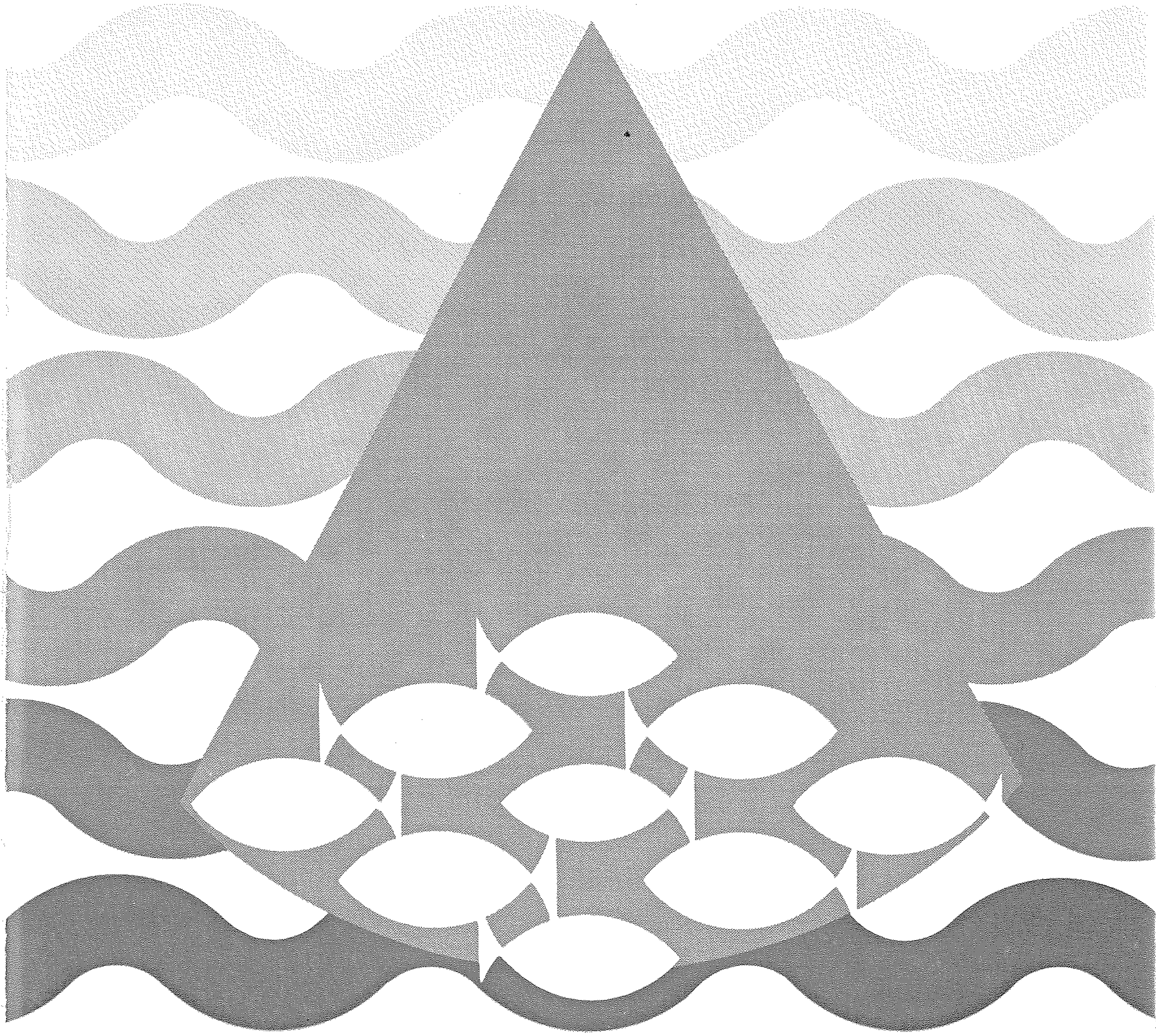
FISKERIDIREKTORATETS SKRIFTER

SERIE HAVUNDERSØKELSER

VOL. 17, No. 13



DIRECTORATE OF FISHERIES, BERGEN, NORWAY
1984

FiskDir. Skr. Ser. HavUnders., 17: 473–481

A COMPARATIVE STUDY ON THE DISPOSITION OF THREE AROMATIC HYDROCARBONS IN FLOUNDER (*PLATICHTHYS FLESUS*)

By

Jan Erik Solbakken, Marit Solberg and Karsten H. Palmork
Institute of Marine Research, Bergen

ABSTRACT

Solbakken, J.E., Solberg, M. and Palmork, K.H. 1983. A comparative study on the disposition of three aromatic hydrocarbons in flounder (*Platichthys flesus*). *FiskDir. Skr. Ser. HavUnders., 17: 473–481.*

The disposition of three aromatic hydrocarbons was studied in flounder (Platichthys flesus). The components, ^{14}C-naphthalene, ^{14}C-phenanthrene and ^{14}C-benzo(a)pyrene, were all given intragastrically. The radioactivity was analysed in several tissues and body fluids at various times after dosing. This study, performed under identical laboratory conditions, made it possible to compare the fate of the three components in flounder. The accumulation was greatest in the case of phenanthrene, whereas the elimination was most efficient with naphthalene. There was a close relationship in the disposition of radioactivity between the liver and bile as well as between blood plasma and muscle. The results indicate that the biliary and urinary excretion is less important with napthalene-derived components than with phenanthrene- and benzo(a)pyrene-derived components. Different factors that might affect the disposition of the aromatic hydrocarbons are discussed.

INTRODUCTION

Several studies describing the disposition of aromatic hydrocarbons in fish have been published during the last decade (e.g. Collier, Thomas and Malins 1978; Dixit and Anderson 1977; Lee, Sauerheber and Dobbs 1972; Melancon and Lech 1978, 1979; Roubal, Collier and Malins 1977; Solbakken andPalmork 1980, 1982; Solbakken *et al.* 1979, Solbakken, Knap and Palmork 1982; Thomas and Rice 1981; Varanasi and Gmur 1980, 1981; Varanasi, Gmur and Treseler 1979; Whittle *et al.* 1977). It is apparent that the large variations in experimental parameters (e.g. route of administration, components, species) make general conclusions difficult. In our laboratory the biological disposition of phenanthrene in several marine organisms has been studied under similar laboratory conditions (Palmork and Solbakken 1980, 1981; Solbakken and Palmork 1980, 1981; Solbakken, Palmork, Neppelberg and Scheline 1979, 1980).

The present study describes the disposition of three aromatic hydrocarbons, naphthalene (Nph), phenanthrene (Phe), and benzo(a)pyrene (BaP), in the flounder *Platichthys flesus*. This is the first comparative study on the uptake and elimination of napthalene, phenanthrene and benzo(a)pyrene in a fish species. Our main interest was to compare the disposition of aromatic hydrocarbons of lower and higher molecular weight (Mw) than Phe with our previous results obtained with the latter compound. Nph (Mw: 128) is more hydrophilic and BaP (Mw: 252) is more lipophilic than Phe (Mw: 178). These characteristics are expected to influence the biological disposition of the components, depending primarily on the lipid content of the tissues.

MATERIALS AND METHODS

Flounder (*P. flesus*) of both sexes were collected from a shellow coastal area near Bergen and kept in an aquarium in the dark for 4 weeks prior to the experiments. They were before and during the experiment fed twice a week with thawed frozen krill (*Meganyctiphanes norvegica*). Dosing was performed as described by Solbakken *et al.* (1979), but the fishes were not anesthetized. The experimental conditions are given in Table 1.

At various times (1, 2, 4, 6 and 14 d and also after 12 h in the Nph and BaP experiments) after the start of the experiments, 5 fish were killed, quickly frozen, and stored at –20°C for up to one week. After thawing, samples of liver, white muscle (taken near the head), bile, urine and blood plasma were taken and analysed for radioactivity.

Bile and urine were sampled by puncturing the bladder with a Pasteur pipette. Blood (approx. 1 mL) was collected from the caudal vein and blood plasma obtained after treatment with heparin (5000 I.U./mL) and centrifugation. Aliquots (approx. 100 mg) of each tissue were digested (Soluene-350) and mixed in 10 mL of Dimilume-30 (Packard Instruments Co.). The radioactivity was determined using a Packrd 300 CD scintillation counter.

RESULTS AND DISCUSSION

The consentrations of radioactivity in different tissues and body fluids are given in Fig. 1. Radioactivity derived from three aromatic hydrocarbons was found in all tissues and body fluids within 12–24 h after dosing. The highest concentrations of radioactivity were found with Phe. There were great variations in the elimination of radioactivity in the different tissues and body fluids. The most distinct decreases were found in liver, blood plasma and

Table 1. The experimental conditions during the studies of the disposition of labelled naphthalene, phenanthrene and benzo(a)pyrene in flounder.

Experimental conditions	Experiment		
	[1(4,5,8)-^{14}C]Naphthalene (Amersham)	[9-^{14}C]Phenanthrene (Amersham)	[7,10-^{14}C]Benzo(a)pyrene (Amersham)
The organisms:			
Mean wet weight ± SD (g)	74 ± 25	79 ± 21	75 ± 20
Total number of fishes	35	25	35
The experimental system:			
Volume of containers (L)	260	260	260
Number of containers	3	2	3
Number of organisms per container	10, 12, 13	12, 13	10, 12, 13
Flowrate of seawater (L/min)	5	5	5
Temperature (°C)	8.5	8.5	8.5
Salinity (‰)	34	34	34
Dosing:			
Spesific activity (mCi/mmol)	5	19.3	21.7
Dose per fish			
μg	9.25	9.25	9.25
μCi	0.36	1.00	0.80
nmole	72	52	37

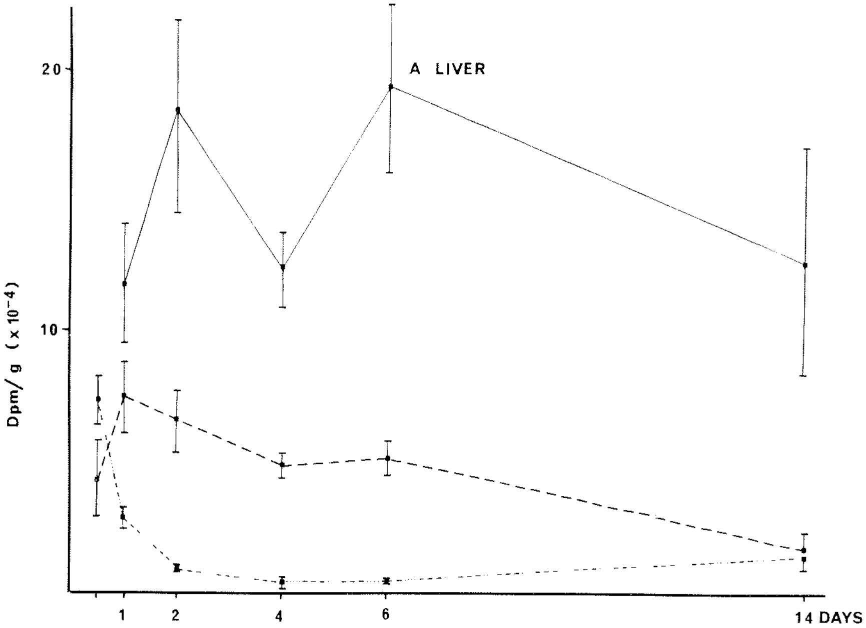
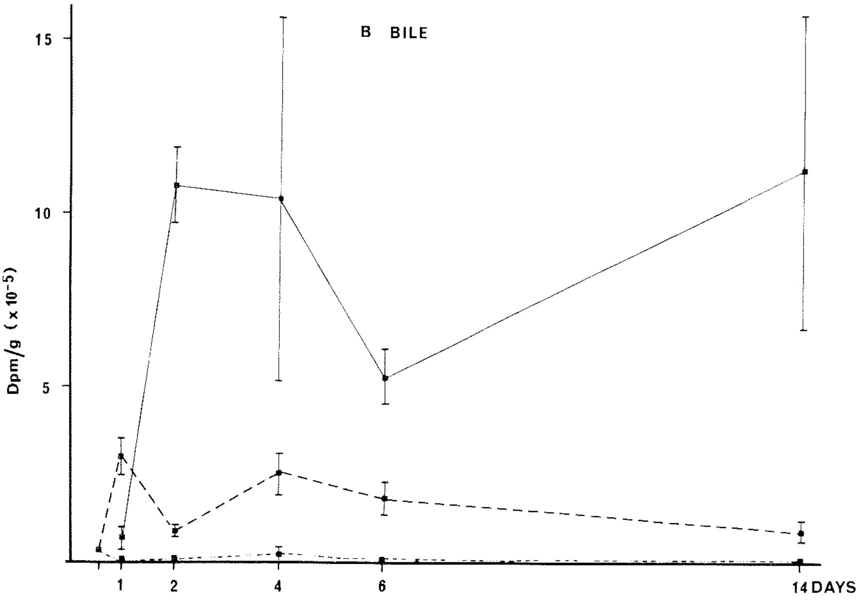
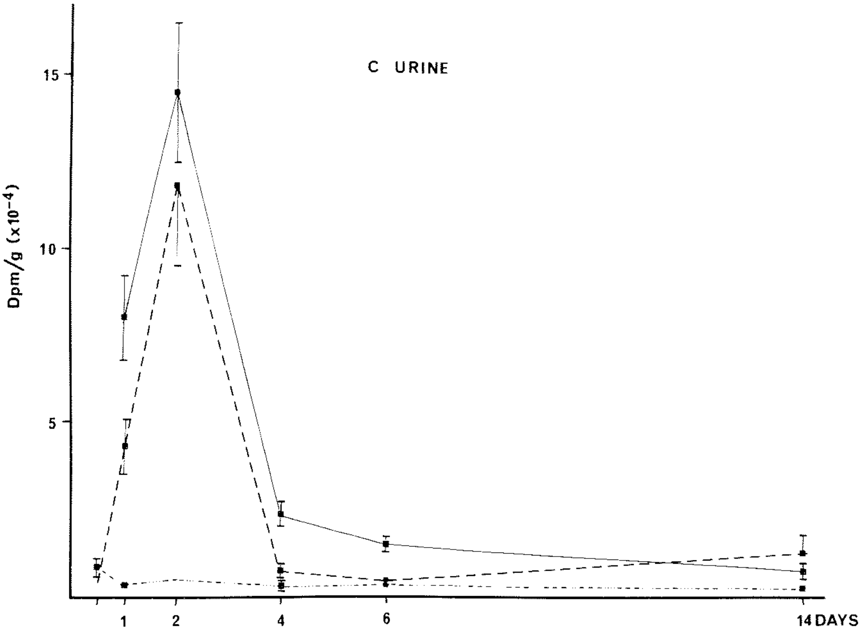
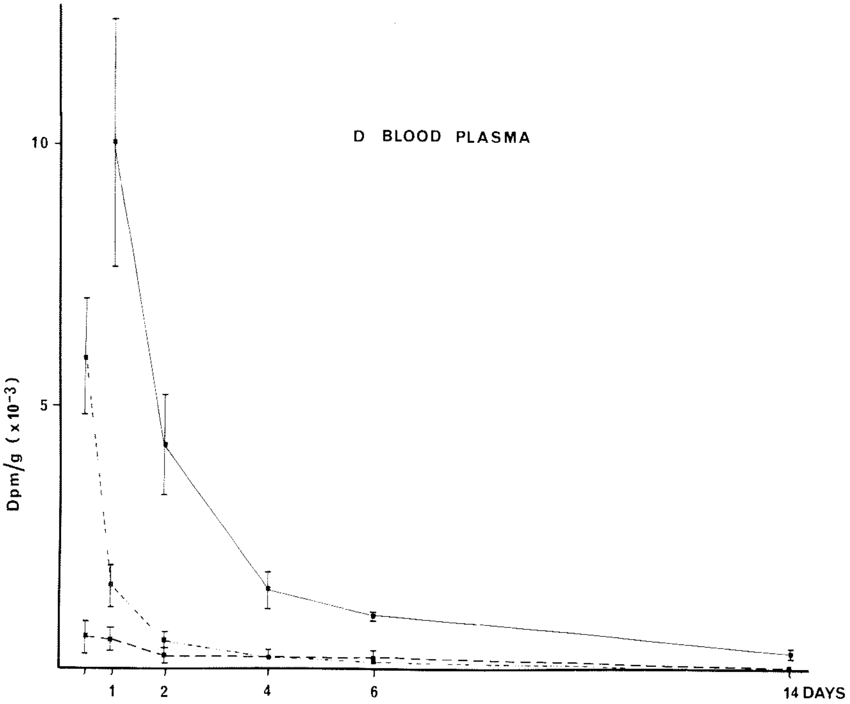
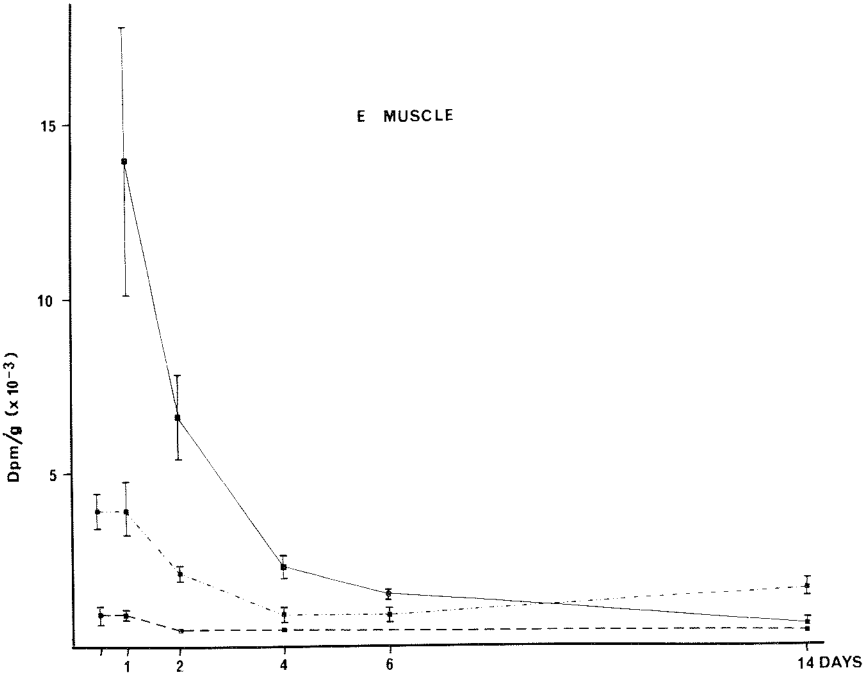


Fig. 1. Time-course of radioactivity in (A) liver, (B) bile, (C) urine, (D) blood plasma and (E) muscle of flounder after oral administration of 9.25 μg ^{14}C-labelled-naphthalene (dot-and-dash line), -phenanthrene (unbroken line), and -benzo(a)pyrene (broken line). Each point represent the mean (±SE) of 5 fish. The values of radioactivity of Nph and BaP have been corrected to correspond to the same specific activity as for Phe (i.e., measured radioactivity of Nph or BaP x $\frac{\text{spes.act. Phe}}{\text{spes.act. Nph or BaP}}$).




C URINE
Dpm/g (x10⁻⁴)
15
10
5
1
2
4
6
14 DAYS


D BLOOD PLASMA
Dpm/g (x10⁻³)
10
5
1
2
4
6
14 DAYS



muscle with Nph, in urine with BaP and in blood plasma, urine and muscle in the case of Phe. The concentrations of Phe-derived radioactivity in the liver and bile did not change markedly over the experimental period (14 d).

The consentrations of the Phe-derived compounds in the liver (Fig. 1A) were nearly twice the concentration of the BaP compounds. The time course of the radioactivity in the Nph-study decreased markedly during the first two days. Thereafter low concentrations of radioactivity were found. In the Phe and BaP study there was only a slight decrease of radioactivity in the liver during the 14 d experimental period. A more efficient elimination of Nph-derived radioactivity was expected since Nph is less lipophilic than Phe and BaP. These results are in accordance with findings from an experiment in which starry flounder (*P. stellatus*) was given labelled BaP and Nph (Varanasi and Gmur 1980). They found that the concentration of BaP-derived radioactivity in the liver 7 d after dosing was 5-fold greater than the corresponding value of Nph. Roubal *et al.* (1977) reported much higher concentrations of radioactivity in the liver of coho salmon (*Oncorhynchus kisutch*) when the fish was given labelled anthracene as compared to naphthalene.

Many authors have previously stressed the importance of biliary excretion of aromatic hydrocarbons (e.g. Lee *et al.* 1972; Solbakken *et al.* 1979, 1980). In the present investigation the time courses for the analyses of the bile and liver samples in the Phe and BaP studies (Fig. 1A and B) were roughly similar, indicating a close relationship between the liver and bile in the excretion of aromatic hydrocarbons.

In urine and bile (Fig. 1B and C) only low concentrations of Nph-derived radioactivity were found in spite of high concentrations in liver and blood plasma. It therefore seems likely that the biliary and urinary excretion are less important in the case of Nph than with Phe and BaP. This is in accordance with results published by THOMAS and RICE (1981). They found that the gills were the most important site of excretion of Nph-derived radioactivity from fish (*Salvelinus malma*) after oral administration. The excretion from the gills was more than 10 times greater than via the urine and intestine.

There was also a great similarity in the results from the blood plasma and the muscle analyses (Fig. 1D,E) which might reflect a close connection between blood and muscle tissue. However, the concentrations were only approximately 5% of the concentrations found in the liver. The fat content of the muscle used in this study was 6.5% of the fat content in the liver which may explain the different concentrations of the lipophilic xenobiotics in these tissues. Low concentrations of radioactivity in muscle of cod (*Gadus morhua*) given a single oral dose of BaP was also reported by CORNER, HARRIS, WHITTLE and MACKIE (1976).

SOLBAKKEN and PALMORK (in prep.) carried out an experiment where Phe was given to flounder in the winter season. The present experiment was performed in the summer, using corresponding laboratory conditions. The temperature was the same in the two experiments. The results, however, were diverging. Solbakken and Palmork found much lower concentrations of radioactivity in liver, muscle, urine, and bile compared to the present study. The elimination was less efficient in the present investigation. Thus, the time course of radioactivity in the liver (Fig. 1A) was closer to a corresponding curve in an experiment performed at 3°C (SOLBAKKEN and PALMORK, in prep.). These differences were probably due to seasonal variations in the biochemistry and physiology of the species, and this stresses the need of more knowledge about such variations to be able to give a meaningful evaluation of the results.

ACKNOWLEDGEMENTS

This investigation was sponsored in part by the Norwegian Marine Pollution Research and Monitoring Programme. The manuscript was reviewed by A.K. Walde, Department of Pharmacology, University of Bergen, and typed by G. Christensen. The Packard 300 CD scintillation counter was partly sponsored by Statoil.

REFERENCES

Collier, T.K., Thomas, L.C. and Malins, D.C. 1978. Influence of environmental temperature on disposition of dietary naphthalene in coho salmon (*Oncorhynchus kisutch*): Isolation and identification of individual metabolites. *Comp. Biochem. Physiol., 61C*: 23–28.

Corner, E.D.S., Harris, R.P., Whittle, K.J. and Mackie, P.R. 1976. Hydrocarbons in marine zooplankton and fish. P. 70–105 in Lockwood, A.P.M. ed. *Effects of pollutants on aquatic organisms,* Vol. 2 Society for Experimental Biology Seminar Series, Cambridge University Press, U.K.

Dixit, D. and Anderson, J.W. 1977. Distribution of naphthalenes within exposed *Fundulus simulus* and correlations with stress behavior. P. 633–636 in Anon. ed. *Proc. 1977 Oil Spill Conference (Prevention, behaviour, control, cleanup).* American Petroleum Institute, Washington D.C.

Lee, R.F., Sauerheber R. and Dobbs, G.H. 1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. *Mar. Biol., 17*: 201–208.

Melancon, M.J. and Lech, J.J. 1978. Distribution and elimination of naphthalene and 2-methylnaphthalene in rainbow trout during short- and long-term exposures. *Arch. Environm. Contam. Toxicol., 7*: 207–220.

Melancon, M.J. and Lech, J.J. 1979. Uptake, biotransformation, disposition and elimination of 2-methylnaphthalene and naphthalene in several fish species. P. 5–22 in Marking, L.L. and Kimerle, R.A. ed. *Aquatic toxicology, ASTM STP 667.* American Society for Testing and Materials.

Palmork, K.H. and Solbakken, J.E. 1980. Accumulation and elimination of phenanthrene in Norway lobster (*Nephrops norvegicus*) following intragastic administration of [9-^{14}C]phenanthrene. *Bull. Environm. Contam. Toxicol., 25*: 668–671.

Palmork, K.H. and Solbakken, J.E. 1981. Distribution and elimination of [9-^{14}C]phenanthrene in the horse mussel (*Modiola modiolus*). *Bull. Environm. Contam. Toxicol., 26*: 196–201.

Roubal, W.T., Collier, T.K. and Malins, D.C. 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). *Arch. Environm. Contam. Toxicol., 5*: 513–529.

Solbakken, J.E. and Palmork, K.H. 1980. Distribution of radioactivity in the chondrichthyes *Squalus acanthias* and the osteichthyes *Salmo gairdneri* following intragastric administration of [9-^{14}C]-phenanthrene. *Bull. Environm. Contam. Toxicol., 25*: 902–908.

Solbakken, J.E. and Palmork, K.H. 1981. Metabolism of phenanthrene in various marine organisms. *Comp. Biochem. Physiol.,70C*: 21–26.

Solbakken, J.E. and Palmork, K.H. (in prep.). Accumulation and elimination of [9-^{14}C]phenanthrene in flounder (Platichthys flesus) at two temperatures.

Solbakken, J.E., Palmork, K.H., Neppelberg, T. and Scheline, R.R. 1979. Distribution of radioactivity in coalfish (*Pollachius virens*) following intragastric administration of [9-^{14}C]phenanthrene. *Bull. Environm. Contam. Toxicol., 23*: 100–103.

Solbakken, J.E., Palmork, K.H., Neppelberg, T. and Scheline, R.R. 1980. Urinary and biliary metabolites of phenanthrene in the coalfish (*Pollachius virens*). *Acta Pharmacol. et Toxicol., 46*: 127–132.

Solbakken, J.E., Knap, A.H. and Palmork, K.H. 1982. The disposition of [9-^{14}C]phenanthrene in a subtropical marine teleost (*Haemulon sciurus*). *Bull. Environm. Contam. Toxicol., 28*: 285–289.

Thomas, R.E. and Rice, S.D. 1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and saltwater Dolly Varden char. P. 425–448 in Vernberg, F.J., Thurberg, F.P., Calabrese, A. and Vernberg, W.B. ed. *Biological monitoring of marine pollutants,* Academic Press, New York.

VARANASI, U., GMUR, D.J. and TRESELER, P.A. 1979. Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder (*Platichthys stellatus*) and rock sole (*Lepidopsetta bilineata*). *Arch. Environm. Contam. Toxicol., 8*: 673–692.

VARANASI, U. and GMUR, D.J. 1980. In vivo metabolism of naphthalene and benzo(a)pyrene by flatfish. In DENNIS, A.J. and COOKE, M. ed. *Proc. fifth internatl. symp. polynuclear aromatic hydrocarbons.* Battelle Press, Ohio.

VARANASI, U. and GMUR, D.J. 1981. Hydrocarbons and metabolites in English sole (*Parophrys vetulus*) exposed simultaneously to [^{3}H]benzo(a)pyrene and [^{14}C]naphthalene in oil-contaminated sediment. *Aquat. Toxicol., 1*: 49–67.

WHITTLE, K.J., MURRAY, J., MACKIE, P.R. HARDY, R. and FARMER, J. 1977. Fate of hydrocarbons in fish. *Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171*: 139–142.

Received 10 April 1983
Printed 2 March 1984

FiskDir. Skr. HavUnders., 17: 483–498.

ESTIMATES OF PUP PRODUCTION, AGE AT FIRST PARTURITION AND NATURAL MORTALITY FOR HOODED SEALS IN THE WEST ICE

By
Njål Oftedal Jacobsen
Institute of Fisheries Biology
University of Bergen, Bergen, Norway[1]

ABSTRACT

Jacobsen, N.O. 1984. Estimates of pup production, age at first parturition and natural mortality for hooded seals in the West Ice. *FiskDir. Ser. HavUnders., 17:* 483–498.

An analysis of the population of hooded seals breeding in the West Ice near Jan Mayen was carried out on the basis of catch statistics for 1946–1978 and age samples comprising a total of 5 094 four years old and older breeding females. A deterministic population model for females without density dependent control was constructed and applied to estimate pup production, age at first parturition and natural mortality. The method was based on minimizing devations between the age structure of the hypothetic population generated by the model and the age composition in samples.

The mean age at first parturition was estimated to 4.9 years. Production was estimated to 95 000 pups in 1956 (samples collected in 1961–1965) and 54 000 pups in 1978 (samples 1972–1978). Combining these figures in the model, instantaneous natural mortality was estimated to 0.12. By projection, pup production in 1979 was estimated to 50 000 pups from a population of 100 000 females. These figures are, however, very sensitive to the estimate of natural mortality.

INTRODUCTION

A study of the population dynamics of hooded seals *(Cystophora cristata)* breeding in the West Ice near Jan Mayen in the Greenland Sea, was made in 1979 (Jacobsen 1979). The study was based on catch statistics for the years 1946–1978 and age composition data from samples of breeding females collected up. to 1978. A brief unpublished summary in English with selected tables and graphs presenting the basis data and pertinent results, has been available since 1980 (Jacobsen MS 1980). However, the summary did not describe the methods used in the analysis.

The West Ice population model has later been applied with slight modifications to available data from hooded seals breeding at Newfoundland (Jacobsen and Øritsland MS 1982), again without an adequate description of the methods. The present paper therefore attempts to describe the method and results from the 1979-analysis of the West Ice data in sufficient detail for evaluation.

---

[1] Present adress: Syftesokbakken 37, N-4040 Madla, Norway

MATERIALS AND METHODS

The data available for this study include statistics of total catches of hooded seals in the West Ice and the Denmark Strait through 1946–1978 (Table 1) and age composition data for a total of 2 580 four years old and older females in Norwegian samples collected on the breeding grounds in the West Ice from 1972 to 1978 (Table 2).

Table 1. Total catches of hooded seals in the West Ice and the Denmark Strait during the years 1946–1978.

Year	West Ice		Denmark Strait
	Pups	1 year +	
1946	8 482	3 083	17 767
47	27 039	12 484	14 130
48	21 242	11 671	16 020
49	48 698	7 782	1 494
1950	49 131	18 568	17 742
51	47 485	35 936	47 607
52	18 098	21 864	16 910
53	21 864	4 160	2 902
54	53 321	12 680	18 292
1955	45 266	11 511	10 283
56	31 564	9 224	12 840
57	13 238	8 951	21 425
58	41 497	23 344	14 950
59	23 305	5 782	6 894
1960	28 213	6 031	8 703
61	47 250	31 770	803
62	29 422	23 398	988
63	20 291	7 456	913
64	23 930	9 407	366
1965	28 787	12 312	—
66	34 016	7 072	748
67	21 390	20 351	371
68	11 795	2 168	20
69	15 870	7 057	—
1970	25 208	12 507	779
71	19 572	10 678	—
72	16 052	4 164	869
73	22 455	3 994	—
74	16 595	9 800	1 201
1975	18 905	8 290	—
76	4 831	2 465	323
77	14 198	4 635	—
1978	16 356	2 680	1 201

Sources: ØRITSLAND 1972, 1976 and unpublished, KAPEL 1975.

Table 2. Age distributions of breeding female hooded seals in Norwegian samples collected in the West Ice during the years 1972–1978.

Age	1972	1973	1974	1975	1976	1977	1978
1			(1)	(5)		(7)	(47)
2				(2)	(1)	(4)	(3)
3	(1)				(1)	(9)	(1)
4	15	24	17	55	18	41	42
5	20	32	23	92	28	81	46
6	13	27	27	136	23	74	67
7	16	18	22	127	36	67	54
8	13	22	14	68	29	69	49
9	4	18	20	41	12	76	43
10	7	17	16	48	11	44	42
11	4	9	14	55	12	18	22
12	7	10	11	29	11	27	16
13	5	9	2	13	7	30	11
14	2	8	3	18	4	9	11
15	2	4	11	21	2	10	7
16	1	5	3	18	4	6	5
17	2	6	2	7	5	5	3
18	3	2	1	13	1	12	5
19	2	4	1	11	1	2	3
20	1	3	—	5	2	6	2
21	1	1	—	6	3	3	3
22	—	2	1	2	1	—	3
23	1	—	1	2	—	5	2
24	2	4	—	5	—	4	1
25	1	2	—	4	—	—	—
26	—	2	—	1	—	3	3
27	1	—	—	3	—	—	2
28	—	—	—	1	—	2	1
29	—	—	—	2	—	2	—
30	—	—	—	—	—	—	—
31	—	—	—	1	—	1	1
32	—	—	—	1	—	1	—
33	—	—	—	1	—	—	—
34	—	—	—	1	—	—	—
Sum	123	229	189	787	210	598	444
(incl. 1–3 years)	(124)	(229)	(190)	(794)	(212)	(618)	(495)

Most of this age material has been studied three or four times independently by two readers, who later came together to establish the age of the animals where there was uncertainty. For age groups 4–10 years 8.4 % of the individual readings showed deviations from the established age. This deviation was 15.7 % for age groups 11–20 and 28.6 % for age groups over 20 years.

Additional age distributions of hooded seals in the West Ice were available for 1 649 breeding females in samples collected by USSR scientists in 1961–1965 (Table 3), and for 314 females in Dutch samples collected in 1973 and 1975 and 551 females in USSR samples collected in 1975–1977 (Table 4).

Table 3. Age distributions of breeding female hooded seals in USSR samples from the West Ice, collected during the years 1961–1965.

Age	1961	1962	1963	1964	1965
1	(7)	(8)	(3)	(3)	(4)
2	(1)	(2)	—	(3)	(5)
3	(5)	(7)	(3)	(6)	(1)
4	16	67	56	18	16
5	16	112	51	49	43
6	15	87	53	38	74
7	27	49	37	23	43
8	13	35	19	17	37
9	20	38	17	7	15
10	17	29	20	13	12
11	7	37	16	12	9
12	10	23	12	10	6
13	8	20	8	4	12
14	4	15	7	6	1
15	4	11	6	10	4
16	4	10	5	10	4
17	—	11	3	3	7
18	3	9	12	7	3
19	2	8	2	5	1
20	5	5	3	6	4
21	1	7	1	2	1
22	—	3	4	3	1
23	3	1	4	2	—
24	1	1	1	1	1
25	2	—	—	—	1
26	2	2	—	—	—
27	—	—	—	1	—
28	1	1	—	—	—
29	—	—	1	—	—
30	—	1	—	—	—
31	—	—	1	—	—
32	—	—	—	—	1
33	—	—	2	—	1
34	—	—	—	—	1
Sum	181	582	341	247	298
(incl. 1–3 years)	(194)	(599)	(347)	(259)	(308)

Table 4. Age distributions of breeding female hooded seals from the West Ice. Dutch and USSR samples collected during the years 1973–1977.

	Dutch samples*)		USSR samples		
	1973	1975	1975	1976	1977
1					
2					
3			(3)		
4	1	15	22	3	16
5	4	25	20	12	33
6	4	39	42	11	33
7	6	41	27	13	36
8	9	31	20	10	34
9	6	17	17	10	26
10	9	17	14	5	19
11	4	12	6	6	11
12	3	15	2	7	8
13	4	10	6	1	5
14	—	6	4	3	4
15	—	5	6	1	3
16	—	2	—	—	4
17	1	1	5	—	3
18	—	5	1	1	3
19	—	1	2	3	2
20	—	2	3	1	2
21	1	3	4	—	1
22	1	2	3	—	1
23	—	3	4	1	1
24	—	1	1	—	—
25	—	1	1	—	1
26	—	1	1	—	—
27	1	1	—	—	1
28	—	1	—	—	—
29	—	—	—	—	—
30	—	—	—	—	—
31	—	—	2	—	—
32	1	1	—	—	—
33	—	—	3	—	—
34	—	1	—	—	—
Sum	55	259	216 (219)	88	247

*) courtesy E. Flipse (†).

POPULATION PROJECTION

A population projection for females was constructed for the years from 1946 to 1986. To initiate the projection it was assumed that pup production in 1940, 1941 and 1942 was 80 %, production in 1943 was 85 %, and production in 1944 was 90 % of pup production in 1945. It was further assumed that the number of 5 years old and older females was equal to pup production in 1944. The 1946 age vector (1–7 +) was then constructed from these assumptions and assumptions about natural mortality, applying a sex ratio at birth of 1:1.

Pup production in 1946 was calculated assuming all females to be recruited to the breeding stock at 7 years of age and assuming a pregnancy rate of 0.95 among mature females. Assumptions of partial recruitment for age groups 4,5 and 6 were also necessary for this calculation.

Recorded West Ice catches were then distributed over age groups, assuming that females constituted 75 % of subadult and adult (1 year +) catches through 1946–1974. Recorded percentages of females in the seasons 1975–1978 are 72.7 %, 54.8 %, 52.3% and 54.6 %. It was also assumed that 94 % of these catches of females consisted of mature seals taken without age selection. The other 6 % were assumed to be evenly distributed over age groups 1–3.

Cathes of moulting hooded seals in the Denmark Strait, which were significant until 1960, were distributed over age groups assuming West Ice seals to make up half of the catches, the other half being assigned to the northwest Atlantic stock. It was also assumed that half of the Denmark Strait catches were one year old and older females taken without age selection.

Having subtracted the age specific catches from the age vector, next year's age vector was calculated using the assumed natural mortality. Repeating this procedure the population was projected forward to 1986, assuming total annual catches of 20 000 pups and 1 500 adult females through the years from 1979 to 1985.

PARAMETER ESTIMATION

The parameters which are necessary to run the projection described above are:

i) pup production in 1945,
ii) natural mortality, and
iii) partial recruitment to the breeding stock of 4, 5 and 6 years old females.

Assuming that the age samples collected from females on the West Ice breeding grounds are representative of the breeding population, these parameters were estimated by comparing the age structure as generated by the model to the observed age group frequencies. Two alternative principles were applied for this comparison:

a) *The least squares principle,* where deviations between observed age frequencies and frequencies from the model were squared and summed. Each sample was weighted according to size. The formula used for this comparison was

$$SQ_j = AT_j \cdot \sum_i \left(\frac{A_{i,j}}{AT_j} - P_{i,j} \right)^2$$

where

AT_j is the sample size in year j,
$A_{i,j}$ is the number of females of age i in the sample from year j, and
$P_{i,j}$ is the fraction of the breeding population being i years old in year j according to the model.

b) *The maximum likelihood principle,* based on the multinomiate distribution. Excluding the multinomiate coefficient which is a constant for each sample, it is shown in APPENDIX I that the likelihood formula can be arranged as

$$LL_j = \sum_i A_{i,j} \cdot \ln P_{i,j}$$

A number of different population projections were generated by varying the input parameters. The set which gave the closest fit to observed age distributions was considered to represent the best estimates.

The two principles turned out to give approximately the same results. Since the maximum likehood method is statistically optimal when the underlying assumptions of the multinomiate distribution are satisfied (see APPENDIX I), only the results from this method have been given. The parameter values which gave a likehood value of 2 units ($\frac{1}{2} \cdot c_{1,0.95}$) below the maximum, have been used as approximate limits of 95 % confidence intervals (Cox and Hinkley 1974). $c_{1,0.95}$ is the upper 0.05 point of the chi-squared distribution with one degree of freedom. These confidence intervals do not include errors introduced by non-random sampling or errors in age determinations, and the true level of confidence is probably considerably smaller than 95 %. However, they should at least indicate the relative level of uncertainties in different estimates.

RESULTS

A summary of the results based on age samples of 3 445 females collected in 1972–1978 (Norwegian, Dutch and USSR samples, Tables 2 and 4), is given in Table 5. The table shows that about 41 % of four years old, 75 % of five years old and 96 % of 6 years old females are recruited to the breeding population, and that these estimates are fairly insensitive to varitions in assumed natural mortality. The corresponding mean age at first parturition was calculated to 4.9 years.

Table 5. Results of population projections with maximum values of the likelihood function for alternative values of natural mortality.

M	Prod. 1945	Likelih. value	Estimates of: Prod. 1968	PREC (4)*	PREC (5)*	PREC (6)*
0.08	64.350	– 8 308.0	54 200	0.40	0.74	0.98
0.10	79 250	– 8 305.7	54 000	0.40	0.74	0.97
0.12	98 900	– 8 303.8	53 600	0.41	0.75	0.96
0.14	125 500	– 8 302.3	53.600	0.42	0.75	0.95
0.16	162 500	– 8 301.2	54 400	0.42	0.74	0.94

*) PREC (i) is the proportion of i years old females being recruited to the whelping population.

While the estimate of production in 1945 depends heavily upon the mortality rate chosen, the production estimate of about 54 000 in 1968 is quite insensitive to variations in mortality. The corresponding 95 % confidence interval is 47 500–64 000.

By the same method, the USSR samples collected in the seasons 1961–1965 (Table 3) gave a production estimate of about 95 000 for 1956 with 85 000 and 113 000 as 95 % confidence limits.

Combining the two production estimates, 95 000 in 1956 and 54 000 in 1968, an estimate of 0.12 was obtained for natural mortality. The projection based on this mortality rate, given in detail in Table 6, shows a population which increased slowly since 1976, with a pup production of 50 000 from a total population of about 100 000 females in 1979 (Figure 1).

Attempts to estimate natural mortality from the 1972–1978 samples alone broke down, giving the impossible rate of M = 0.20. With such a high natural mortality the population would decline even in the absence of hunting. The lower 95 % confidence limit is slightly lower than 0.14.

DISCUSSION

In order to start a population projection, it is necessary to make assumptions not only about production, but also about the age distribution in the starting year. Since there was no hunting during the Second World War, it seems likely that production must have increased in this period, especially towards the end of the period when the first unhunted years classes started to recruit the whelping population. This is the background for the assumption that production in 1940, 1941 and 1942 was 80 %, in 1943 85 % and in 1944 90 % of the production in 1945. As the pregnancy rate is quite high and the mean age at first parturition is close to 5 years, it was further assumed that the number of

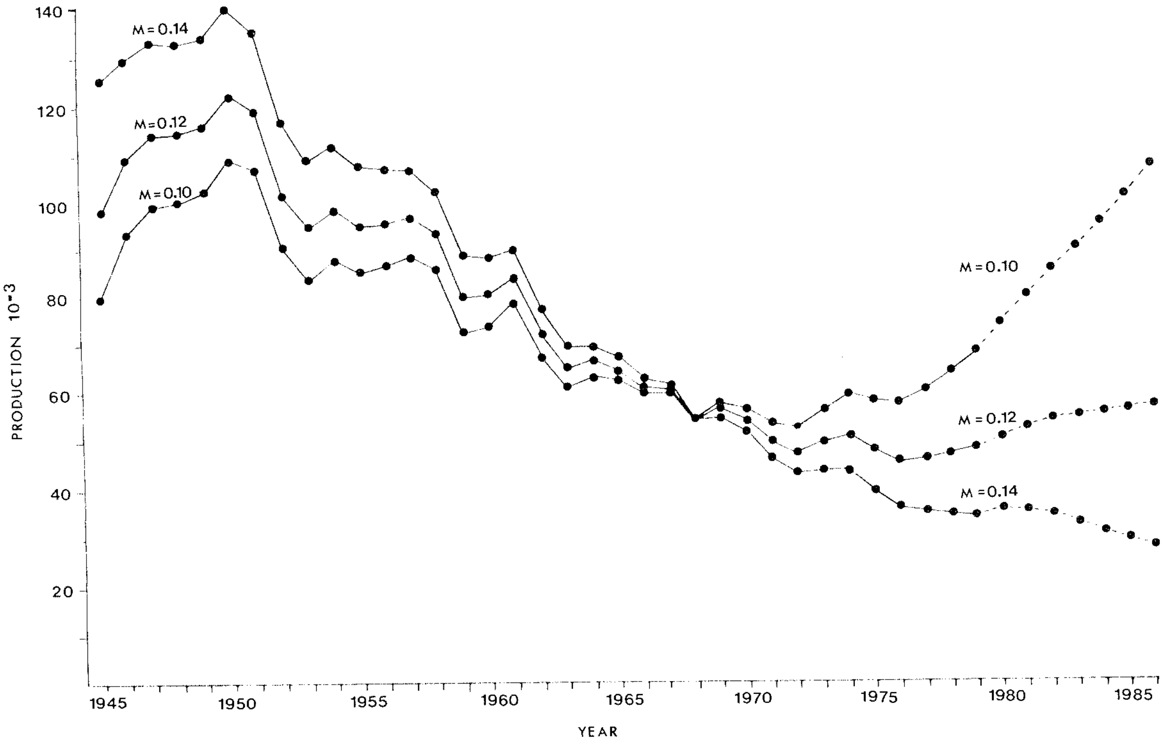


Fig. 1. Production estimates of hooded seals in the West Ice for the years 1945–1986 for three alternative values of natural mortality, M (age samples 1972–1978). Annual catches in 1979–1985 were assumed to be 20 000 pups and 1 500 adult females.

Table 6. Outprint from the population model, representing the estimated number of females in each age group for the years 1946–1986. This projection is based on instantaneous natural mortality 0.12 and a

YEAR	0	1	2	3	4	5	6	7
1946	54541.	43858.	35009.	29325.	24479.	21711.	19256.	70018.
1947	57213.	44612.	38149.	30443.	25494.	21147.	18632.	16457.
1948	57341.	38752.	38853.	33200.	26460.	21620.	17468.	15137.
1949	58199.	41437.	33671.	33759.	28825.	22441.	17892.	14234.
1950	61523.	30022.	36594.	29717.	29795.	25062.	19211.	15168.
1951	59929.	32778.	25909.	31635.	25643.	24863.	20149.	15083.
1952	51115.	32094.	27144.	21355.	26180.	19737.	17631.	13534.
1953	47357.	37309.	27599.	23297.	18267.	21387.	15271.	13171.
1954	49396.	32306.	32917.	24336.	20534.	15948.	18483.	13113.
1955	47828.	20164.	27861.	28392.	20947.	17175.	12926.	14684.
1956	48014.	22346.	17494.	24231.	24695.	17718.	14110.	10425.
1957	48527.	28587.	19352.	15124.	20995.	20944.	14683.	11524.
1958	46668.	37169.	24495.	16543.	12903.	17605.	17173.	11871.
1959	40133.	22988.	32004.	20986.	14072.	10432.	13344.	12481.
1960	40261.	25259.	20107.	28023.	18349.	12109.	8825.	11168.
1961	42077.	23197.	22040.	17528.	24460.	15734.	10200.	7351.
1962	36159.	16365.	20127.	19102.	15105.	19419.	11292.	6840.
1963	32541.	19022.	14178.	17508.	16601.	12181.	14388.	7909.
1964	33355.	19863.	16743.	12454.	15403.	14235.	10163.	11799.
1965	32609.	18972.	17479.	14714.	10913.	13122.	11722.	8190.
1966	30776.	16155.	16662.	15339.	12887.	9177.	10535.	9135.
1967	30664.	12211.	14212.	14662.	13490.	11052.	7657.	8639.
1968	26806.	17711.	10551.	12324.	12723.	10865.	8160.	5337.
1969	28193.	18543.	15680.	9329.	10902.	11159.	9440.	7049.
1970	27261.	17968.	16353.	13813.	8180.	9337.	9275.	7699.
1971	24995.	13000.	15742.	14312.	12063.	6786.	7313.	6999.
1972	23979.	13489.	11388.	13820.	12552.	10071.	5373.	5595.
1973	25168.	14149.	11882.	10023.	12175.	10842.	8523.	4489.
1974	25738.	12363.	12496.	10485.	8836.	10563.	9233.	7174.
1975	24041.	15468.	10801.	10919.	9141.	7403.	8444.	7161.
1976	22962.	12939.	13612.	9473.	9577.	7735.	6015.	6684.
1977	23612.	18223.	11442.	12038.	8370.	8395.	6719.	5196.
1978	24130.	14646.	16119.	10105.	10634.	7284.	7190.	5697.
1979	24789.	14148.	12925.	14228.	8909.	9298.	6310.	6192.
1980	26063.	13117.	12521.	11437.	12592.	7814.	8080.	5451.
1981	27140.	14247.	11607.	11079.	10117.	11051.	6797.	6989.
1982	27941.	15202.	12609.	10268.	9799.	8882.	9620.	5886.
1983	28320.	15912.	13456.	11157.	9080.	8606.	7736.	8335.
1984	28650.	16248.	14086.	11908.	9868.	7975.	7497.	6706.
1985	29125.	16541.	14384.	12467.	10535.	8669.	6950.	6500.
1986	29792.	16963.	14644.	12731.	11030.	9255.	7556.	6028.

pup production in 1945 of 98 900. It includes estimates of total production of about 95 000 in 1956 and about 54 000 in 1968.

8	9	10	11	12	13	14	15	16+
0.	0.	0.	0.	0.	0.	0.	0.	0.
59793.	0.	0.	0.	0.	0.	0.	0.	0.
13327.	48422.	0.	0.	0.	0.	0.	0.	0.
12298.	10828.	39340.	0.	0.	0.	0.	0.	0.
12044.	10406.	9162.	33288.	0.	0.	0.	0.	0.
11855.	9414.	8133.	7161.	26017.	0.	0.	0.	0.
10024.	7879.	6256.	5405.	4759.	17291.	0.	0.	0.
10042.	7438.	5846.	4642.	4010.	3531.	12829.	0.	0.
11296.	8613.	6379.	5014.	3981.	3440.	3028.	11003.	0.
10378.	8940.	6816.	5049.	3968.	3151.	2722.	2397.	8708.
11801.	8341.	7185.	5478.	4058.	3189.	2532.	2188.	8925.
8491.	9612.	6793.	5852.	4462.	3305.	2597.	2062.	9051.
9291.	6846.	7749.	5477.	4718.	3597.	2664.	2094.	8960.
8557.	6697.	4935.	5586.	3948.	3401.	2593.	1921.	7968.
10424.	7147.	5594.	4122.	4666.	3297.	2841.	2166.	8259.
9283.	8665.	5941.	4650.	3426.	3878.	2741.	2361.	8666.
4863.	6142.	5733.	3930.	3076.	2267.	2566.	1813.	7295.
4730.	3369.	4255.	3971.	2723.	2131.	1570.	1777.	6310.
6464.	3873.	2754.	3478.	3246.	2225.	1742.	1283.	6610.
9469.	5188.	3108.	2210.	2791.	2605.	1786.	1398.	6335.
6346.	7337.	4020.	2408.	1712.	2162.	2018.	1384.	5991.
7466.	5187.	5996.	3285.	1968.	1399.	1767.	1650.	6028.
5953.	5145.	3574.	4132.	2264.	1356.	964.	1218.	5290.
4605.	5137.	4440.	3084.	3566.	1954.	1170.	832.	5616.
5728.	3742.	4174.	3607.	2506.	2897.	1587.	951.	5240.
5768.	4291.	2803.	3127.	2702.	1877.	2170.	1189.	4638.
5319.	4384.	3261.	2131.	2377.	2054.	1427.	1650.	4428.
4663.	4433.	3653.	2718.	1776.	1981.	1712.	1189.	5066.
3770.	3916.	3723.	3068.	2283.	1491.	1663.	1438.	5243.
5532.	2907.	3019.	2871.	2366.	1760.	1150.	1283.	5159.
5641.	4357.	2289.	2378.	2261.	1863.	1386.	906.	5074.
5767.	4867.	3760.	1975.	2052.	1951.	1608.	1196.	5159.
4397.	4881.	4119.	3182.	1672.	1737.	1651.	1361.	5378.
4901.	3782	4199.	3543.	2737.	1438.	1494.	1420.	5797.
5343.	4229.	3264.	3623.	3058.	2362.	1241.	1289.	6228.
4711.	4617.	3655.	2821.	3131.	2642.	2041.	1072.	6496.
6046.	4075.	3994.	3161.	2440.	2708.	2286.	1766.	6547.
5095.	5234.	3528.	3458.	2737.	2112.	2345.	1979.	7196.
7218.	4412.	4532.	3055.	2994.	2370.	1829.	2030.	7945.
5808.	6252.	3822.	3926.	2646.	2593.	2053.	1584.	8640.
5633.	5033.	5418.	3311.	3402.	2293.	2247.	1779.	8860.

5 years old and older females in 1944 (the year classes up to and including 1939) was equal to the production the same year. As errors in assumed age distribution in 1945 will have a minor effect on the age distribution after 1960 when the age samples become available, these assumptions are not discussed any further.

The assumed pregnancy rate of 0.95 is based on research by ØRITSLAND (1964, 1975) and BORN (1982), whose estimates by various methods fall in the range from 0.918 to 0.98. The assumption of a 1:1 sex ratio at birth is based on counts by ØRITSLAND (1964) and ØRITSLAND and BENJAMINSEN (1975a). A total of 2 434 pups were controlled, and 50.4% of these were males.

Data about sex composition of the subadult and adult catches through 1946–1974 are sparse, the assumption that 75 % of these catches were females is in agreement with ØRITSLAND and BENJAMINSEN (1975b). Though the proportion of subadults in the catches vary from year to year (see age samples), in order to simplify the model it was assumed that the catches included 6% immature animals, 2% of each of age groups 1–3 years.

There is no evidence to justify the assumption that West Ice seals make up half of the Denmark Strait catches. Low catches in this area since 1961, however, probably make this a non-critical assumption.

The age samples from the Denmark Strait show 46.8 % females in 1958–60 (ØRITSLAND 1964) and 41.9 % in 1970–1978 (ØRITSLAND unpubl.). As some animals sink after being shot and are not recorded in the catch statistics, it has been assumed that 50 % of this catch are females. All age groups except the pups, which do not moult, seem to be well represented in these age samples, and this is the basis for the assumption of no age selection in the Denmark Strait.

The sexual maturity data obtained using the population projection agree well with results from ovary analyses (ØRITSLAND 1964 and 1975, JACOBSEN 1979, BORN 1982). These analyses all indicate a mean age at first parturition of about five years. This supports the critical assumption that the age samples are representative of the breeding population.

Provided that this assumption holds, it is shown in APPENDIX II that the expected value of the square function (SQ_j) for an individual sample should be about 0.9. For the best projection (Table 6) the 1972–1978 samples gave a mean SQ-value of 1.16, which must be considered acceptable. For the Soviet samples collected in 1961–1965, however, the mean SQ-values was 2.54, and it was not possible to generate projections with acceptably low SQ-values. Whether this lack of fit between the Soviet samples and the projection is caused by non-random sampling, errors in age determinations or factors inherent in the model, is not known, but it does indicate that less confidence can be held in the production estimate of 95 000 for 1956 than in the estimate of 54 000 for 1968.

Table 7. Sealing regulations for hooded seals in the West Ice since the introduction of quotas in 1971 (ØRITSLAND MS 1982).

Season	Opening data	Closing data	Total quotas				Scientific permits
			Overall	Pups	Females[1]	Males	
1971	20 March	5 May	30 000				
1972–74	23 »	»	30 000				
1975	22 »	» »	31 800				1 800
1976	» »	» »	34 500			5 000	
1977	» »	» »		33 500	2 500	10 000	
1978	» »	» »		31 500	1 000	»	500 females
1979	» »	» »		23 600	1 520	»	
1980[2]	» »	12 »		20 000	max 2% of pups[3]	free	400 females 200 subad.
1981	» »	» »		20 000	» [4]	»	400 pups
1982	» »	» »		20 000	»[4]	»	400 females

[1] Breeding females protected since 1969, but killing for compelling safety reasons is permitted.

[2] Subadults protected.

[3] One pup deducted for each female taken.

[4] Two pups deducted for each female taken.

The estimate of 0.12 for natural mortality must be taken only as an indication of the real value. When a population is projected through a fixed point, changes in assumed natural mortality are compensated by changes in estimated recruitment trends. This self-compensational effect makes it possible to construct projections with quite different natural mortalities and different trends in stock size which have very similar age structures, since apparent mortality is equal to real mortality plus recruitment change. When the maximum likelihood method is applied, the confidence interval therefore tends to be very large. In addition to this even a minor overrepresentation of young animals in the samples will give substantially overestimated natural mortalities. This might explain the "impossible" estimate of 0.20 from the 1972–1978 age samples.

Figure 1 shows that an error as small as 0.01–0.02 in the estimate of natural mortality will have a considerable effect on the production level when the population is projected from 1968 up to the present date. Because of the uncertainty inherent in this estimate, it is not possible to give an exact assessment of the status of the West Ice population of hooded seals. However, on the basis of preliminary results from this study, total catch quotas for pups and females were gradually reduced by 44 % from 1977 to 1980 (Table 7), and adult females are now given nearly complete protection. Though it is not clear whether the population has been increasing of decreasing during the last decade, there should be no reason to fear depletion of the stock under the present management policy.

Monitoring of catches and biological sampling should be continued to ensure that significant changes are discovered in time to maintain the population in a robust state.

ACKNOWLEDGEMENTS

Unpublished material and data were put at my disposal for this study by the Sea Mammal Section of the Institute of Marine Research, Directorate of Fisheries, Bergen, through T. Øritsland who also edited the manuscript. B. Bergflødt of the Sea Mammal Section contributed greatly by assisting in age determinations. T. Benjaminsen, Ø. Ulltang and C.J. Rørvik of the Institute of Marine Research, and M. Aksland of the Institute of Fisheries Biology, University of Bergen, gave valuable comments and advice during the study. J. Cooke of the International Institute for Environment and Development, London, suggested the procedure for calculating confidence limits. Unpublished Soviet age distributions were released through the Norwegian-Soviet Sealing Commission. Finally I acknowledge with gratitude that E. Flipse, Amsterdam, released his then unpublished age composition data before his tragic death in August 1979.

REFERENCES

BORN, E. W. 1982. Reproduction in the female hooded seal, *Cystophora cristata* Erxleben, at South Greenland. *J. Northw. Atl. Fish. Sci., 3:*57–62.

COX, D. R. and HINKLEY, D. V. 1974. *Theoretical statistics.* Chapman and Hall, London. 511 p.

JACOBSEN, N. O. 1979. *Produksjon, alder ved første gangs kasting og naturlig dødelighet hos klappmyss,* Cystophora cristata *(Erxleben, 1777), i Vesterisen.* Cand.real.thesis Institute of Fisheries Biology, University of Bergen, 1979. 78 p. [Mimeo.]

JACOBSEN, N. O. MS 1980. Pup production, age at first pupping and natural mortality of hooded seals in the West Ice. *NAFO Sci. Coun. Work. Pap., 80* (XI/56): 1–9. [Mimeo.]

JACOBSEN, N. O. and ØRITSLAND, T. MS 1982. Provisional estimates of production and natural mortality for Newfoundland hooded seals. *Rep. Inst. Marine Res. Bergen, 6 juni 1982.* 5 p. [Mimeo.]

KAPEL, F. O. MS 1975. Data on the catch of harp and hooded seals 1954–1974, and long-term fluctuations in seal hunting in Greenland. *ICNAF Work. Pap., 75* (XII/9): 1–14. [Mimeo.]

ØRITSLAND, T. 1964. Klappmysshunnens forplantningsbiologi [The breeding biology of female hooded seals]. *Fisken Hav., 1964* (1): 1–15.

ØRITSLAND, T. MS 1972. Norwegian catches of hooded seals *(Cystophora cristata)* in the Denmark Strait 1945–1960. *Rep. Inst. Marine Res., Bergen, 22 February 1972.* 1 p. [Mimeo.]

ØRITSLAND, T. 1975. Sexual maturity and reproductive performance of female hooded seals at Newfoundland. *ICNAF Res. Bull., 11:* 37–41.

ØRITSLAND, T. 1976. The management of harp and hooded seals in the West Ice. *Coun. Meet. int. Coun. Explor. Sea, 1976* (N:17):1–12. [Mimeo.]

ØRITSLAND, T. MS 1982. Summary of West Ice sealing regulations and catches of seals. *Note, Inst. Marine Res., Bergen 25 May 1982.* 5 p. [Mimeo.]

ØRITSLAND, T., and BENJAMINSEN, T. 1975a. Additional data on the sex ratio, age composition and mortality of Newfoundland hooded seals, with an estimate of pup production and sustainable yield. *ICNAF Res. Doc., 75* (122): 1–22. [Mimeo.]

ØRITSLAND, T., and BENJAMINSEN, T. 1975b. Rapport om norske selundersøkelser og norsk selfangst i det nordøstlige Atlanterhav i 1975. *Selfangstkommisjonen for den nordøstlige del av Atlanterhavet, Oslo 1975:* 1–30. [Mimeo.]

Received 19 April 1983
Printed 2 March 1984

APPENDIX I

The likelihood function

According to standard statistical theory, the likelihood function for the multinomiate distribution may be written as

$$l = \frac{n!}{\prod_{i=1}^{s} x_i} \prod_{i=1}^{s} P_i^{x_i}$$

where n is the number of trials, x_i is the number of times an individual trial results in the event A_i and P_i is the probability that an individual trial will result in the event A_i.

If it is assumed that ages of individual animals are independent of each other (no segregations within the whelping area and small sample sizes compared to total populations), then the age samples will show å multinominate distribution, and with the symbols defined on page 489, the formula above may be written as

$$l_j = \frac{AT_j!}{\prod_{i=4}^{16} A_{i,j}} \prod_{i=4}^{16} P_{i,j}^{A_{i,j}}$$

For a given age sample the first term of this expression, the multinomiate coefficient, will be a constant and may be omitted in the calculations.

We may then write

$$\ln l^* = \sum_{i=4}^{16} A_{i,j} \cdot \ln P_{i,j}$$

This simplified function is referred to in the text as the likelihood function, LL_j.

The likelihood value for several age samples is found by summing the values for each sample.

APPENDIX II

Expected value of the square function

We assume that a certain population projection corresponds to the population, so that $E\left(\frac{A_{i,j}}{AT_j}\right) = P_{i,j}$. (Symbols explained on page 489.)

For this projection we have:

$$E(SQ_j) = E(AT_j \cdot \sum_i \left(\frac{A_{i,j}}{AT_j} - P_{i,j}\right)^2)$$

$$= AT_j \cdot \sum_i \; E\left(\frac{A_{i,j}}{AT_j} - P_{i,j}\right)^2$$

$$= AT_j \cdot \sum_i \; \mathrm{Var}\left(\frac{A_{i,j}}{AT_j}\right)$$

$$= \frac{1}{AT_j} \cdot \sum_i \mathrm{Var}\, A_{i,j}$$

$$= \frac{1}{AT_j} \cdot \sum_i AT_j \cdot P_{i,j}\,(1-P_{i,j})$$

$$= \sum_i (P_{i,j} - (P_{i,j})^2) = 1 - \sum_i (P_{i,j})^2$$

The value of the term $\sum_i (P_{i,j})^2$ will vary from year to year, but trail calculations have shown it to be close to 0.10 for this population. Thus, the expected value for the square function for one sample is close to 0.90, provided that sampling is random and that the projection correspond to the true population.

FiskDir. Skr. Ser. HavUnders., 17: 499-500.

A NOTE ON THE PREDOMINANCE OF NON-K-REGION METABOLITES OF PHENANTHRENE FOUND IN BONY FISHES

By

Jan Erik Solbakken and Karsten H. Palmork
Institute of Marine Research, Bergen

The metabolism of phenanthrene in marine organisms and rats has been intimately studied in our laboratory (Solbakken *et al.* 1980, Solbakken and Palmork 1981). We found a different main metabolite between bony fishes and cartilaginous fishes, crustaceans and mammals. The K-region metabolite, 9, 10-dihydro-9, 10-dihydroxyphenanthrene is previously found to be the main metabolite in mammals (e.g. Boyland and Wolf 1950, Chaturapit and Holder 1978). We also found the K-region metabolite most abundant in rats, crustaceans and cartilaginous fishes (Solbakken and Palmork 1981). However, in a study using coalfish, *Pollachius virens,* the non-K-region metabolite, 1,2-dihydro-1,2-dihydroxyphenanthrene was found to be the main metabolite (Solbakken *et al.* 1980) This is later on also reported in other experiments using rainbow trout, *Salmo gairdneri,* and flounder, *Platichthys flesus,* (Solbakken and Palmork 1981).

In an experiment to see if changes in dose, sampling time and temperature were due to the different pathway of the metabolism of phenanthrene, flounders and bluestriped grunts, *Haemulon sciurus,* were given phenanthrene intragastrically. The total weights were 143±33 and 444±113 g ($\bar{x}$±SD) of the flounders and the grunts, respectively. The method is previously described by Solbakken *et al.* (1980). Samples of urine and bile from six fish were combined and analysed for conjugated and hydroxylated metabolites by using gaschromatography-masspectrometry (see Solbakken *et al.* 1980). The temperature, the magnitude of the dose and the sampling times are given in Table 1.

The results show no variation in the main metabolite; the 1,2-dihydro-1,2-dihydroxyphenanthrene (non-K-region) was the main metabolite of phenanthrene in urine and bile. In all samples more than 98% of the metabolites were conjugated as sulfate or glucuronide conjugates, and the main metabolite represented more than 89% of the metabolites. The K-region metabolite,

Table 1. The experimental conditions.

	Dose (mg/fish)		
Temperature	25	5	0.5
°C	Sampling time (days after dosing)		
	Flounder *(Platichthys flesus)*		
0	*2,4*	–	–
4	*3*	–	–
9	1,2,7	2	2
	Grunts *(Haemulon sciurus)*		
25	2	–	–

9,10-dihydro-9,10-dihydroxyphenanthrene varied from 1 to 9% of the metabolites, and the 1-hydroxyphenanthrene from 0.1 to 0.9%. Only small amounts of the other metabolites were found.

These results show that the composition of metabolites is not affected by changes in dose, temperature and at which time after dosing the samples were analysed. It therefore seems likely that the difference in the main metabolites between bony fishes and cartilaginous fishes, crustaceans and mammals is due to genetic variations among the species.

REFERENCES

BOYLAND, E. and WOLF, G. 1950. Metabolism of polycyclic compounds. 6. Conversion of phenanthrene into dihydroxydihydrophenanthrenes. *Biochem. J.*, *47* : 64–69.

CHATURAPIT, S. and HOLDER, G.M. 1978. Studies on the hepatic microsomal metabolism of (^{14}C)penanthrene. *Biochem. Pharmac.*, *27* : 1865–1871.

SOLBAKKEN, J.E. and PALMORK, K.H. 1981. Metabolism of phenanthrene in various marine organisms. *Comp. Biochem. Physiol.*, *70C* : 21–26.

SOLBAKKEN, J.E., PALMORK,, K.H. NEPPELBERG, T. and SCHELINE, R.R. 1980. Urinary and biliary metabolites of phenanthrene in the coalfish *(Pollachius virens)*. *Acta Pharmacol. et Toxicol.*, *46* : 127–132.

Received 15 June 1983
Printed 2 March 1984