STANDARD SAMPLING PROCEDURE FOR BARENTS SEA CAPELIN. A DESCRIPTION OF STANDARD SAMPLING TECHNIQUE AND METHODS APPLIED TO IMPROVE THE REPRESENTATIVENESS OF THE SAMPLES.

By

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ABSTRACT

The various types of samples, the observations made on each fish, and the coding procedure for these observations are described. The standard procedure of otolith studies on the capelin stock is described in detail, including dissection out, rinsing, embedding, reading and measuring. A description of the present ageing method used at the IMR in Bergen is included.

In addition, a method for using the samples to construct representative absolute distributions is presented, based on weighing the samples relative to the acoustic abundance estimate. An appendix describes the format in which the coded information is stored for computer treatment.

1. INTRODUCTION

The capelin stock is sampled for the purpose of biological investigation. Different types of samples are taken, according to the purpose of the survey, and additional samples may be taken from commercial catches. The most frequent type of sample is the "standard biological capelin sample", and only this sample is considered in the present paper.

2. SAMPLING PROCEDURE

2.1 Sampling on scientific cruises

When a capelin catch is onboard the vessel, a random sample of 100 fish is taken, and the sample is procesed immediately. On a sampling form, specially designed for later punching of the data, the following details on the fishing station are recorded:

position,
species,
vessel name,
station no.,
sampling gear,
year,
month,
date, and
depth.

For every fish in the sample the volume to nearest ml is measured by immersing the fish in water in a graduated cylinder, and the total length is measured to the nearest 1/2 cm below (from tip of snout to end of lower lobe of caudal fin). Fish above 10 cm are opened, and the sex, maturity stage, and the degree of stomach fullness are determined. Usually the sagittae are removed for all fishes in the sample, and used for ageing purposes. A description of the ageing technique is given in section 3.

The maturity scale used is one modified after Nikolsky (1963). (See Table 1). This maturity scale is based on macroscopic criteria only. In addition, a new maturity scale developed by Forberg (1983) has been used for females since 1980, (Forberg and Tjelmeland 1984). This scale is based on microscopic inspection of whole oocytes. This scale has ten stages, some of them with up to three subdivisions. It allows for a more precice staging of the females, especially during periods of fast maturation.

The stomach fullness is determined using a scale with six stages (Monstad 1971), of which

1 - empty stomach
2 - very litle content
3 - some content
4 - stomach full, but not expanded
5 - stomach expanded (food visible from outside)
6 - stomach everted

TABLE 1 MATURITY SCALE

Code	Stage	Description	Females	Males
1	Juvenile(a)	Gonads threadlike, sexes can hardly be separated.		
2	Juvenile(b)	Gonads increasing in volume. Sex can be determined.	Ovaries transparent, without colour.	Testes transparent, without colour.
3	Maturing(a)	Gonads opaque, blood vessels can be seen.	Ovaries with yellow/white grains.	Testes white or with white spots.
4	Maturing(b)	Gonads increasing in vol. Blood vessels distinct.	Ovaries pink or yellowish white filling up 2/3 or more of body cavity.	Testes light grey or white No milt-drops appear under pressure.
5	Maturing(c)		Ovaries occupy whole of body cavity.Most eggs transparent	Testes grey. Milt run with some pressure.
6	Spawning	Runny gonads		
7	Spent	Gonads emptied. Some residual eggs and sperm may occur		
8	Spent/ recovering	Gonads small and collapsed		

2.2 Sampling from commercial catches

At the start of each fishing season a sampling program is initiated, which provides samples from the commercial catches. These samples are of two types, length samples and standard biological samples (Section 2.1). From each catch a length sample of about 100 fishes is taken. This is done routinely in both fishing seasons from all catches which are landed at meal and oil factories. In the winter season, two factories are selected, one in the eastern, the other in the western part of the fish landing area, at which a sample of 100 specimens is taken from about every fourth catch, frozen and sent to the Institute of Marine Research in Bergen. Not all these samples are actually processed, but emphasis is put on getting a good spatial coverage during the fishing season. In the summer season this procedure would not provide samples with adequate quality, so samples are then frozen onboard the scouting vessels operating in the fishing area. The catches meant for human consumption represents a special problem as these catches are mainly processed onboard the fishing vessels, and samples are therefore not accessible.

The frozen samples are processed according to the procedure described for the samples taken onboard scientific vessels, but the maturity determination according to the new microscopic criteria may be difficult and is often omitted on frozen material.

The length samples from each catch are the basis for the fishery statistics delivered on a monthly basis, together with the total volume of the catch and length-age and weight-age keys. These statistics are broken down to number or weight per age-group by weighing the length sample to give the total length distribution for the whole catch and then applying the appropriate key. The keys are constructed on the basis of the biological samples taken in the fishing area the catches originate from.

3. AGEING TECHNIQUE

The ageing of the fish sampled from the capelin stock is essential to the assessment model in use. Only by reliable ageing can a good estimate for the abundance of each age-group be achieved, which in turn is a prerequisite for reliable estimates of growth, mortality and maturity.

Otoliths have been preferred in age reading of capelin. This is partly because scales do not develop during the first year of life, and the otolith zones are also relatively well pronounced. Of the three otolith pairs only the saccular otoliths - the sagittae - are used for ageing purposes.

This section describes the standard technique of handling the otoliths, from when they are removed from the fish, to when they are embedded and prepared for reading. A description of the ageing is also included, together with a discussion of the ageing technique which has been and is now in use at the Institute of Marine Research in Bergen.

3.1 Dissection of the fish and preparation of otolith samples

As mentioned earlier, a standard sample of capelin usually contains 100 fish. With some few exceptions, otoliths are taken from the whole sample. In some cases only a part of the sample is aged, usually when there has been frequent sampling and/or uniform length distributions in an area. In these situations a random subsample of 20-40 specimens is aged.

The sagittae are removed from the underside of the cranium by a scalpel, after removal of the gills and sectioning of the labyrinth organ. This is usually quite easy, but operating under a dissecting microscope may be helpful when working with small 0-group fish and larvae. After rinsing in water, the otoliths are placed convex side up in cavities in specially designed otolith plates. The rinsing may be done either by rubbing the otoliths between the wet fingers or by placing them in waterfilled holes in plastic mouldings designed for this purpose. In this case, the otoliths are rinsed by means of a fine brush and then placed on the otolith plates. Each hole in these plates is labeled with a number corresponding to the fish's number in the sample. The otoliths are then left to dry. This dehydrating of the otoliths improves the readability, while too early embedding will cause the otoliths to turn opaque after storing. Sufficient drying time will depend upon air temperature, and the size of the otoliths.

The otoliths are embedded in the synthetic mounting medium Eukitt which makes them become partly transparent, the winter rings discernable. The otolith plates, each with ten pairs of otoliths, are labelled for easy reference (Fig 1).

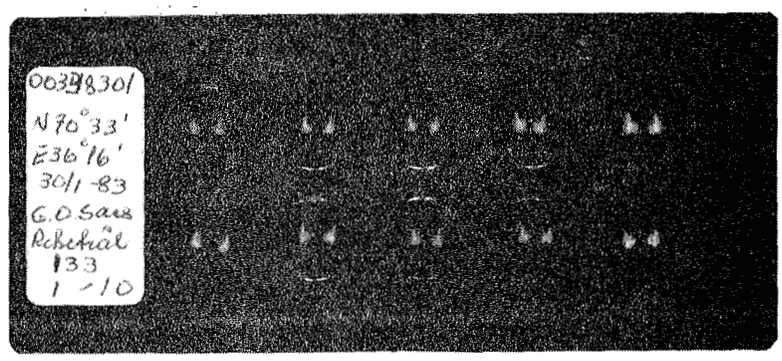

Figure 1. Otolith plate with embedded capelin otoliths.

Reading of the otoliths is done under a dissecting microscope, using about 40 X magnification and reflected light. The otolith plates are black, and so the winter rings appears dark because they are transparent The zones of summer growth however, are opaque due to the high calsium to protein ratio, and thus appear white under the microscope. A scale mounted in one of the oculars is used for measuring the growth zones.

3.2 The reading and measuring of growth zones

The following description is an excerpt from sampling instructions used at the Institute of Marine Research (Anon 1984).

The age is given as the number of winter rings. However, all fishes shall by convention shift their age on January 1, so the age of a fish is not its exact lifespan, but the number of winters it has experienced during its lifetime. This implies some modification of the rule sited above. If an otolith with a hyaline margin is found in late autumn, i.e. its winter ring has started to form, this outer winter ring should not be counted. Also, if an otolith is found in spring without a winter ring in the margin, the margin shall be counted as a winter ring.

The measurements of the growth zones are undertaken not only for the purpose of growth studies, but also to assess the validity of the ageing. The measuring is done according to fig. 1 along a line perpendicular to the longest radius through rostrum. The radius in each winterring is measured from the center of the otolith to the inner margin of the winterring. As the inner margin of the first winter ring is often indistinct, this ring is measured to its outer margin. In addition, the total radius to the otolith edge is measured. The measurements are in eye-piece units which at the magnification most often in use, (40X), corresponds to 0.025 mm. As other microscopes with slightly different magnifications are sometimes used, the measurements are calibrated against a ruler for each sample taken, and the calibration factor is written on the sampling form.

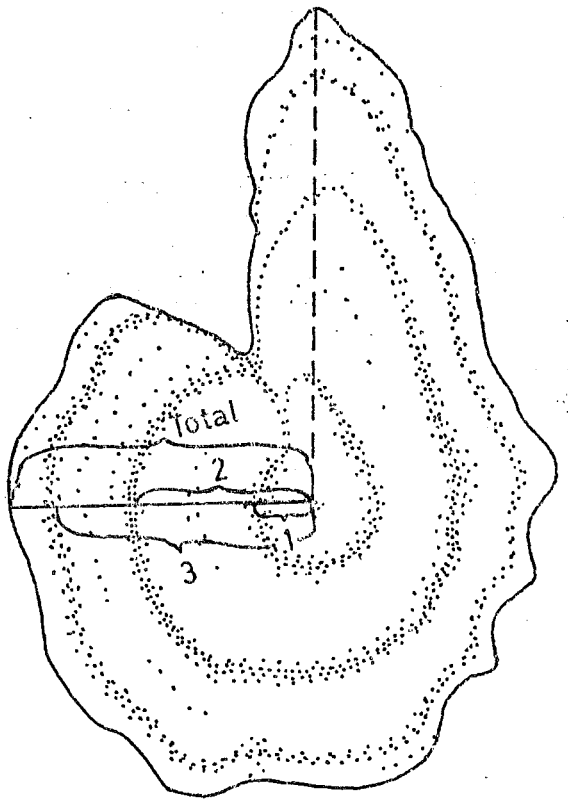

Figure 2. Capelin otolith showing the measurement taken. 1, 2 and 3 are radia in first, second and third winter rings respectively. Total means total otolith radius.

As not all the otoliths are equally easily read and measured, a code for readability is set. Its use is explained below.

3.3 Readability scale

An otolith in which the winter rings appear clearly is given readability 0. This implies that the age is set equal to the number of winter rings, and the radii in these rings are measured. In case of false rings, this readability shall be used if these rings can easily be discriminated from the winter rings.

Two kinds of otoliths are given readability 1. Otoliths in which the age can be given with a high rate of accuracy, but where the winter rings are impossible to measure in the desired direction belong in this group. (See fig. 1). This could be caused by split rings, broken otoliths etc. The age is set as the number of winter rings, but no measurements are made.

Secondly, readability 1 is used when the number of winterrings clearly seen is thought not to represent the true age of the fish, because the first ring seen is unexpectedly large, and the central part of the otolith is so thick that the true first ring may have disappeared. In such cases, usually involving older fish, the age is set one year older than the number of winterrings seen, and the radia in these rings are measured, but the first ring seen is measured as the second ring and so forth.

Readability 2 is given in cases where an age cannot be given with any confidence. The reason may be false rings which cannot be discriminated from winterrings, broken otoliths, opaque or crystalline otoliths etc. Often a "minimum age" can be given, and in such cases this age is given as the minimum number of rings which one believes represents winter growth. This is done to show the ratio of older fish among those which cannot be aged. The fish with readability 2 are not used when constructing age-distributions. No measurements of radii are made on otoliths with readability 2. This readability is also used when both otoliths are missing.

3.4 Discussion of the ageing method

As the life cycle of capelin is relatively short, and the winter rings are usually distinct, the age reading of capelin is probably quite reliable. However, problems do occur, which may be divided into two categories; (1) the thickening of the otolith which causes the first ring to disappear in older otoliths, and (2) false rings which may be present quite frequently, and most pronounced in otoliths more than three years old.

The ageing method described here aims to reduce the number of wrongly aged otoliths, and is developed in light of these different problems. Various ways of reading and interpreting capelin otoliths are described in the literature (see Bailey et al., 1977; Hamre, 1977; Prochorov, 1968; Tempelmann, 1968).

Judging from these papers, and from experience gained during routine ageing, the first translucent zone in the otolith is the main problem in ascertaining age. The question is whether a translucent ring or area in the center of the otolith should be counted as a first winter ring, regardless of its size. Different methods have been considered for solving this problem, but as yet there seems to be no conclusive support for any method covering all the north Atlantic capelin stocks. Growth considerations were used to assess the validity of ageing by Bailey et al. (1977) in Canadian waters and by Hamre (1977) for the Barents Sea. By backcalculating lengths at deposition of the first ring and comparing them with lengths from sampling, Bailey reached the conclusion that the first ring was a true winterring, but a second ring was found to be deposited during the process of metamorphosis, which normally occured during its second year of life. This "metamorphic check" was found in 77% of the one and two years old fish and 44% of adults, this decrease being interpreted as an obfuscation of the check by the increasing opacity observed in older otoliths. The absence of the metamorphic check in some otoliths could result from its deposition simultaneously with the first or second annulus.

Hamre (1977), working with Barents Sea capelin, studied this problem during the summer of 1976. Both 0- and 1-group capelin were sampled and length at deposition of first ring was backcalculated. He found that even the smallest fish with one ring, with a modal length of 5.8 cm in early July and a mean length at deposition of the first ring of 4.2 cm, were far too big to be the offspring of that year's spawning. It was found that even at the border of the larval drift towards the north and east the mean length of the larvae was less than two cm. He concluded that the first ring, even with a radius as small as 0.05 mm, reflects the first year growth and should be counted as the first winter ring. Hamre located a group of one year old fish with small first rings in the southeastern Barents Sea in 1976, comprising about

10% of the 1975 yearclass. It seems reasonable to believe that this group was the offspring from late spawners, and thus the phenomenon of small first rings is probably linked to the magnitude of late spawning.

It is difficult to compare the ageing methods used on the capelin stocks on either side of the North Atlantic. Differences in growth rate, spawning time and duration of spawning activity make any direct comparison of otoliths uncertain.

At present, the ageing method discussed above for the Barents Sea capelin is used at the IMR in Bergen. This method, to count each translucent zone as a winter ring, was also supported by Prochorov (1968). Individual fishes have been detected which seem to contradict these findings, e.g. fishes of six to seven cm length with two clear translucent rings caught in the autumn, and also samples within 1-groups of very uniform length-distribution where some individuals have an extra ring inside and are thus aged two years.

The consequences of false ageing are difficult to assess, but are of course linked to how many fishes are involved relative to the total number of aged fishes. The primary effect of giving a fish a false age is to transfer the fish to an age-group above (if a false ring is counted) or below (if a ring is overlooked). This will in turn affect the analysis of the stock/recruitment relationship. In addition to the effects on stock assessment, false ageing will introduce noise and/or bias to growth studies.

Observations indicate that some individuals probably get a false age, but as this probably is a rare event, the practical consequences are small. However, it must be concluded that the problem of ageing is not yet completely solved for the Barents Sea capelin stock, and further validation of the method in use is needed.

Appendix 1 gives some details on punching and storage of the data.

4. METHODS APPLIED TO IMPROVE THE REPRESENTATIVENESS OF THE DATA

The data listed here can be processed further by a computer, resulting in different kinds of distributions, of which the most commonly used are age, length, weight, sex, maturity and otolith growth zone width. These distributions may be computed on the basis of yearclass, age, year,season, or area, or whichever combination is desirable.

However, when we want to compare these distributions for different yearclasses, we encounter statistical problems, how representative are the samples taken in different areas for the total stock or a yearclass? To be specific; is it statistically correct to pool all the samples and let them represent the total stock? It would be correct if the relative number of fishes in the samples matched the relative number of fishes in the subpopulation inhabiting the subarea for which the sample is considered representative; or if there was no geographical variation in the biological parameters. The last assumption does not hold for capelin, and the first is also unreasonable, at least when the samples are taken on a research survey. This because the samples will be more or less evenly distributed over the whole distribution area, even when the major part of the stock is residing in a small portion of this area. Pooling the samples would then give too much weight to the fishes in the less dense populated areas.

This problem can however be circumvented by weighing the samples according to the abundance of fish in the subareas. We are now using a procedure which makes use of the acoustic estimate of the number of fish as a weight for the samples. As yet the technique is only used for data sampled on the joint Soviet/Norwegian cruise in September, because this is the only cruise in which the whole stock is sampled and assessed acoustically. The method and the assumptions on which it is based are briefly outlined below.

The Barents Sea is divided into statistical subareas called locations, (Fig. 3), which are the basic divisions used for the abundance estimation. The 0-group capelin is not covered by the integrating system, and is thus not included in the abundance estimate. The number of fish one year old and older in each length-group in one location is then:

$$N(l) = \frac{p(l)}{\int \frac{p(l)}{C(l)}} M A \qquad (1)$$

where p(l) = the length distribution of fish in the location.
C(l) = a constant depending on the instruments used, the fish species and length.
M = mean integrator output for the location.
A = area of location.

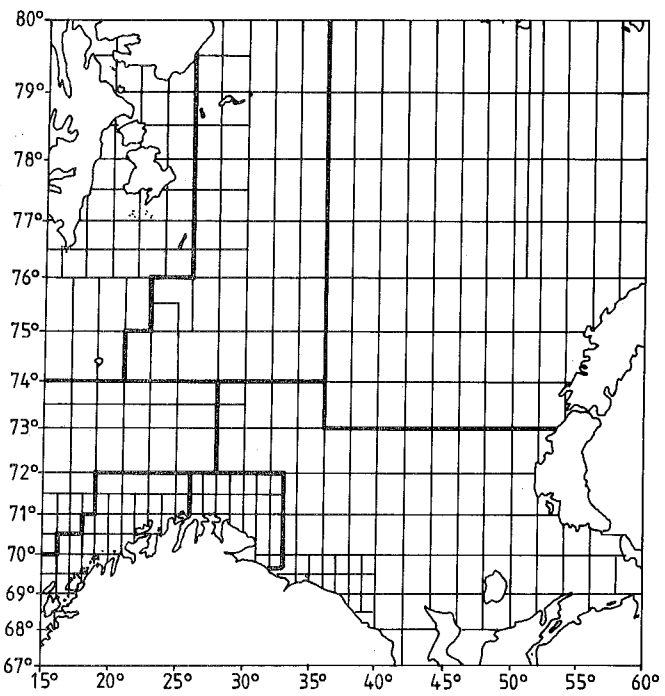

Figure 3. Map showing the Barents Sea with the statistical squares mentioned in the text.

The length-dependent part of the C-value is for capelin found to be $l^{-1.91}$. This gives:

$$N(l) = \frac{p(l)}{\sum_l p(l)\, l^{1.91}} M\ A\ C \qquad (2)$$

where C is the length-independent part of the C-value.

In some cases we want to extract a part of the stock (e.g. age two and older) from the stock of age one and older. The absolute length distribution of a subgroup of fish N_i is given by:

$$N_i(l) = \frac{p_i(l)}{\sum_l p(l)\, l^{1.91}} M\ A\ C \qquad (3)$$

where $p_i(l)$ is the length distribution of subgroup no. i and p(l) is the total length distribution.

We use one or more samples to construct the length distribution p(l). We call the length distribution in each sample $q_j(l)$, and give each sample a weight V_j. To make the contribution from each sample to p(l) independent of the sample size, we normalize the length distribution in each sample by dividing by the total number of fish in the sample, q_j. This gives:

$$p(l) = \sum_j V_j \frac{q_j(l)}{q_j} \qquad (4)$$

We may think of the fish in the location as comprized of as many subpopulations as there are samples. If we let one sample be representative for one subpopulation, the absolute length distribution of the subgroup i in subpopulation j is:

$$N_{ij}(l) = \frac{\sum_j V_j \frac{q_{ij}(l)}{q_j}}{\sum_l \left(\sum_j V_j \frac{q_j(l)}{q_j} \right) l^{1.91}} M\ A\ C \qquad (5)$$

or

$$N_{ij}(1) = \sum_j \frac{M\ A\ C\ \frac{V_j}{q_j}}{\sum_I \left(\sum_j \frac{V_j}{q_j} q_j(1)\ 1^{1.91}\right)} q_{ij}(1) \qquad (6)$$

The appropriate weight for each sample in the location is then:

$$\text{Weight} = \frac{M\ A\ C\ \frac{V_j}{q_j}}{\sum_I \sum_j \frac{V_j}{q_j} q_j(1)\ 1^{1.91}} = \frac{M\ A\ C\ \frac{V_j}{q_j}}{\sum_j \frac{V_j}{q_j} \sum_I q_j(1)\ 1^{1.91}} \qquad (7)$$

This can be thought of as the sample's (or the subpopulation it represents) contribution to the integrator value in the location. If the sample is used also in other locations, the sample's weight in each of the locations have to be added to get the total weight for each sample.

When the weighed samples are used to construct distributions, the parameter value belonging to each fish is counted as many times as the weight for that sample states. If, for example, we construct the length distribution of fishes two years old and older for a subarea, the parameter value for each fish in a sample with weight 10 would count twice as much as that for a fish in a sample with weight 5.

Weighing the samples has two effects. The distributions will be representative for the population inhabiting the area, and the number of fish is the actual number estimated by the acoustical method.

The weighing procedure presented here rests on the following assumptions:

1) The age and length distributions of the fishes one year old and older in a sample is the same as that in the location which the sample is used to represent.

2) The quantity N in equation (1) is the number of fishes one year old and older in the location.

Computer programs have been made, which, by input of M for the different locations, their area, the samples used and their weight of representativeness in each location, will, after going through the files of biological data, produce a list of the numbers of all the samples used and their appropriate weight based on the chosen subgroup. By input of this list to the programs computing the distributions of the different biological parameters, these distributions will be weighed accordingly.

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APPENDIX 1

The sampling form is shown in Figure 1. The same form is used also for other pelagic species, so all the columns are not exclusively for capelin.

In the upper right-hand corner of the form, some details are given which are not stored in the computer. This is species, location no., vessel name, fishing station no., gear, preservation technique, and name of person responsible for taking the sample, reading and measuring the otoliths. However, some of these data are given in coded form on the A-record .(See below).

The data stored on magnetic storing media are organized in two types of records, an A-record for each sample and a B-record for each fish in the sample. The following tables show the format of these records.

Format of A-record.

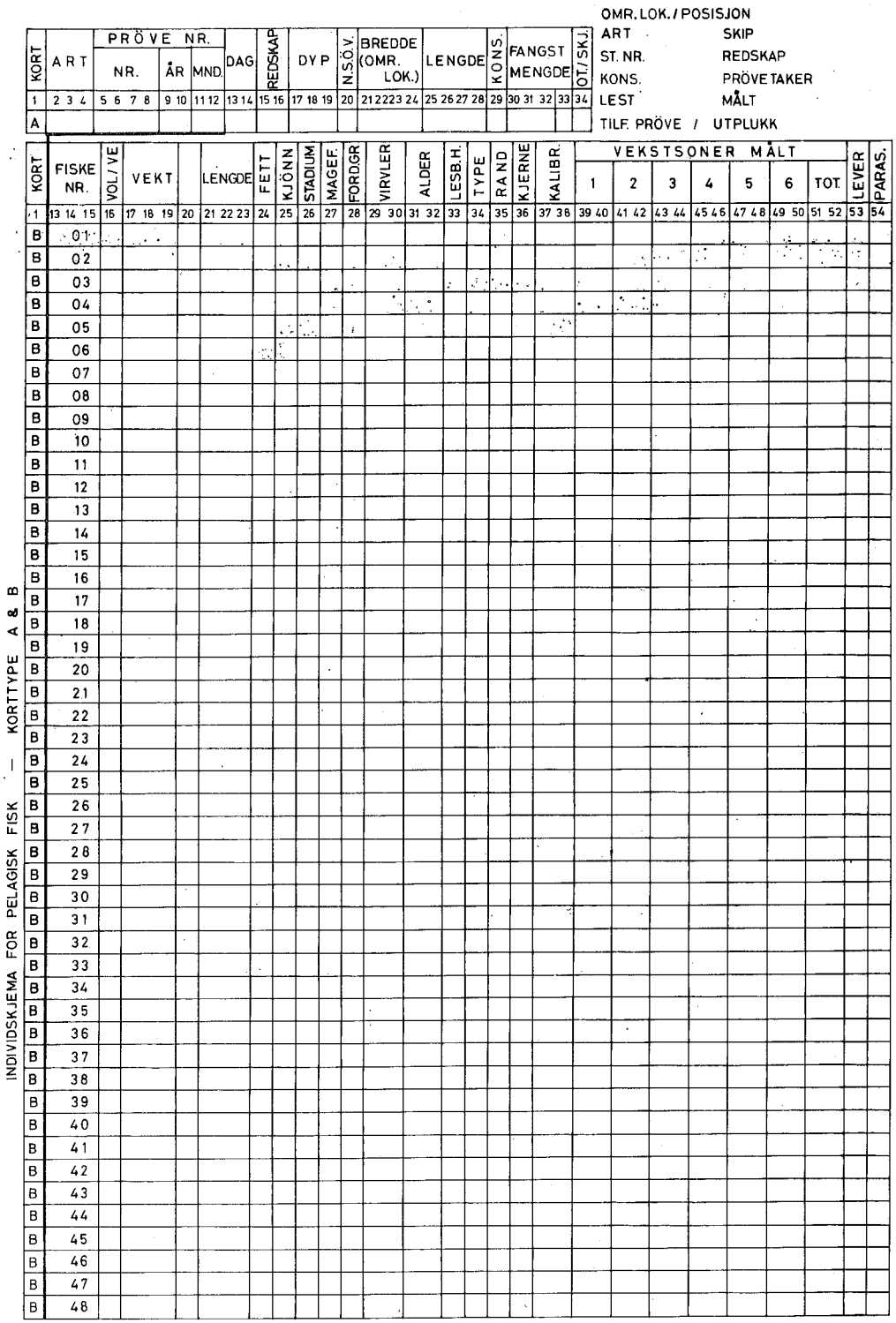
Position	Content
1	A
2-4	Species code (B02 for capelin)
5-8	Sample no.
9-10	Year (last two digits)
11-12	Month
13-14	Day
15-16	Gear code
17-19	Depth
20	Position code
21-24	Latitude
25-28	Longitude
29	Preservation code
30-33	Weight of catch
34	Ageing code (2 denotes otoliths taken)

The sample no. (5-8) consists of a serial no. and a specific sample no. The serial no. bears information on vessel and type of sample (random sample or other special types of samples). The sample no. starts from 01 each month, and so the complete sample no. consists of all the positions 5-12. Consequently, the first random sample taken onboard the G.O. Sars in september 1984 will get the no. 00018409. The depth (17-19) is the fishing depth.

Format of B-record.

Position	Content
1	B
2-12	Copy of pos. 2-12 of record a
13-15	The fish's number in the sample
16	Weight or volume code (1=volume, 2=weigth)
17-19	Weight or volume of fish in g or ml
20	Not used for capelin
21-23	Length in mm
24	Not used for capelin
25	Sex: 0=not determined, 1=female, 2=male
26	Maturity according to the old maturity scale
27	Stomach filling degree
28	Not used for capelin
29-30	Maturity of females according to the new scale
31-32	Age
33	Readability code
34-36	Not used for capelin
37-38	Ocular micrometer calibration (no. of EPU in 2 mm)
39-50	Radia in up to 6 winter rings in EPU
51-52	Total radius in EPU
53-54	Not used for capelin

After punching, some test programs are run on the data, to search for obvious errors obtained during coding or punching of the data. The data are then stored in files, each file containing all capelin data sampled during one year. During the autumn cruise, the data are punched onboard the G.O.Sars, so the data are available for computer treatment upon termination of the cruise.



KORT	ART	PRÖVE NR. NR.	ÅR	MND.	DAG	REDSKAP	DYP	N.S.Ö.V.	BREDDE (OMR. LOK.)	LENGDE	KONS.	FANGST MENGDE	OT./SKJ.
1	2 3 4	5 6 7 8	9 10	11 12	13 14	15 16	17 18 19	20	21 22 23 24	25 26 27 28	29	30 31 32	33 34
A													

OMR. LOK. / POSISJON
ART SKIP
ST. NR. REDSKAP
KONS. PRÖVETAKER
LEST MÅLT
TILF. PRÖVE / UTPLUKK

INDIVIDSKJEMA FOR PELAGISK FISK — KORTTYPE A & B

KORT	FISKE NR.	VOL/VE	VEKT	LENGDE	FETT	KJÖNN	STADIUM	MAGEF.	FORDGR	VIRVLER	ALDER	LESB.H.	TYPE	RAND	KJERNE	KALIBR.	VEKSTSONER MÅLT 1	2	3	4	5	6	TOT.	LEVER	PARAS.
1	13 14 15	16	17 18 19	20 21 22 23	24	25	26	27	28	29 30	31 32	33	34	35	36	37 38	39 40	41 42	43 44	45 46	47 48	49 50	51 52	53	54
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Appendix Figure 1. The data form used for standard samples of capelin.