum of all measured weights should approach the sum of computed weights. Since the weight of all the length-frequency individuals together may also be determined, the sum of weight of these individuals is printed too. The sum of weights measured for the l-N distribution is not printed by MUSSEL, but has to be filled in by hand by the user afterwards (e.g.

in a spreadsheet).

A.2.3.2 Results of the length-frequency distribution (l-N data)

The results of the length-frequency distribution including the calculated weights and input data are printed to a file with extension .OUT. The name is derived from the input-file. When there are no l-N data, then this output-file is *not* produced. In Table A.V part of such a file is shown. Total measured weight always equals 0, and has to be filled in by the user, e.g. in a spreadsheet, when post-processing the results. Total computed weight of the length-frequency distribution sample equals 0 when length-weight data were absent. Then, the percentage contribution of each size class to the total number of animals in the sample is printed.

Then, a large table gives measured and computed results for each animal size. Printed are: measured and computed numbers, computed numbers for each size class, individual weight for each animal, total weight for all animals (= individual weight times number of animals) and the total weight per size class (= individual weight times number of animals in that size-class). The sum of 'total weights per animal' equals the 'Wtot-calc' data in the heading of this table.

A.2.3.3 Results of the length-weight (l-W) computation

Results of the length-weight (l-W) computation are printed to a file with extension .GEW, and a name derived from the input-file. In Table A.VI an example is given. Parameter values concern: a-value ('alfa-value') for the situation with a fixed b-value ('beta-value'), and it's standard deviation ('aSig-value'). Then, alfaD and betaD denote the results that come from the estimation of both a and b. 'Wtot-meas' and 'Wtot-calc' denote the measured and computed total sample weights; 'Weight-LSQ' is the finally found minimum sum-of-squares value Σ_i(measured weight$_i$ - computed weight$_i$)^2.

Then, in a large table, results are given per length:

- meas-W : measured weight
- calc-W : calculated weight

Table A.V Results computation of length-frequency distribution (.OUT file)

"Identifier"	"S23_1_840516 "
"Wtot-meas"	0.000E+000
"Wtot-calc"	0.000E+000
"N-% c-1"	9.226E-001
"N-% c-2"	2.025E+000
"N-% c-3"	9.705E+001

"length"	N-measured	N-computed	N-class1	N-class2	N-class3	G-indiv	G-tot	Weight-c1	Weight-c2	Weight-c3
1	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.459E+121	0.000E+000	0.000E+000	0.000E+000
2	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.520E+121	0.000E+000	0.000E+000	0.000E+000
3	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.578E+121	0.000E+000	0.000E+000	0.000E+000
4	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.646E+121	0.000E+000	0.000E+000	0.000E+000
5	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.716E+121	0.000E+000	0.000E+000	0.000E+000
6	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.782E+121	0.000E+000	0.000E+000	0.000E+000
7	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.831E+121	0.000E+000	0.000E+000	0.000E+000
8	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.893E+121	0.000E+000	0.000E+000	0.000E+000
9	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	1.948E+121	0.000E+000	0.000E+000	0.000E+000
10	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	2.006E+121	0.000E+000	0.000E+000	0.000E+00
11	0.000E+000	5.013E-013	5.013E-013	0.000E+000	0.000E+000	0.000E+000	2.055E+121	0.000E+000	0.000E+000	0.000E+000
12	0.000E+000	3.084E-006	3.084E-006	0.000E+000	0.000E+000	0.000E+000	2.144E+121	0.000E+000	0.000E+000	0.000E+000
13	0.000E+000	3.664E-002	3.664E-002	0.000E+000	0.000E+000	0.000E+000	2.310E+121	0.000E+000	0.000E+000	0.000E+000
14	1.000E+000	8.401E-001	8.401E-001	0.000E+000	0.000E+000	0.000E+000	2.426E+121	0.000E+000	0.000E+000	0.000E+000
15	0.000E+000	3.719E-002	3.719E-002	0.000E+000	0.000E+000	0.000E+000	2.546E+121	0.000E+000	0.000E+000	0.000E+000
16	0.000E+000	3.178E-006	3.178E-006	0.000E+000	0.000E+000	0.000E+000	2.697E+121	0.000E+000	0.000E+000	0.000E+000
17	0.000E+000	5.242E-013	5.242E-013	0.000E+000	0.000E+000	0.000E+000	2.895E+121	0.000E+000	0.000E+000	0.000E+000
18	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	3.014E+121	0.000E+000	0.000E+000	0.000E+00
19	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	0.000E+000	3.134E+121	0.000E+000	0.000E+000	0.000E+00
20	0.000E+000	1.130E-016	0.000E+000	0.000E+000	1.130E-016	0.000E+000	3.299E+121	0.000E+000	0.000E+000	0.000E+000
21	0.000E+000	1.362E-015	0.000E+000	0.000E+000	1.362E-015	0.000E+000	3.411E+121	0.000E+000	0.000E+000	0.000E+000
22	0.000E+000	1.057E-013	0.000E+000	9.056E-014	1.513E-014	0.000E+000	3.529E+121	0.000E+000	0.000E+000	0.000E+000
23	0.000E+000	1.460E-008	0.000E+000	1.460E-008	1.548E-013	0.000E+000	3.636E+121	0.000E+000	0.000E+000	0.000E+000
24	0.000E+000	1.176E-004	0.000E+000	1.176E-004	1.460E-012	0.000E+000	3.762E+121	0.000E+000	0.000E+000	0.000E+000
25	0.000E+000	4.734E-002	0.000E+000	4.734E-002	1.269E-011	0.000E+000	3.863E+121	0.000E+000	0.000E+000	0.000E+000
26	1.000E+000	9.525E-001	0.000E+000	9.525E-001	1.016E-010	0.000E+000	4.060E+121	0.000E+000	0.000E+000	0.000E+000
27	1.000E+000	9.577E-001	0.000E+000	9.577E-001	7.495E-010	0.000E+000	4.310E+121	0.000E+000	0.000E+000	0.000E+000
28	0.000E+000	4.812E-002	0.000E+000	4.812E-002	5.095E-009	0.000E+000	4.576E+121	0.000E+000	0.000E+000	0.000E+000

Table A.VI Results of computation of length-weight relationship (.OUT file)

"alfa-value"	1.159E-006
"aSig-value"	4.594E-007
"beta-value"	2.800E+000
"alfaD-value"	2.841E-005
"betaD-value"	2.562E+000
"Wtot-meas"	1.222E+001
"Wtot-calc"	1.214E+001
"Weight-LSQ"	2.460E-001

"length"	"meas-W"	"calc-W"	"cWmin-pop70	cWmax-pop70	cWmin-pop90	cWmax-pop90	cWmin-pop95	cWmax-pop95	cWmin-ind70	cWmax-ind70	cWmin-ind90	cWmax-ind90	cWmin-ind95	cWmax-ind95"
3.300E+001	1.966E-001	2.070E-001	-9.977E+302	9.977E+302	-9.977E+302	9.977E+302	-9.977E+302	9.977E+302	-5.104E+303	5.104E+303	-5.104E+303	5.104E+303	-5.104E+303	5.104E+303
3.300E+001	1.593E-001	2.070E-001	-9.977E+302	9.977E+302	-9.977E+302	9.977E+302	-9.977E+302	9.977E+302	-5.104E+303	5.104E+303	-5.104E+303	5.104E+303	-5.104E+303	5.104E+303
3.400E+001	1.824E-001	2.250E-001	-1.017E+303	1.017E+303	-1.017E+303	1.017E+303	-1.017E+303	1.017E+303	-5.107E+303	5.107E+303	-5.107E+303	5.107E+303	-5.107E+303	5.107E+303
3.400E+001	2.363E-001	2.250E-001	-1.017E+303	1.017E+303	-1.017E+303	1.017E+303	-1.017E+303	1.017E+303	-5.107E+303	5.107E+303	-5.107E+303	5.107E+303	-5.107E+303	5.107E+303
3.500E+001	2.887E-001	2.441E-001	-1.038E+303	1.038E+303	-1.038E+303	1.038E+303	-1.038E+303	1.038E+303	-5.112E+303	5.112E+303	-5.112E+303	5.112E+303	-5.112E+303	5.112E+303
3.500E+001	2.566E-001	2.441E-001	-1.038E+303	1.038E+303	-1.038E+303	1.038E+303	-1.038E+303	1.038E+303	-5.112E+303	5.112E+303	-5.112E+303	5.112E+303	-5.112E+303	5.112E+303
3.600E+001	2.677E-001	2.641E-001	-1.062E+303	1.062E+303	-1.062E+303	1.062E+303	-1.062E+303	1.062E+303	-5.117E+303	5.117E+303	-5.117E+303	5.117E+303	-5.117E+303	5.117E+303
3.600E+001	2.422E-001	2.641E-001	-1.062E+303	1.062E+303	-1.062E+303	1.062E+303	-1.062E+303	1.062E+303	-5.117E+303	5.117E+303	-5.117E+303	5.117E+303	-5.117E+303	5.117E+303
3.700E+001	2.916E-001	2.851E-001	-1.089E+303	1.089E+303	-1.089E+303	1.089E+303	-1.089E+303	1.089E+303	-5.122E+303	5.122E+303	-5.122E+303	5.122E+303	-5.122E+303	5.122E+303
3.700E+001	6.070E-001	2.851E-001	-1.089E+303	1.089E+303	-1.089E+303	1.089E+303	-1.089E+303	1.089E+303	-5.122E+303	5.122E+303	-5.122E+303	5.122E+303	-5.122E+303	5.122E+303
3.800E+001	2.939E-001	3.073E-001	-1.119E+303	1.119E+303	-1.119E+303	1.119E+303	-1.119E+303	1.119E+303	-5.129E+303	5.129E+303	-5.129E+303	5.129E+303	-5.129E+303	5.129E+303
3.800E+001	3.055E-001	3.073E-001	-1.119E+303	1.119E+303	-1.119E+303	1.119E+303	-1.119E+303	1.119E+303	-5.129E+303	5.129E+303	-5.129E+303	5.129E+303	-5.129E+303	5.129E+303
3.900E+001	4.846E-001	3.304E-001	-1.151E+303	1.151E+303	-1.151E+303	1.151E+303	-1.151E+303	1.151E+303	-5.136E+303	5.136E+303	-5.136E+303	5.136E+303	-5.136E+303	5.136E+303
3.900E+001	2.865E-001	3.304E-001	-1.151E+303	1.151E+303	-1.151E+303	1.151E+303	-1.151E+303	1.151E+303	-5.136E+303	5.136E+303	-5.136E+303	5.136E+303	-5.136E+303	5.136E+303
4.000E+001	3.600E-001	3.547E-001	-1.186E+303	1.186E+303	-1.186E+303	1.186E+303	-1.186E+303	1.186E+303	-5.144E+303	5.144E+303	-5.144E+303	5.144E+303	-5.144E+303	5.144E+303
4.000E+001	2.181E-001	3.547E-001	-1.186E+303	1.186E+303	-1.186E+303	1.186E+303	-1.186E+303	1.186E+303	-5.144E+303	5.144E+303	-5.144E+303	5.144E+303	-5.144E+303	5.144E+303
4.100E+001	3.473E-001	3.801E-001	-1.225E+303	1.225E+303	-1.225E+303	1.225E+303	-1.225E+303	1.225E+303	-5.153E+303	5.153E+303	-5.153E+303	5.153E+303	-5.153E+303	5.153E+303
4.100E+001	4.565E-001	3.801E-001	-1.225E+303	1.225E+303	-1.225E+303	1.225E+303	-1.225E+303	1.225E+303	-5.153E+303	5.153E+303	-5.153E+303	5.153E+303	-5.153E+303	5.153E+303
4.200E+001	4.002E-001	4.066E-001	-1.266E+303	1.266E+303	-1.266E+303	1.266E+303	-1.266E+303	1.266E+303	-5.163E+303	5.163E+303	-5.163E+303	5.163E+303	-5.163E+303	5.163E+303
4.200E+001	3.229E-001	4.066E-001	-1.266E+303	1.266E+303	-1.266E+303	1.266E+303	-1.266E+303	1.266E+303	-5.163E+303	5.163E+303	-5.163E+303	5.163E+303	-5.163E+303	5.163E+303
4.300E+001	4.730E-001	4.343E-001	-1.311E+303	1.311E+303	-1.311E+303	1.311E+303	-1.311E+303	1.311E+303	-5.174E+303	5.174E+303	-5.174E+303	5.174E+303	-5.174E+303	5.174E+303
4.300E+001	3.540E-001	4.343E-001	-1.311E+303	1.311E+303	-1.311E+303	1.311E+303	-1.311E+303	1.311E+303	-5.174E+303	5.174E+303	-5.174E+303	5.174E+303	-5.174E+303	5.174E+303
4.400E+001	4.181E-001	4.632E-001	-1.359E+303	1.359E+303	-1.359E+303	1.359E+303	-1.359E+303	1.359E+303	-5.186E+303	5.186E+303	-5.186E+303	5.186E+303	-5.186E+303	5.186E+303
4.400E+001	3.687E-001	4.632E-001	-1.359E+303	1.359E+303	-1.359E+303	1.359E+303	-1.359E+303	1.359E+303	-5.186E+303	5.186E+303	-5.186E+303	5.186E+303	-5.186E+303	5.186E+303
4.500E+001	5.602E-001	4.933E-001	-1.411E+303	1.411E+303	-1.411E+303	1.411E+303	-1.411E+303	1.411E+303	-5.200E+303	5.200E+303	-5.200E+303	5.200E+303	-5.200E+303	5.200E+303
4.500E+001	5.159E-001	4.933E-001	-1.411E+303	1.411E+303	-1.411E+303	1.411E+303	-1.411E+303	1.411E+303	-5.200E+303	5.200E+303	-5.200E+303	5.200E+303	-5.200E+303	5.200E+303
4.600E+001	6.044E-001	5.246E-001	-1.466E+303	1.466E+303	-1.466E+303	1.466E+303	-1.466E+303	1.466E+303	-5.215E+303	5.215E+303	-5.215E+303	5.215E+303	-5.215E+303	5.215E+303
4.600E+001	4.631E-001	5.246E-001	-1.466E+303	1.466E+303	-1.466E+303	1.466E+303	-1.466E+303	1.466E+303	-5.215E+303	5.215E+303	-5.215E+303	5.215E+303	-5.215E+303	5.215E+303
4.700E+001	5.780E-001	5.572E-001	-1.524E+303	1.524E+303	-1.524E+303	1.524E+303	-1.524E+303	1.524E+303	-5.232E+303	5.232E+303	-5.232E+303	5.232E+303	-5.232E+303	5.232E+303
4.700E+001	6.864E-001	5.572E-001	-1.524E+303	1.524E+303	-1.524E+303	1.524E+303	-1.524E+303	1.524E+303	-5.232E+303	5.232E+303	-5.232E+303	5.232E+303	-5.232E+303	5.232E+303
4.800E+001	4.621E-001	5.910E-001	-1.586E+303	1.586E+303	-1.586E+303	1.586E+303	-1.586E+303	1.586E+303	-5.250E+303	5.250E+303	-5.250E+303	5.250E+303	-5.250E+303	5.250E+303
4.800E+001	5.152E-001	5.910E-001	-1.586E+303	1.586E+303	-1.586E+303	1.586E+303	-1.586E+303	1.586E+303	-5.250E+303	5.250E+303	-5.250E+303	5.250E+303	-5.250E+303	5.250E+303

- cWmin-pop70 - cWmax-pop95 : the 70, 90 and 95 % confidence regions for the computed relationship, regarding the total sample (the 'population'). This means: how reliable is the result as a representation of the length-weight relationship in this sample.
- cWmin-ind70 - cWmax-ind95 : the 70, 90 and 95 % confidence regions for the computed relationship, regarding an individual of the given weight. This means: how certain can one be that an animal of a certain size has the weight as computed using the present length-weight parameters.

A.2.3.4 Results of the confidence contour computations

The results of the confidence contours are printed in files with extension .UNC. The name is derived from the input-files for the length-frequency data and the length-weight data, respectively. When confidence contours are not asked, the file(s) is (are) not produced. Both files look almost identical. In Table A.VII a part of such a file is shown. When MUSSEL has not been successful, 'no success' is printed. This may be caused by the fact that too few animals were present in a size class (note that MUSSEL does not even start computing confidence contours for parameter from a certain size-class when less than 10% of total number is in that size class), or that the computation yields other extreme results. Each table starts with 'n1' and 'n2', that denote the parameters for which the confidence contours are computed. The "real" value (x,y0) for both parameters is also printed, as is the sum-of-squares ('F-value') and the alfa-value for the ({1.0 - alfa} · 100 = confidence region) contour. The results have such a format that, after the file is imported into a spreadsheet, results for all alfa-values can easily be plotted. *Note, that in the spreadsheet, for the graphs it is required to empty the fields with quotes alone!*

Table A.VII Example of (part of) a file with uncertainty results.

```
"n1-number"                1
"n2-number"                2
"x,y0"    "    "    0.000E+000"    "    "    "    "    "    0.000E+000
"F-value"              1.450E+002
"alfa-value"           5.000E-002
"No success"  "        "


"n1-number"                1
"n2-number"                2
"F-value"              1.409E+002
"alfa-value"           1.000E-001
"No success"  "        "


"n1-number"                7
"n2-number"                8
"x,y0"    "    "    4.033E+001"    "    "    "    "    "    3.617E+000
"F-value"              1.450E+002
"alfa-value"           5.000E-002
"x,y0"    "    "    3.958E+001  3.579E+000
"x,y0"    "    "    3.959E+001  3.523E+000
"x,y0"    "    "    3.964E+001  3.400E+000
"x,y0"    "    "    3.964E+001  3.400E+000
"x,y0"    "    "    3.964E+001  3.400E+000
"x,y0"    "    "    3.968E+001  3.346E+000
"x,y0"    "    "    3.968E+001  3.346E+000
"x,y0"    "    "    3.968E+001  3.346E+000
"x,y0"    "    "    3.972E+001  3.293E+000
"x,y0"    "    "    3.977E+001  3.243E+000
"x,y0"    "    "    3.978E+001  3.239E+000
"x,y0"    "    "    3.984E+001  3.196E+000
"x,y0"    "    "    3.984E+001  3.192E+000
"x,y0"    "    "    4.000E+001  3.115E+000
"x,y0"    "    "    4.009E+001  3.089E+000
"x,y0"    "    "    4.019E+001  3.074E+000
"x,y0"    "    "    4.033E+001  3.070E+000
"x,y0"    "    "    4.038E+001  3.074E+000
"x,y0"    "    "    4.048E+001  3.089E+000
"x,y0"    "    "    4.058E+001  3.115E+000
"x,y0"    "    "    4.067E+001  3.150E+000
"x,y0"    "    "    4.083E+001  3.239E+000
"x,y0"    "    "    4.084E+001  3.243E+000
"x,y0"    "    "    4.089E+001  3.289E+000
"x,y0"    "    "    4.090E+001  3.293E+000
"x,y0"    "    "    4.095E+001  3.346E+000
"x,y0"    "    "    4.095E+001  3.346E+000
"x,y0"    "    "    4.095E+001  3.346E+000
"x,y0"    "    "    4.100E+001  3.400E+000
"x,y0"    "    "    4.100E+001  3.400E+000
"x,y0"    "    "    4.100E+001  3.400E+000
"x,y0"    "    "    4.106E+001  3.523E+000
"x,y0"    "    "    4.108E+001  3.579E+000
"x,y1"    "    "    4.108E+001  3.848E+000
"x,y1"    "    "    4.106E+001  3.903E+000
"x,y1"    "    "    4.100E+001  4.026E+000
"x,y1"    "    "    4.100E+001  4.026E+000
"x,y1"    "    "    4.100E+001  4.026E+000
"x,y1"    "    "    4.095E+001  4.079E+000
"x,y1"    "    "    4.095E+001  4.079E+000
"x,y1"    "    "    4.095E+001  4.079E+000
"x,y1"    "    "    4.090E+001  4.131E+000
"x,y1"    "    "    4.089E+001  4.136E+000
"x,y1"    "    "    4.084E+001  4.180E+000
"x,y1"    "    "    4.083E+001  4.185E+000
```

Table A.VIII Example of log-file output. In this case: only length-weight parameter estimation results.

```
After    1 iterations the sum of squares is   1.4042E+000
Alfa            Beta
 1.1849E-005 2.8000E+000

After    8 iterations the sum of squares is   1.3615E+000
Alfa            Beta
 2.0332E-005 2.6620E+000

After   13 iterations the sum of squares is   1.3384E+000
Alfa            Beta
 2.6998E-005 2.5928E+000

After   18 iterations the sum of squares is   1.3252E+000
Alfa            Beta
 3.2411E-005 2.5477E+000

After   23 iterations the sum of squares is   1.3163E+000
Alfa            Beta
 3.7017E-005 2.5146E+000
```

A.2.3.5 Output of intermediate results to a LOG-file

Intermediate results from the parameter estimation routine are printed to a log-file (.LOG). The Iprint-parameter (MUSSEL.CFG, section A.1) determines the print frequency. The file-name is derived from the length-frequency data-file; when not present, from the length-weight data-file. In Table A.VIII an example of such a file is given. When a program execution error occurs, no extra output to this file is printed. See section A.4.

A.3 Further processing of results

After a MUSSEL-run, the ASCII-formatted results have to be post-processed using a spreadsheet tool (Lotus-123, -Symphony, Quattro-Pro, etc.); the result-files can be imported, and further computations can be done. Also, results can be combined with results from other samples. Graphical tools (from these spreadsheets, or from separate programs such as Harvard Graphics, DrawPerfect, etc.) are suited to produce graphs.

A.4 Error handling

When an error occurs during program execution, MUSSEL stops. Reasons for errors may be:

1- the user supplies a data- or configuration file that is not correct.

2- during execution extreme situations occur resulting in messages as 'range error' or 'floating point overflow'. A 'range error' may be due to a wrong length (length is over 100), or too many weight data (maximum is 1400).

When read errors occur (reading MUSSEL.CFG, BBSTUDEN.GEG, BBISHER.GEG, length-frequency and length-weight data-files) MUSSEL may stop execution without notice. In that case the program expects further input from these files (Control-Break exits MUSSEL). Otherwise, an error message precedes the end of the execution. When no error message follows, the user is advised to check the data files carefully. When an execution stops after 'range error' or 'floating point overflow', a large data-file called MUSSEL.PMD is produced, while displaying 'writing post-mortem dump'. This data-file contains all values of variables at the moment the error occurred. This MUSSEL.PMD is several hundreds of Kb large. Be sure that enough disk-space is available! This .PMD file can be studied using Logitech's MODULA-2 post-mortem debugger (Logitech, 1987), which is *not* available for the MUSSEL-user since it is part of Logitech's MODULA-2 development system. If necessary, this .PMD file can be checked by the author in order to find and to correct the errors.

A.5 Support

MUSSEL is developed by the author. Some support can be offered. After consultation, slight adaptations may be possible in some cases. The software program itself is free, and available upon request for Dfl. 25.= per copy to cover copy and transportation costs.