Variability by Readers and Method of Preparation in Dall's Porpoise Age Determination

by

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READER VARIABILITY IN DALL'S PORPOISE AGE DETERMINATION

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ABSTRACT

Considerable variation was detected between readers in age estimation of the Dall's porpoise using cemental growth layers. The major discrepancy came from differences in interpretation of layers formed in porpoise at less than 160 cm in body length. Two readers counted 2-4 layers in these animals, but the third reader counted 1-2 layers. An additional (smaller) disagreement was detected for older individuals. There were also differences between methods of preparation in the counts of growth layers by the readers. Since these disagreements between readings cause significant error in life history analysis, further clarification of the source of the differences and a search for a suitable calibration method for converting the layer counts into absolute age are needed.

INTRODUCTION

Age of Dall's porpoise has been estimated using counts of growth layers in decalcified and stained thin sections of the teeth, and has been used in analyzing the life history and behavior of the species (Kasuya, 1978; Newby, 1982; Miyazaki, 1984; Kasuya and Shirago, in press). Recognizing the importance of results of such analyses for population management, and lacking confirmation of agreement between readers of the counts, the Scientific Sub-Committee of the Ad Hoc Committee on Marine Mammals of the International North Pacific Fisheries Commission recommended in 1984 that two experiments be conducted, i.e., comparison of between-reader differences using selected tooth slides, and examination of the effect of different preparation techniques on the readability of the teeth.

This study analyzes the between-reader and between method of preparation differences of age determination of the Dall's porpoise using tooth sections prepared by three participants and circulated between them for counts of growth layers.

MATERIALS AND METHODS

A total of 125 decalcified and haematoxylin stained tooth sections (65 female and 53 male dalli-types, and 2 female and 5 male true-types) were prepared by three readers, G - Method 3A and B, K - Method 1, and M - Method 2, and circulated among them for age determination (Table 1). The locality and method of collection were: 21 *dalli*-types from the salmon mothership gillnet fishery catch in the northwestern North Pacific in June-July 1979, 1981 and 1982 (Method 3A) and 45 *dalli*-type from the mothership fishery in the Bering Sea (Method 3B) and 30 hand-held harpoon samples of *dalli*-types (Method 1) collected in the offshore western North Pacific during the Hoyomaru No. 12 cruise in August-September 1983, and 30 *dalli*- and *truei*-types (Method 2) selected from the offshore northwestern North Pacific harpoon samples taken during the *Hoymomaru No. 12* cruise in August-September 1982, the land-based salmon gillnet fishery in June-July 1982 and Japanse coastal harpoon fishery for the Dall's porpoise in 1982 (for location of samples taken during these *Hoyomaru No. 12* cruises see Kasuya and Shirago, in press). Selection of these samples was made arbitrarily by each contributor to cover both sexes,and as wide an age range as possible. Thus they were not a random sample of either the population, or catch. The body length frequencies of the samples show different peaks (Figure 1). In the Bering Sea sample the mode is at 155-160 cm with a second peak at 170-175 cm. The gillnet sample from south of the Aleutians has the mode at 170-175 and second peak at 150-160 cm. Finally the harpoon sample has the mode at 180-185 cm but one-third of the sample occurs at lengths 160-175 cm and there are no animals smaller than 160 cm.

Teeth were decalcified, stained with haematoxylin, and longitudinal sections prepared. Each preparation method was slightly different. Readers K and M used the method described in Kasuya (1978), i.e. teeth were thinly polished longitudinally before decalcification in formic acid and staining, producing only one section from each tooth. Reader G first decalcified a whole tooth in RDO (a commercial decalcifying agent obtained from Dupage Kinetic Laboratories, Inc., Plainfield, Illinois, U.S.A.), sectioned each tooth using a freezing microtome, and stained them. Each tooth produced several sections and readers chose the most readable.

Each reader counted growth layers in both dentine and cementum, or only in the latter tissue. Reading was done without knowledge of the biological data of the samples, such as body length, sex and reproductive status. After reading the slides, all readers considered the cemental layer counts more reliable and used these for the best estimate of ages. Although two readers indicated the best count for each tooth, one reader (G) gave a possible range of values, or two equal alternatives for 5 teeth. In these cases, we selected one of the two figures or used the middle value of the indicated range. Of the three readers, K and M had previous experience analyzing their own age determination data but reader G did not.

Comparison of methods

For comparisons between readers, samples prepared by each scientist by their method were analyzed separately to eliminate possible differences related to the sampling area, composition of the sample, and methods of preparation. To test the hypothesis that there was no difference between readers for each sample, a nonparametric randomized block analysis (Friedman's test) was used. Samples from males and females were combined for each method and only specimens from the *dalli*-type were used.

Comparisons between methods of preparation could only be made by inference since samples were collected from different areas that apparently have different age structures (Kasuya and Jones, 1984).

RESULTS

Comparison between readers

There were significant differences between the counts of growth layers in each of the four samples ($P<0.001$, Friedman's Test)(Table 2). Although there was still a significant difference, the best agreement between readers occurred with Method 1.

To compare the precision between readers, an index of Average Percent Error (APE) in the ages (Beamish and Fournier, 1981) was also calculated (Table 3). Method 1 had the smallest APE (11.54%), implying that the readers were more consistent in reading these teeth.

Agreement between readers was also examined by plotting scatter plots of growth layer counts by two readers for each animal (Fig. 2). The diagonal line indicates complete agreement between readers. In general, reader K tends to count fewer layers and reader M more layers. It can be seen that there is better agreement between the readers (counts are more similar, more points falling on the 45° line) in Method 1. In addition, the sample prepared by G from Bering Sea animals (Method 3B) showed reasonable agreement between counts by readers G and M (16 out of 45 animals).

There is some indication that the preparation method used by K results in somewhat more consistency in the counts than in the other preparations. This may be related in part to there being only one tooth section to read per animal, rather than several sections as in the case of teeth prepared by G. Perhaps with a large number of sections it becomes more difficult to find a section with all layers represented. Therefore readers may examine different sections, having different numbers of layers present.

Analyses suggest that the interpretation of growth layers by reader K for porpoises below 1 layer (of reader K) is different from that of the other readers. Readers M and K seem to have similarly interpreted growth layers laid down before the age of about 4 layers (their reading). Data suggest that, for individuals over 2-3 layers (reading by K, or 4-5 layers by G and M), G counted all the layers in the tooth (including layers deposited in juvenile period) in the same way as K did, while M retained his own standard for all the growth stages.

Age frequency

Although the ranges of the estimated ages were similar among the three readers, being 1-13(G), 1-15(K) and 1-17(M) layers, the mode was considerably different between readers (Fig. 1). Counts by G and M produced a similar pattern (mode at 3-4 layers), but the distribution was more diffuse in the latter's readings. Counts by K had a mode at 1 layer.

Mean growth curve

The scatter plot of body length on age is shown in Fig. 3. The correlation between age and body length varies among readers, and is almost absent in the juvenile age data of some readers.

Since all readers gave the same reading (0 layers) for two smallest individual (90 and 95 cm), it is possible that the discrepancy between readers is in age estimation of older individuals. To test this, the mean number of layers for four body length groups was calculated for each reader (dalli-types and sexes combined):

Body length	Sample size	G	K	M
140-159 cm	29	2.9	1.2	2.8
160-179 cm	43	3.4	2.5	4.0
180-199 cm	34	4.8	4.4	5.9
200-235 cm	9	8.2	7.4	9.1

The magnitude of the between-reader difference in the smallest group was retained even in the larger length groups in the case of K and M but decreased in magnitude for G compared with K. The major disagreement between K and the other readers is due to interpretation of growth layers deposited during the juvenile stage, below 160 cm in body length. The readers M and G gave very close reading for the smallest length group, but disagreed in the larger individuals, suggesting a change in interpretation by the readers for growth layers in the teeth of older individuals (see Comparison between readers).

Female sexual maturity

Correlation between age and number of corpora in the ovaries was not high in any of the three readers (Fig. 4). Ages of the youngest and oldest immature female was 2 and 6 for G, 1 and 8 for K and 2 and 7 layers for M. The peak of ages for immature females was also different between readers. Reader G aged the majority of them at 2-4 layers (peak at 3 layers), K at 1-2 layers (peak at 1), and M at 2-4 layers (peak at 2).

Correlation between age and proportion of sexually mature females was highest in reader M (r=0.96, age 1-9 layers), followed by K (r=0.94, age 0-6), and G (r=0.92, age 1-7). The estimates give age 6 (G), 3 (K) and 5 layers (M) as the youngest age where more than 50% of females are sexually mature (Fig. 5). The highest figure was twice the smallest value.

Male sexual maturity

Males having testis at or over 40 grams were defined as sexually mature (Kasuya and Jones, 1984) (Fig. 5). The age of the youngest mature and the oldest immature male were 2 and 7 for G, 2 and 5 for K and 2 and 13 layers for M. Reader G aged the majority of immature individuals at 2-4 layers (peak at 3 layers), K at 1-3 layers (peak at 1), and M at 1-4 (peak at 2 and 3).

Correlation between the proportion of sexually mature males and age declined in the order of K (r=0.97, 1-7 layers), G (r=0.71, 1-8 layers) and M (r=0.61, 1-9 layers). The ages where the proportion of sexually mature males exceeded 50% were 5 (G), 4 (K) and 5 layers (M) (Fig. 5).

DISCUSSION

The analyses indicate that there is a large disagreement between readers in the counting of growth layers in Dall's porpoise teeth, particularly of immature animals. This causes problems in the life history analyses of Dall's porpoise and the present understanding of the early postnatal growth of the species has to be questioned. Recent studies recognized two peaks in the body length frequency at 100 and 150 cm in samples collected in the summer season, and interpreted them as representing age classes 0 and 1 layer (year), respectively (Newby, 1982; Kasuya and Jones, 1984; Kasuya and Shiraga, in press). The first peak (100 cm) represents newborn calves. In the present study the other group (150 cm) was considered to represent either 1 or 2-4 layers, depending on who the reader was. If the latter peak really represents ages of 2-4 layers as suggested by some of the readers, we have to consider either that the readers counted accessory growth layers which are not annual in deposition, or that the deposition is annual and the growth of juveniles is much slower than has been considered. The latter also implies that a large proportion of one-year-old individuals might be segregating outside the present sampling areas. Comparison of the Dall's porpoise growth and reproductive pattern to other odontoceti may throw some light on this question.

If these problems in interpretation of growth layers deposited while the porpoises are about 160 cm or less in body length is resolved, then a major problem of age determination is solved. However, there would still remain the problem of the accuracy of reading layers deposited at this size.

To improve agreement between readers and increase the reliability of age determination, the readers must meet to discuss their methods. Material should be available so that the readers can simultaneously count layers and thereby develop more consistency among them.

To determine whether one method is better than the others and can be produced reliably by all the scientists, tooth specimens from a sample of animals should be exchanged, prepared and read by each of the scientists.

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TABLE 1.--List of materials and individual readings of Dall's porpoise teeth.

Specimen no.	Body length (cm)	No. corpora or testis/ wt.*	No. growth layer read by G	K	M	Location**	Preparation method***
Females, Dalli-type							
SDT 124	95	0	0	0	0	2	3A
SDT 025	140	0	4	2	5	2	3A
SDT 103	148	0	3	1	2	2	3A
LMT 44	149	0	4	1	5	1	3A
B10715-1	150	0	4	1	4	2	2
LMT 52	152	0	2	1	2	1	3A
SDT 076	152	0	2	1	1	2	3A
SDT 107	153	0	2	1	2	2	3A
ALH 61	154	0	3	1	3	1	3A
YA 007	154	0	3	1	2	1	3B
SM 012	155	0	4	1	4	1	3B
LMT 42	155	0	4	2	4	1	3A
JM 159	156	0	4	1	2	1	3B
JM 167	158	0	2	1	2	1	3B
JM 135	158	0	4	1	4	1	3B
820726-2	158	0	2	1	3	2	2
MM 172	159	0	3	2	3	1	3B
ALH 57	159	0	2	1	2	1	3A
1983-7	160	0	3	3	4	3	1
820720-1	161	0	3	2	6	2	2
23705	162	0	2	1	3	2	2
1983-19	164	0	2	2	3	3	1
1983-73	164	1	3	2	3	3	1
SM 030	165	0	4	1	4	1	3B
YA 015	165	1	6	6	6	1	3B
1983-118	165	0	3	2	2	3	1
1983-44	167	1	3	3	3	3	1
1983-69	168	0	1	1	1	3	1
1983-12	168	1	2	3	2	3	1
1983-114	170	0	3	2	5	3	1
YA 006	170	0	3	1	4	1	3B
LMT 57	172	0	3	1	3	1	3A
SM 008	172	0	4	4	4	1	3B
SM 028	172	0	6	8	7	1	3B
1983-90	172	1	4	3	5	3	1
SM 031	173	0	3	1	2	1	3B
SDT 136	173	0	5	5	7	2	3A
820721-3	174	1	7	3	7	2	2
820728-3	174	1	3	3	4	2	2
1983-43	175	0	3	2	2	3	1
SM 23	178	0	5	3	4	1	3B
LMT 36	180	2	3	4	5	1	3A

TABLE 1.--List of materials and individual readings of Dall's porpoise teeth.--continued

Specimen no.	Body length (cm)	No. corpora or testis/ wt.*	No. growth layer read by G	K	M	Location**	Preparation method***
Females, Dalli-type (continued)							
1983-98	180	1	5	5	5	3	1
1983-75	181	1	2	3	3	3	1
LMT 051	181	2	2	2	4	1	3A
820528-1	181	1	2	2	3	2	2
820724-2	181	2	3	2	4	2	2
1983-11	182	0	5	4	6	3	1
1983-54	183	1	2	2	2	3	1
SM 007	183	4	4	3	3	1	3B
820727-1	183	1	3	1	4	2	2
SM 17	185	3	6	2	7	1	3B
1983-42	186	3	5	5	6	3	1
MN 9	187	1	4	5	6	1	3B
MM 178	189	6	10	8	7	1	3B
LMT 054	190	2	5	4	6	1	3A
820719-1	191	3	6	4	9	2	2
MN 19	193	5	5	4	6	1	3B
ST 206	198	4	7	10	11	1	3B
MM 165	200	3	6	5	5	1	3B
LMT 049	201	3	8	8	10	1	3A
JM 143	206	4	10	2	7	1	3B
SM27Mand	210	6	6	7	9	1	3B
SM27 Max	210	6	8	8	9	1	3B
MN 168	235	5	13	12	12	1	3B
Males, Dalli-type							
SDT 127	90	0.6	0	0	0	2	3A
TCN 356	143	-	2	1	1	2	3A
LMT 39	152	1.3	5	2	5	1	3A
GJC 15	152	6.1	4	1	3	2	3A
820722-2	154	6.3	3	1	4	2	2
SDT 84	155	4.4	3	1	1	2	3A
SDT 084	155	4.4	2	1	1	2	3A
820723-4	156	5.5	1	1	1	2	2
LMT 40	157	0.5	4	1	4	1	3A
LMT 58	157	0.7	3	2	4	1	3A
GJC 33	159	5.2	3	1	2	2	3A
LMT 35	159	0.7	2	1	3	1	3A
820726-3	159	4.7	1	1	1	2	2
21399	160	8.0	3	1	3	1	2
820723-3	161	7.4	3	2	8	2	2
1983-106	164	8.0	3	3	5	3	1
YA 008	167	0.9	2	1	2	1	3B

TABLE 1.--List of materials and individual readings of Dall's porpoise teeth.--continued

Specimen no.	Body length (cm)	No. corpora or testis/ wt.*	No. growth layer read by G	K	M	Location**	Preparation method***
Males, Dalli-type (continued)							
LMT 41	168	1.5	4	2	3	1	3A
SDT 109	169	6.6	3	1	2	2	3A
SDT 118	170	7.7	2	1	3	2	3A
1983-119	170	9.5	2	2	2	3	1
YA 009	171	0.9	4	3	5	1	3B
GJC 109	171	-	4	2	3	2	3A
820505-1	172	11.7	4	3	6	2	2
1983-16	172	26.5	2	2	2	3	1
SDT 126	174	9.5	3	2	2	2	3A
1982-6	175	21.0	7	3	13	3	2
ALH 55	175	0.9	1	1	2	1	3A
1983-48	175	9.0	2	2	1	3	1
1983-55	177	78.0	4	5	5	3	1
SDT 079	177	54.7	4	3	3	2	3A
820728-2	178	11.5	6	2	8	2	2
1983-61	179	25.0	3	5	3	3	1
1983-64	180	116.0	5	4	5	3	1
1983-56	181	128.0	9	9	9	3	1
1983-81	182	90.0	5	5	6	3	1
LMT 056	183	29.2	5	4	6	1	3A
SDT 75	183	10.1	4	1	4	2	3A
1983-10	184	166.0	4	5	5	3	1
1982-62	184	171.3	5	2	6	3	2
1982-73	186	261.3	4	2	5	3	2
820727-3	186	13.3	3	2	5	2	2
1983-63	188	137.0	2	3	2	3	1
1983-77	189	19.0	4	5	4	3	1
1983-47	189	169.0	5	7	6	3	1
GJC 39	190	173.0	5	5	5	2	3A
1983-6	191	124.0	3	4	4	3	1
LMT 34	191	40.4	13	13	17	1	3A
1982-64	191	137.2	8	8	15	3	2
1983-93	197	138.0	3	3	3	3	1
TCN 305	200	220.5	7	9	9	2	3A
21384	201	-	3	1	4	3	2
TWC 80	213	311.4	13	15	17	2	3A
Females, Truei-type							
216 A 10	170	-	2	2	2	3	2
A 3	183	-	3	2	3	3	2

TABLE 1.--List of materials and individual readings of Dall's porpoise teeth.--continued

Specimen no.	Body length (cm)	No. corpora or testis/ wt.*	No. growth layer read by G	K	M	Location**	Preparation method***
Males, Truei-type (continued)							
17	165	-	4	1	7	3	2
A 14	181	-	3	2	4	3	2
216 A 7	184	-	3	3	7	3	2
1982-82	187	13.8	2	3	3	3	2
216 A 13	197	-	3	2	3	3	2

* Weight of single testis in grams.

** 1: Gill net sample (Bering Sea); 2: Gill net sample (South of Aleutian Islands); 3: Harpoon sample (South of Aleutian Islands).

*** Method 1 prepared by K, Method 2 by M and Methods 3A and B by G.

Table 2.--Comparisons between readers using Friedman's test for each method preparation.

Preparation Method	Test Statistic	Significance level	n
1	4.9143	0.0857	30
2	37.52	7.1226×10^{-9}	22
3A-FCZ	15.5161	4.2728×10^{-4}	21
3B-Bering Sea	43.2133	$4.1338 \times ^{-10}$	45

Table 3.--Index of average percent error (APE) in the age of each reader for each method of preparation.

Preparation Method[1]	APE[2]	n
1	11.54%	30
2	38.70%	22
3A	24.74%	21
3B	24.24%	45

[1] Method 1 prepared by K; Method 2 by M; Methods 3A and B by G.

[2] $$APE = 100 \quad 1/N \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \right]$$

where N = number of teeth aged

R = number of times each tooth was aged

X_{ij} = the ith age determination of the jth tooth

X_j = average age of the jth tooth

Figures

1. Body length frequencies of *dalli*-type Dall's porpoise in this study.

2. Agreement between pairs of readers (K vs G, K vs M and G vs M) for Method 1, 2, 3A and 3B. Diagonal line designates complete agreement between readers. Each point represents one animal, numbers indicate animals with same values.

3. Body length versus number of growth layers (age). Closed circle = *dalli*-type; open circle = *truei*-type. Solid line connects mean body lengths. Horizontal dotted line = mean body length at attainment of sexual maturity (males = 182.5 cm; females = 170.5 cm (Newby 1982; Kasuya and Shiraga in press)).

4. Number of corpora in ovaries (females) or testis weight (males) versus age. Each circle equals one individual. Open circle = *truei*-type; closed circle = *dalli*-type. Horizontal dotted line = mean weight of testis at attainment of sexual maturity (40 cm) (Kasuya and Jones 1984).

5. Relationship between proportion of sexually mature individuals and age in *dalli*-type Dall's porpoise.

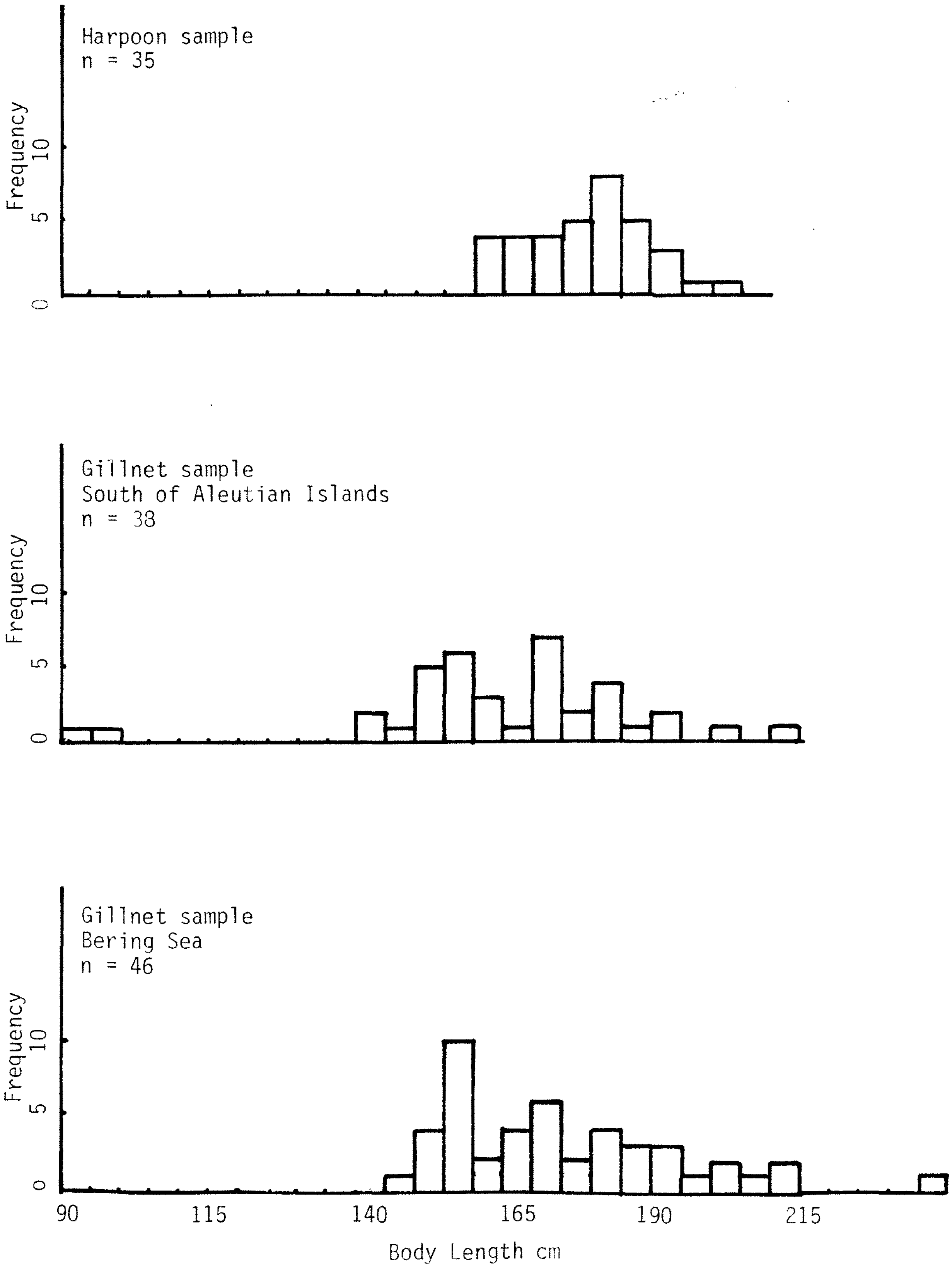
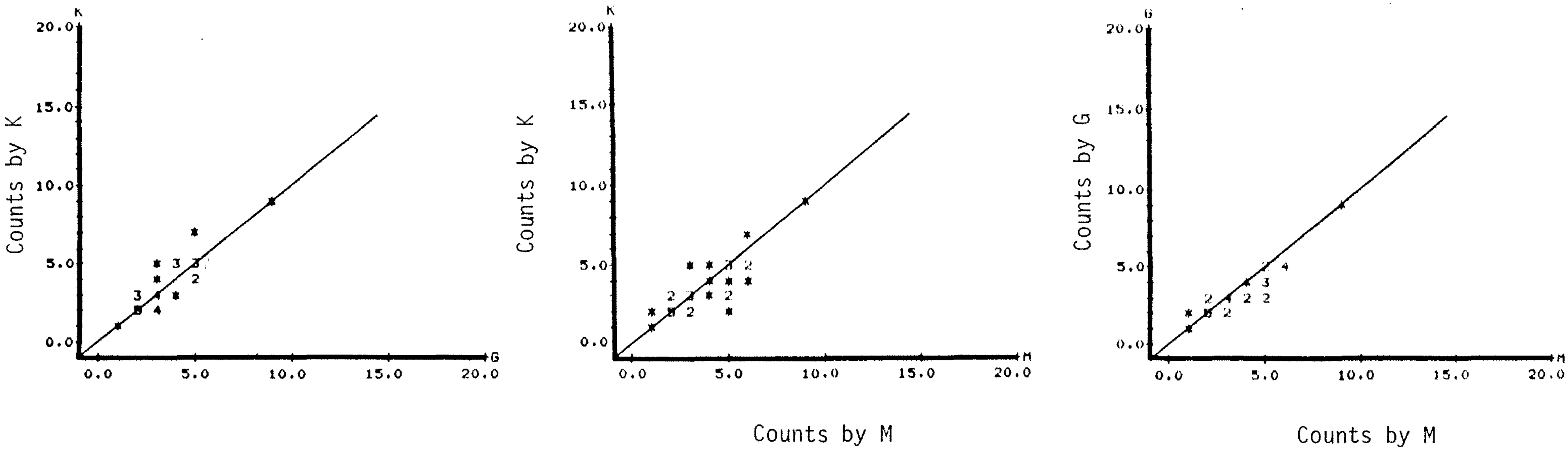

Harpoon sample
n = 35
Frequency
0
5
10
Gillnet sample
South of Aleutian Islands
n = 38
Frequency
0
5
10
Gillnet sample
Bering Sea
n = 46
Frequency
0
5
10
90
115
140
165
190
215
Body Length cm

Figure 1.

METHOD 1



METHOD 2

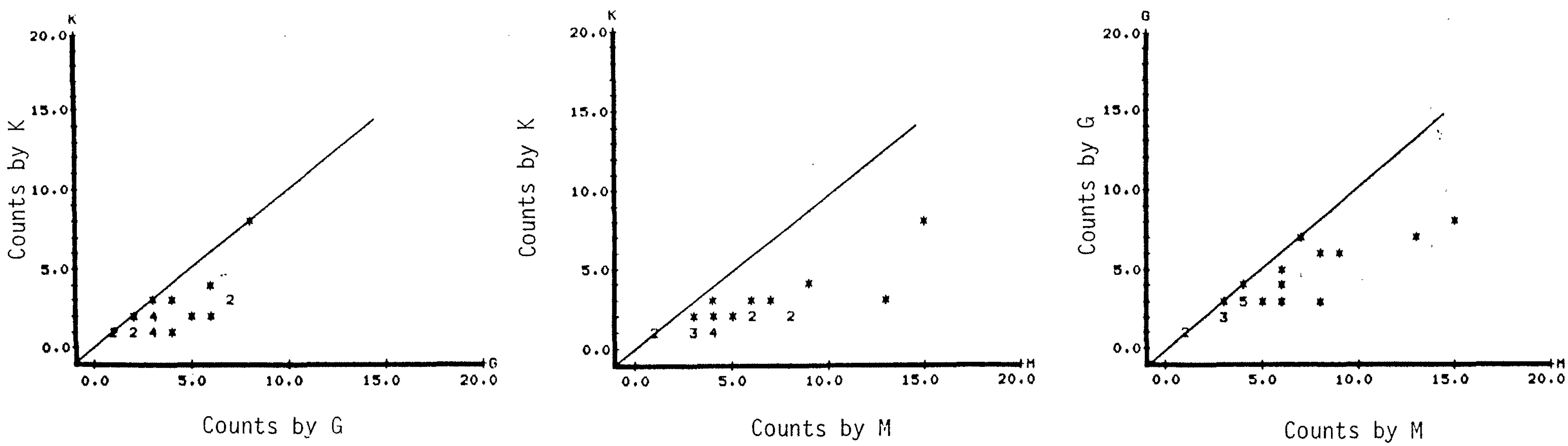
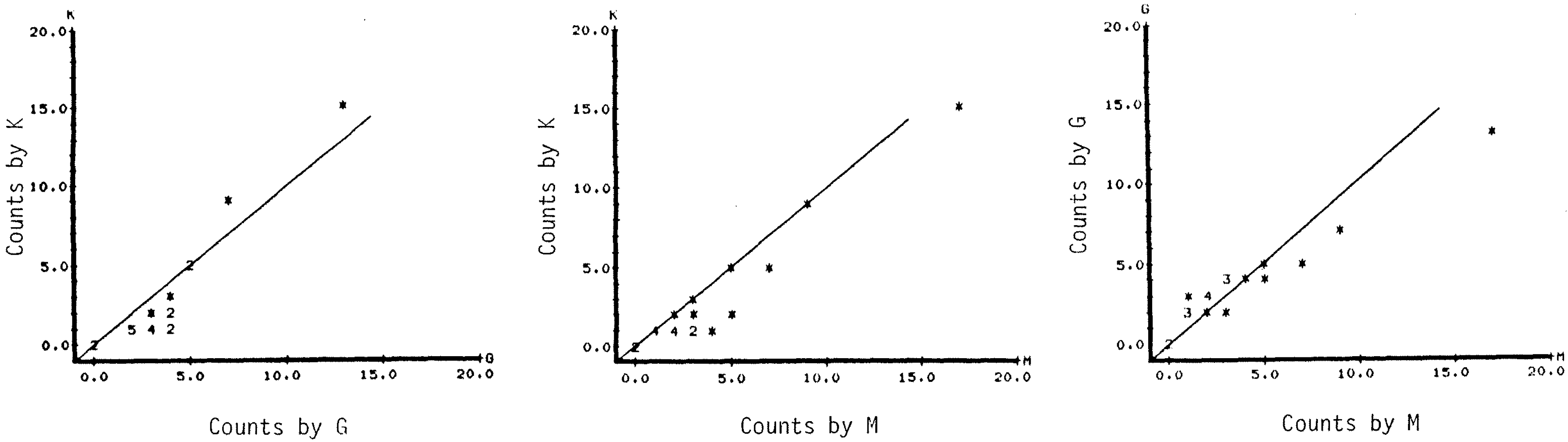


Figure 2.

METHOD 3A



METHOD 3B

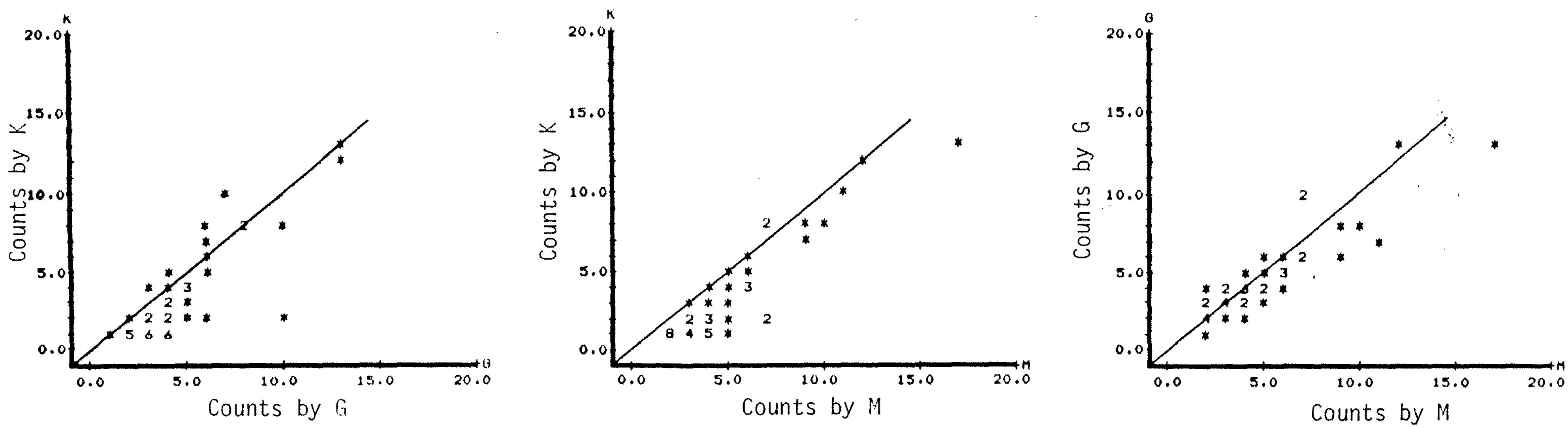


Figure 2.

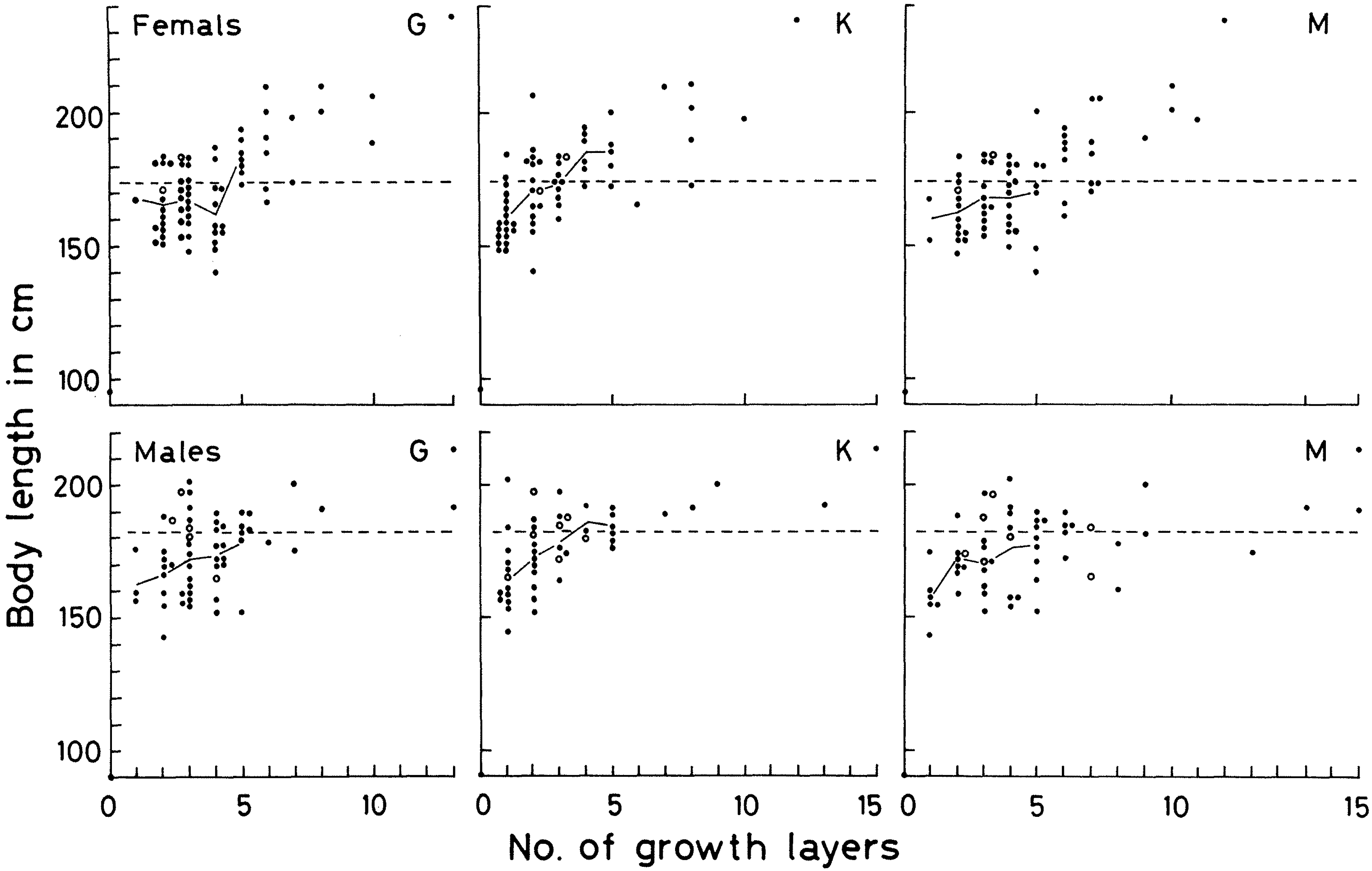

Femals
G
K
M
Males
G
K
M
Body length in cm
200
150
100
0
5
10
15
No. of growth layers

Figure 3.

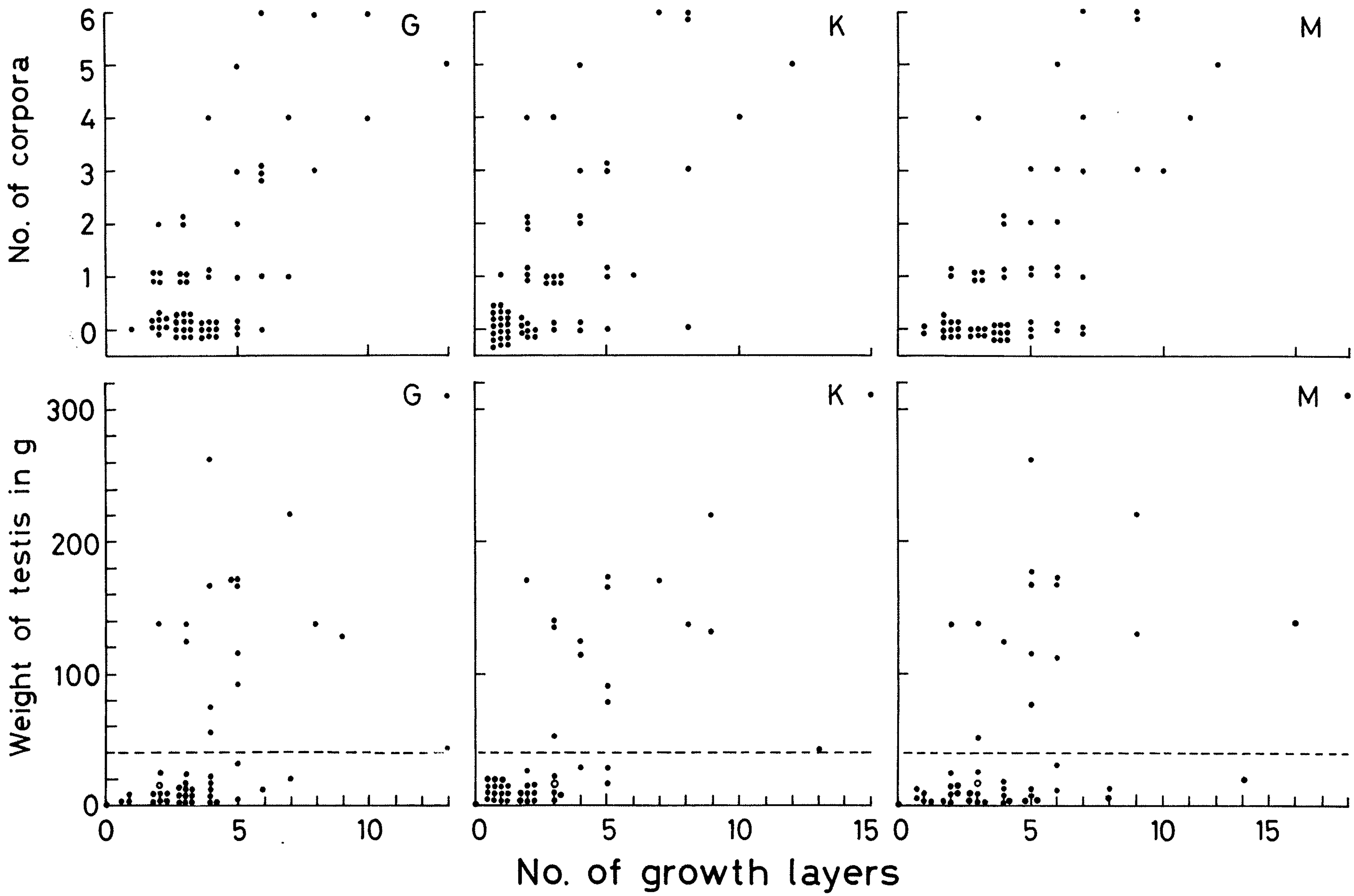

G
K
M
No. of corpora
0
1
2
3
4
5
6
Weight of testis in g
0
100
200
300
No. of growth layers
0
5
10
15

Figure 4.

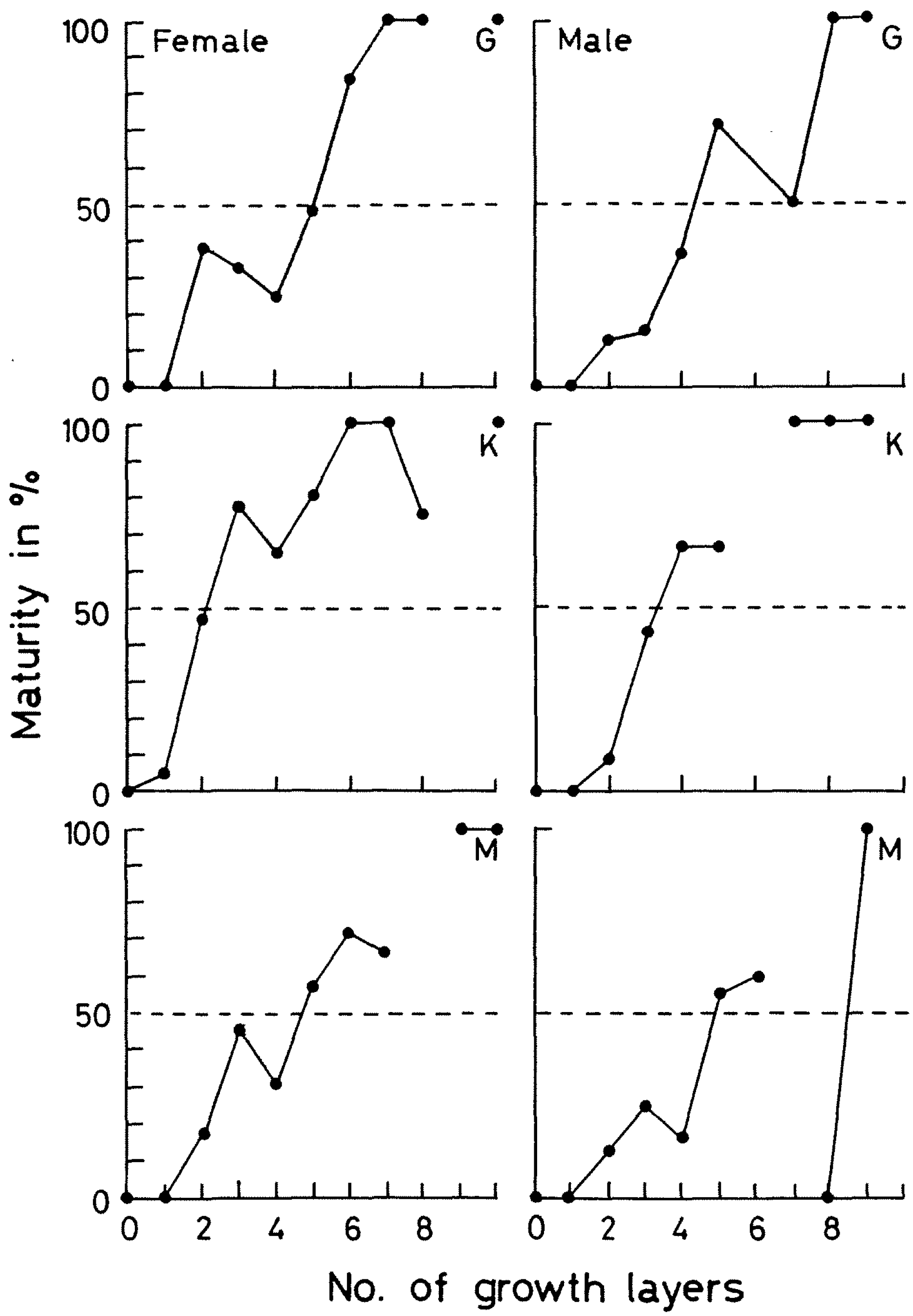

Female
Male
G
K
M
Maturity in %
100
50
0
0
2
4
6
8
No. of growth layers

Figure 5.