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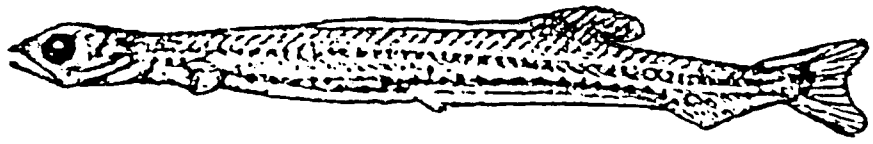
THE USE OF MULTIVARIATE MORPHOMETRICS TO DETERMINE THE NUTRITIONAL CONDITION OF MARINE FISH LARVAE

by

D.Reichow, C.Largiadèr, Ch.Klingenberg, C.Clemmesen, R.Froese and B.Ueberschär .

Institut für Meereskunde
Düsternbrooker Weg 20
D-2300 Kiel 1



A B S T R A C T

The usefulness of multivariate morphometrics to distinguish between fed and starved fish larvae was tested on laboratory reared herring larvae (Clupea harengus). Linear Discriminant Analysis was used to obtain a linear function which separates the two groups of larvae maximally. The calculations were based on twelve morphometric characters, taken individually by means of an image analysing system. A statistically significant separation of fed and starved larvae was obtained. The most important characters and the number of characters necessary for separation are outlined.

I N T R O D U C T I O N

Mortality within the larval stages of marine fishes determines subsequent recruitment to the adult stock (EHRLICH 1974). Thus, fluctuations in year class strength can be related to fluctuations of the survival rates of larval fish stages. One of the important factors which influence the survival rate is considered to be starvation of the larvae (CUSHING 1975, HUNTER 1976, LASKER 1975). The determination of the nutritional condition of fish larvae is therefore an important precondition for the prediction of larval survival and subsequent recruitment. Different methods, such as biochemical investigations (BUCKLEY 1979, CLEMMESEN 1988, UEBERSCHÄR 1988), histological methods (EHRLICH et al 1976, THEILACKER 1986), and application of length-weight relationship (EHRLICH et al 1976) have been used to determine the nutritional condition of fish larvae.

The use of a video based image analysing system for precise morphometric measurements, which are then analysed by multivariate statistics, is a new approach to distinguish between fed and starved larvae. In fisheries science multivariate morphometrics in combination with discriminant analysis has been used successfully to distinguish different fish stocks (MENG & STOCKER 1984, MISRA 1985), to study geographic morphometric variation in a species (SAILA & FLOWERS 1969), or to build a numerical identification key (FROESE 1988) to identify species of fish larvae. The investigation presented here gives an example of the application of multivariate morphometrics on laboratory reared fed and starved herring larvae.

M A T E R I A L & M E T H O D S

The study was based on laboratory reared herring larvae (Clupea harengus), which were kept under different controlled feeding conditions. At the age of 33 days a random sample of 39 well fed larvae (food density: 5 Brachionus plicatilis and 1 Artemia salina nauplius/ml) and a sample of 34 larvae deprived of food for six days was taken. Immediately after sampling, the larvae were fixed in a 4% formaldehyde/seawater solution to reduce their shrinkage (HAY 1981).

For the morphometric measurements an image analysing system was used and the following 12 parameters were measured:

1.	standard length	= SL
2.	prepectoral length	= PL
3.	width at pectorals	= WP
4.	width at anus	= WA
5.	preanal length	= PAL
6.	preorbital length	= POL
7.	diameter of eye	= DE
8.	depth above eye	= DAE
9.	depth behind anus	= DBA
10.	depth above pectorals	= DAP
11.	depth of gut	= DG
12.	interorbital distance	= ID

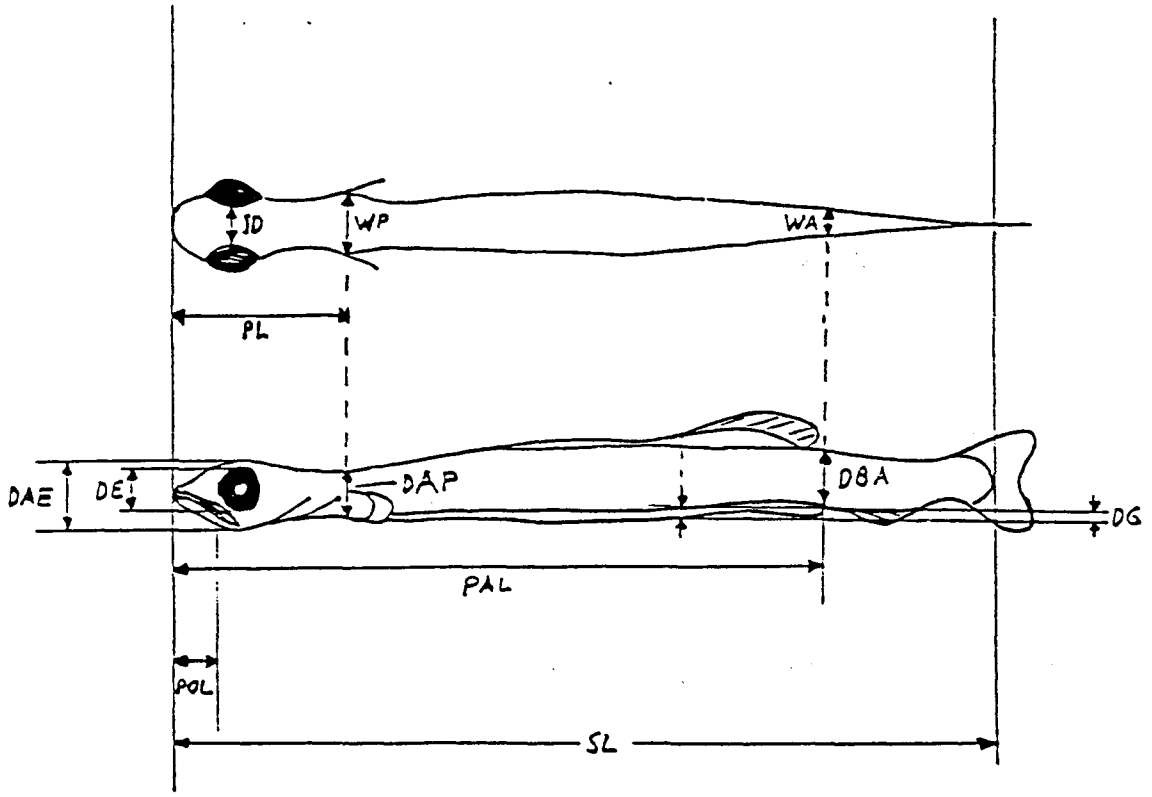


Fig. 1: Measurements performed on the fish larvae

The multivariate analyses were carried out using the SAS statistical software package (Anon. 1985). All measurements were transformed to common logarithms to approximate multivariate normality and linear relationships. Since the standard length of the larvae varied considerably in both groups, all other measurements were corrected for length according to the following equation (FROESE 1988):

Equation No. 1

$$AM = OM - (RC * (SL - MSL))$$

where AM=adjusted measurement, OM=original measurement, RC=regression coefficient between character and standard length, SL=individual standard length, MSL=overall mean standard length.

The aim of the linear discriminant analysis is to produce a linear function which gives the maximal separation of predefined groups, using all characters of each element simultaneously. In this linear discriminant function each larva is represented by a discriminant value, which is obtained through a linear combination of the 11 parameters of each individual. For quantification of the separation of both groups the standard distance Dp was used:

Equation No. 2

$$D_p = \sqrt{\frac{(n_1+n_2)(n_1+n_2-2)}{n_1 n_2} * \frac{R_p^2}{(1-R_p^2)}}$$

where Dp=standard distance, ni=number of elements in each group, R2=square of the coefficient of correlation.
(For details see FLURY & RIEDWYL 1988).

Based on the calculated linear discriminant function individuals with unknown group membership can be classified into the correct group with high probability provided that a significant separation of both groups was obtained.

Redundant variables are eliminated by means of successive backward elimination (FLURY & RIEDWYL 1988).

R E S U L T S

Based on the F-Statistics a significant separation of fed and starved herring larvae can be found. The successive backward elimination of the 11 parameters shows that the 4 characters DAE, DAP, PL and POL (Abbr. see Fig. 1) are eliminated first due to their minor influence on the separation of the groups. Figures 2 and 3 show the frequency distribution of the discriminant value, which represents each individual in the discriminant function, before (Fig. 2) and after (Fig. 3) eliminating the first four redundant characters. Both figures demonstrate a significant discrimination of the groups.

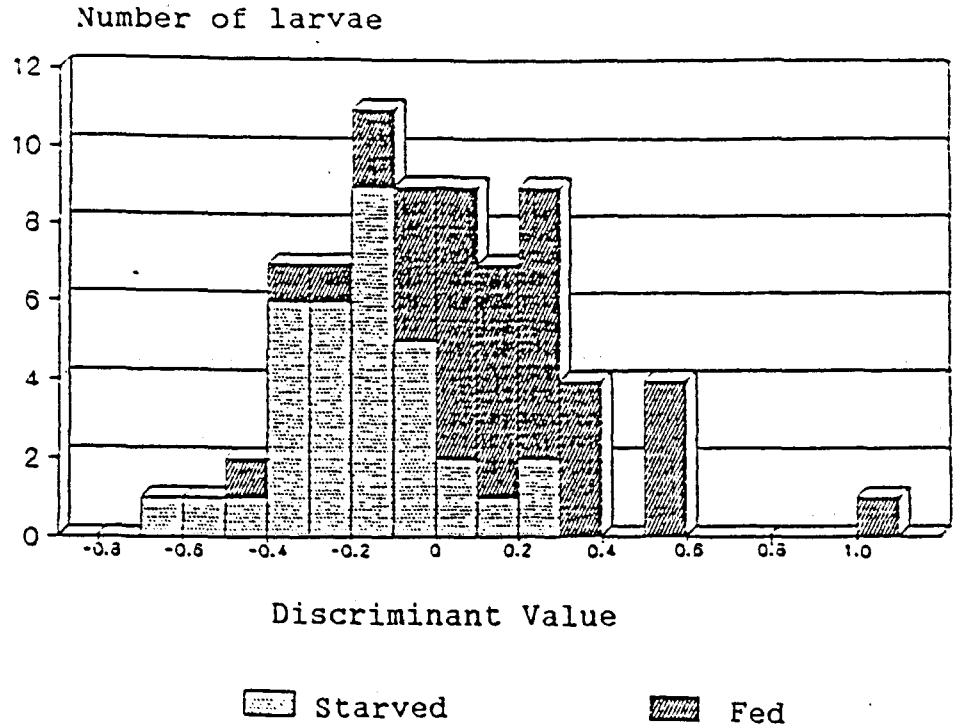


Fig. 2: Frequency distribution of the discriminant value of fed and starved herring larvae calculated with all 11 measurements

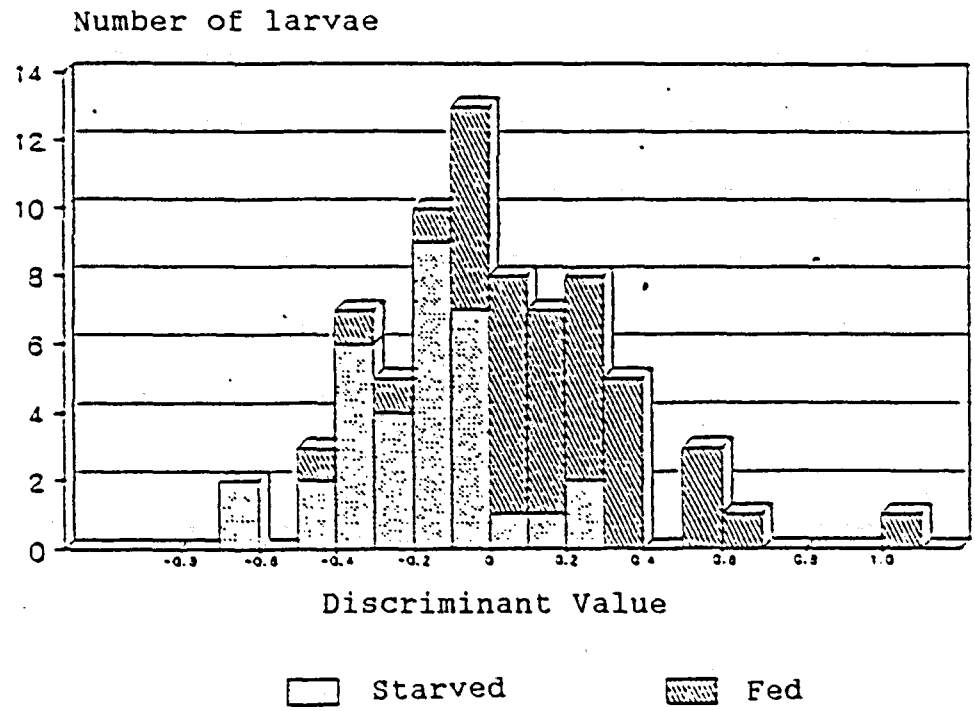


Fig. 3: Frequency distribution of the discriminant value of fed and starved herring larvae based on 7 measurements

The standard distance Dp (Equation No.2), as a quantitative criterion for the separation of the groups, shows a significant decrease after the fourth elimination step using successive backward elimination (Fig. 4).

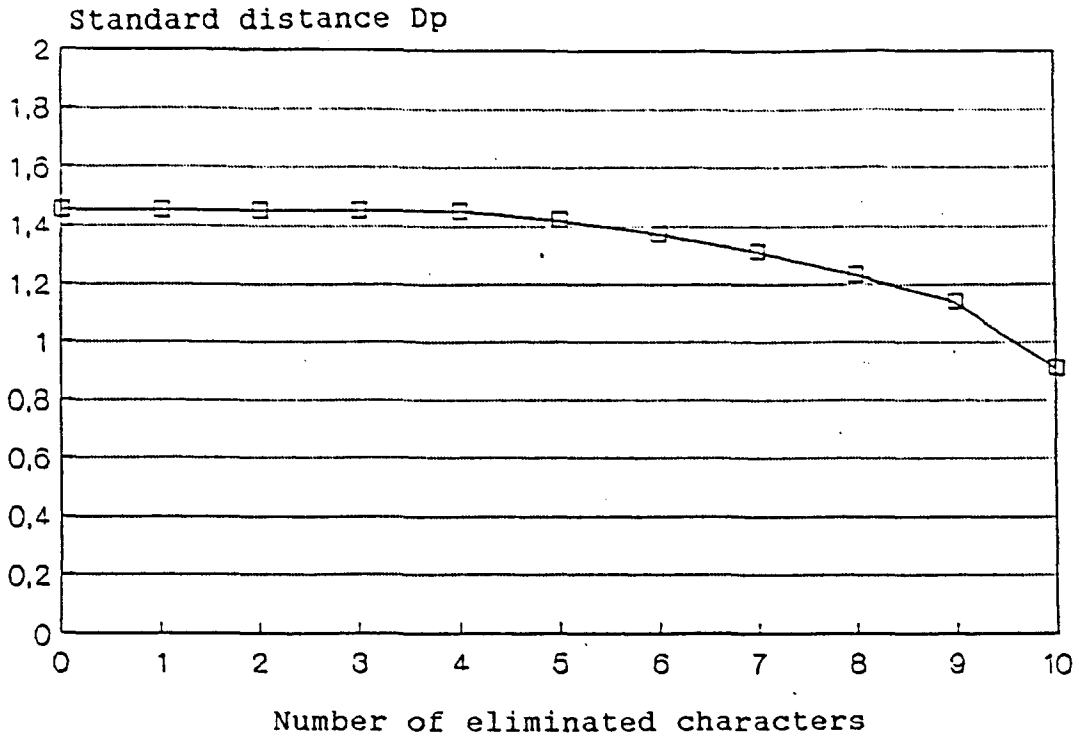


Fig. 4: Change of standard distance between starved and fed group of herring larvae at successive backward elimination.

Table 1 shows the sequence of elimination of the characters and the value of the standard distance after each elimination step.

Tab. 1: Change of standard distance (Dp) and sequence of elimination of characters at successive backward elimination.

Dp	Elimin. charact.	Elimination step
1.45	-	0
1.45	Depth above eye	1
1.45	Depth above pectorals	2
1.45	Prepectoral length	3
1.45	Preorbital length	4
1.42	Preanal length	5
1.37	Interorbital distance	6
1.31	Width at anus	7
1.23	Depth behind anus	8
1.14	Depth of gut	9
0.91	Width at pectorals	10
0.00	Diameter of eye	11

The most important character for the separation of the groups is the diameter of the eye, which is eliminated last. Compared to

a maximal standard distance of 1.45 obtained with all measures, this measure alone results in a standard distance of 0.91.

By using the discriminant function as found with linear discriminant analysis the percentages of correct classification of larvae for the two different conditions are 79.5% for the fed larvae and 85.3% for the starved larvae (Table 2).

Tab.2: Classification of larvae based on the preliminary calculated discriminant function (Total number of larvae and percentages)

From group	Classified into group		
	Fed	Starved	Total
Fed	31	8	39
%	79.49	20.51	100
Starved	5	29	34
%	14.71	85.29	100

D I S C U S S I O N

The results indicate that morphometrics in combination with a discriminant function analysis is a useful tool to distinguish between fed and starved herring larvae. This method is sensitive enough to reflect the changes in body proportions of the larvae after being exposed to different feeding conditions.

The most powerful measurement for separation was the eye diameter of the larvae, followed by the measure width at pectorals and the depth of the gut. A difference between depth of gut of both groups was already visible under the binocular, the one from the starved group being smaller. This might be due to the degeneration of gut tissue and surrounding connective tissue found in starved fish larvae (EHRLICH & BLAXTER 1976). Latest investigations show the midgut cell height to define the nutritional status of fish larvae (THEILACKER & WATANABE 1989). In future investigations additional characters should be measured to test their influence on the discrimination of fed and starved larvae. The characters DAE, DAP, PL and POL (Abbrev. see Fig. 1) are assumed to be of minor significance for the separation.

The lower percentage of correct classification of larvae from the fed group is probably due to ill or starving larvae, which regularly occur, despite of food supply. This was also found in biochemical investigations dealing with the nutritional condition of fish larvae (HAKANSON 1989, CLEMMESEN & UEBERSCHÄR pers. comm.).

The usefulness of multivariate morphometrics for discrimination of starved and fed larvae, as it is shown for a relatively small random sample of laboratory reared herring larvae in this study, has to be tested in large scale experiments, involving different species as well. Before the method can be used in field investigations, it needs experimental evaluation with different age groups.

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