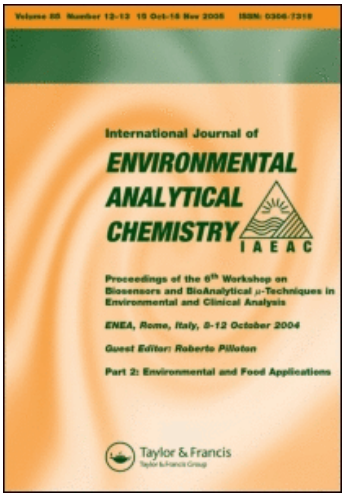

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Musial, Charles J. and Uthe, John F.(1983) 'Widespread Occurrence of the Pesticide Toxaphene in Canadian East Coast Marine Fish', International Journal of Environmental Analytical Chemistry, 14: 2, 117 — 126
To link to this Article: DOI: 10.1080/03067318308071613
URL: http://dx.doi.org/10.1080/03067318308071613

Intern. J. Environ. Anal. Chem., 1983, Vol. 14, pp. 117–126
0306-7319/83/1402-0117 $18.50/0

Printed in Great Britain

Widespread Occurrence of the Pesticide Toxaphene in Canadian East Coast Marine Fish

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(*Received July 5, 1982; in final form September 2, 1982*)

Chromatographic and chemical confirmatory evidence is presented for the presence of residues of toxaphene, a polychlorinated camphene pesticide, in herring (*Clupea harengus harengus*) and cod (*Gadus morhua*) from widely-separated areas of the Canadian east cost. Toxaphene residues were not detected in a sample of deep-sea scallops (*Placopecten magellanicus*). Toxaphene was determined by capillary gas chromatography following a combination of chromatography and fuming nitric-concentrated sulfuric acid cleanup, a procedure which greatly simplified the capillary gas chromatograms and eliminated many co-extractives. Concentrations in the fish tissues ranged from 0.4 to 1.1 μg/g on a wet weight basis and from 2.4 to 12 μg/g on a fat weight bais. These data indicate widespread contamination of the marine environment by chlorinated camphenes.

INTRODUCTION

The occurrence of residues of toxaphene (polychlorinated camphene, PCC) in the spawn of fish from widely-separated parts of the Western Hemisphere has recently been reported by Zell and Ballschmiter.[1] The same authors reported finding PCC components in the livers of Antarctic cod (*Dissostichus eleginoides*), indicating that PCC contamination of even the most pristine areas of the aquatic ecosphere has occurred.

PCC is known to exhibit toxicity to various species of fish. Toxic effects in fish include decreased viability of ova of brook trout (*Salvelinus fontinalis*) which had been exposed to 0.068 μg PCC/L water and decreased bone collagen synthesis resulting in depressed growth and fragile, easily broken backbone in brook trout fry,[2] and inhibition of shell deposition of American oysters (*Crassostrea virginica*) at 3.1 μg PCC/L

water.[3] The acute toxicity of PCC to fish is similar to that of endrin and endosulfan, two organochlorine pesticides lethal to fish at very low concentrations in water.[7] In this regard, the 24 h LD_{50} for goldfish (*Carassius auratus*) of the PCC technical mixture is an order of magnitude less than that of the two most toxic components isolated to date, toxicant A (a mixture of two octachlorobornanes) and toxicant B (a heptachlorobornane).[4]

Introduced in the mid-1940s, PCC soon gained wide acceptance for the control of a variety of insect pests and, due to the restrictions placed on the use of the other organochlorine pesticides, use of PCC is expected to increase. Production estimates of PCC for the United States for 1980 were of the order of 105 million kg.[5] In contrast, the U.S. produced only 47 million kg of DDT in 1967 and 63 million kg in 1968, representing the peak production of DDT in the years immediately before it was banned.[16]

In view of the fact that PCC bioaccumulates in fish,[2,3,6] the lack of knowledge of which PCC components are present in marine fish, and the extreme toxicity of certain of these components to fish, it is essential that baseline concentrations in marine fish be established. The present work describes a simple procedure using nitric acid–sulfuric acid treatment and capillary gas chromatography for the estimation of PCC in marine fish, and presents several results obtained using this procedure to indicate that widespread PCC contamination of the marine environment is occurring.

Experimental

Extraction, cleanup and nitration: Herring (*Clupea harengus harengus*) were taken near Halifax, Nova Scotia, in the summer of 1979 and the oil was extracted from the homogenized fillets of 25 commercial-sized individuals by a process previously described.[8] A similar sample was taken from the Gulf of St. Lawrence in the summer of 1981. Cod (*Gadus morhua*) were caught by otter trawl in the Gulf of St. Lawrence in the fall of 1979 and the livers of 60 fish of various sizes (14–70 cm fork length) were removed and homogenized; 0.5 g of each liver was extracted by a procedure previously described.[9] The deep-sea scallops (*Placopecten magellanicus*) were taken from Georges Bank in the fall of 1981 and in size represented the range of a commercial catch. The gonads, meats (adductor muscles), and rims (gills and digestive tract) of 25 individuals were separated and these three tissues were separately pooled, homogenized, and the oil extracted as described.[9]

The extracted oils were cleaned up by column chromatography, employing Florisil as described previously,[9] with the following modification: In addition to the hexane fraction (FI), a second fraction of 100 ml (FII) and a third fraction of 25 ml (FIII) of dichloromethane in

hexane (3:7, V/V) were collected from the Florisil column. FII was screened for PCC by packed column gas chromatography (GC) with electron capture detection (ECD). This fraction also contained other organochlorine compounds such as the DDT group and α-HCH. The removal of these compounds was necessary to obtain a clear capillary GC profile of PCC residues. The compounds were removed by the nitration procedure of Van Hove Holdrinet,[15] with the following simplification: The extract was taken just to dryness under nitrogen in a 15 ml glass centrifuge tube and 1.0 ml of the fuming nitric:concentrated sulfuric acid (1:1, V/V) (Fisher Scientific) mixture was added. After heating 1/2 h in a 70°C water bath, the tube was cooled in crushed ice and 5.0 ml of glass distilled water were added slowly while the tube was alternately cooled in the icebath and agitated on a vortex mixer. This mixture was then extracted twice with 5.0 ml of glass distilled hexane (Caledon Laboratories) with transfer of the hexane layer by disposable pipet into a second 15-ml tube. The combined hexane extracts were reduced to 1.0 ml under nitrogen and applied to a small glass column (550 mm L × 6 mm ID) plugged with a piece of glass wool and containing 1.0 g Florisil (activated and deactivated as described above).[9] The Florisil was topped with ca. 1 cm anhydrous sodium sulfate (Fisher Scientific) as drying agent. The following fractions were collected: SFI—10.0 ml hexane, SFII—5.0 ml hexane.

Gas chromatography: Capillary gas chromatography (CGC) was performed on a 30-m fused silica column (Hewlett–Packard) coated with OV-101 and installed in a Hewlett–Packard model 5730 A gas chromatograph fitted with a model 18740 capillary kit and a ^{63}Ni electron capture detector (ECD); 3.0 ul injections of hexane solutions were made in the splitless mode with the following conditions: delay—40 s, hold oven temperature for 2 min at 70°C then to 210°C at 16° min^{-1}, hold to end of run (terminated at about 50 min). Carrier gas was helium flowing at 1.2 ml min^{-1} and make-up gas for the ECD was 95% argon/5% methane (Matheson) flowing at 35 ml min^{-1}.

RESULTS AND DISCUSSION

The elution pattern of PCC from the large and small Florisil columns, before and after nitration respectively, is shown in Table 1. In the current study, we concentrated on fractions FII: SFI and FII:SFII since these contained the major portion of the PCC residues. Based on the total amount of PCC "spike" added to the herring sample, FII:SFI contained approximately 40% of the spike and FII:SFII contained some 12% of the original spiked amount, yielding a combined recovery of 52% for these

TABLE I
Elution pattern (%) of PCC from Florisil columns before nitration (F) and after nitration (SF)

FI		FII		FIII	
15.2		83.8		1.0	
SFI	SFII	SFI	SFII	SFI	SFII
99.2	0.8	77.1	22.9	66.8	33.2

two fractions. FII:SFI contained components the retention times of which corresponded to published retention times of toxicants A and B (peaks 15 and 8, Figure 2B) on a similar capillary column.[18] Work is underway to confirm the presence of toxicants A and B by GC-MS.

While the recovery of 52% is somewhat low, the advantage of simplified chromatograms more than outweighs this disadvantage. Nitration alters the relative peak heights of some of the PCC components and the chromatography on Florisil effects a partial separation of some of the components. This can be considered as confirmatory evidence of the presence of these components.

Figure 1 illustrates the complexity of the capillary chromatograms of FII of herring fillet (curve A) and FII of cod liver (curve B) extracts. The retention times of various commonly occurring co-extracted organochlorine pesticides are given, as well as a chromatogram of toxaphene (curve C). The possibility of misidentification of peaks is clearly indicated, if gas chromatography is used as the sole means of peak identification. The negative peaks, most evident in curve A, should also be noted. These are usually caused by long-chain hydrocarbons, some of which are of biogenic origin; the role of these negative peaks in the analysis of polychlorinated biphenyls in marine fish has been discussed in a previous paper.[10]

Figure 2 gives the capillary chromatograms of FII:SFI of the herring extract after nitration and cleanup (Figure 2A) and the comparable PCC fraction after nitration (Figure 2B). The anticipated differences in relative peak heights between the standard and the partly metabolized, "weathered" sample are evident but this does not hide the great similarity between the two chromatograms.

A comparison of Figures 1C and 2B indicates some simplification of the chromatogram of the PCC standard due to the combination of chromatography on Florisil plus nitration. Many of the PCC components pass through the nitration procedure with unchanged retention times which is good evidence of the presence of these components in the samples.

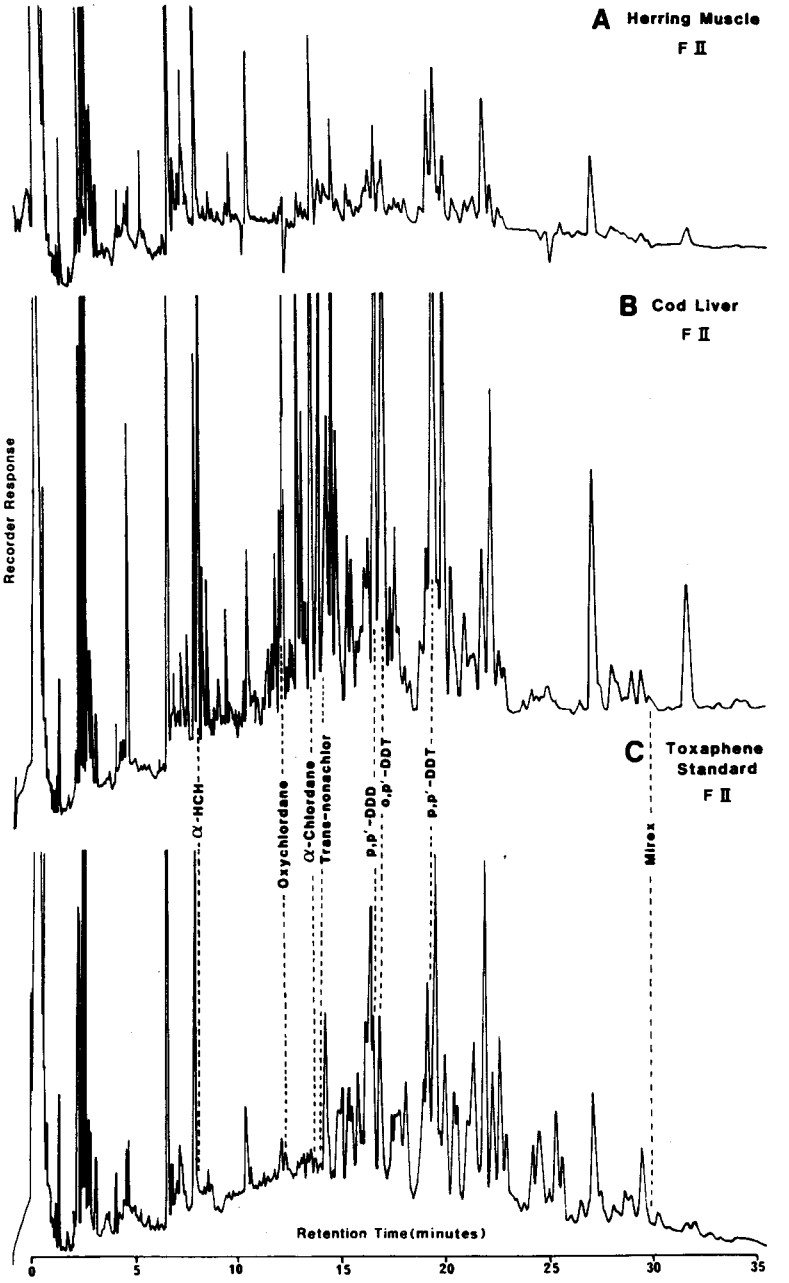

FIGURE 1 Capillary gas chromatograms of (A) herring muscle and (B) cod liver after initial Florisil cleanup, and (C) PCC standard under the same conditions.

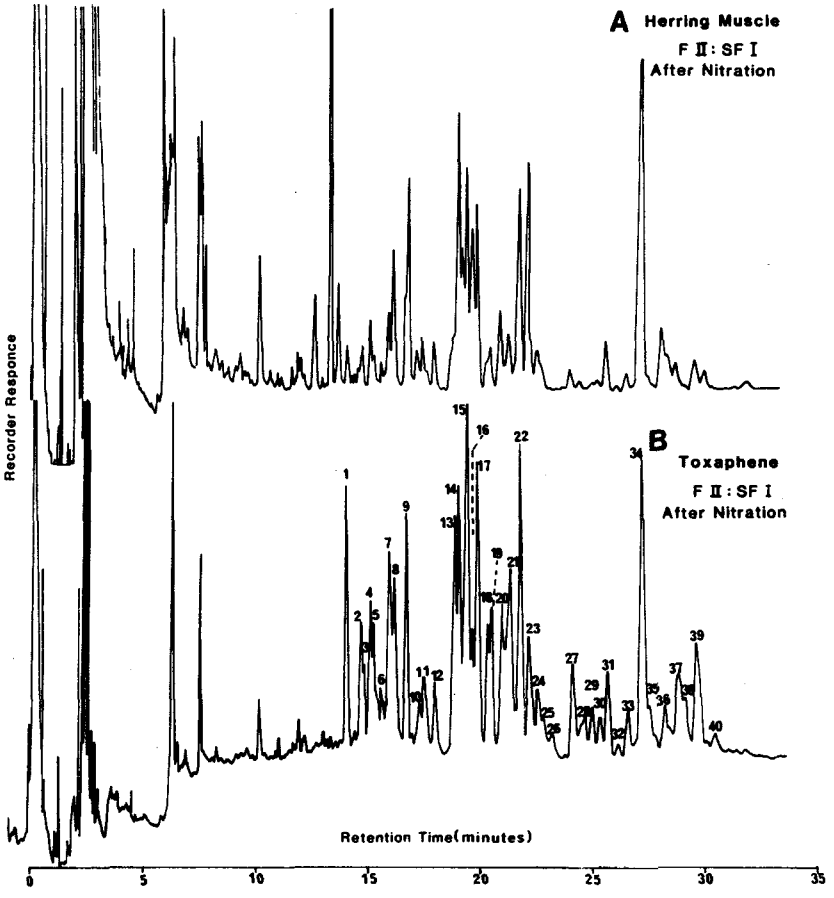

FIGURE 2 Capillary gas chromatograms of (A) herring muscle and (B) PCC standard after nitration and secondary Florisil cleanup.

Figure 3 illustrates the capillary chromatogram of the nitrated cod liver extract (Figure 3A) again compared with the appropriate fraction of PCC standard (Figure 3B) and the procedural blank (Figure 3C). The simplification of the cod liver chromatogram is apparent (compare to Figure 1B). While the cod liver chromatogram does not match that of the PCC standard as well as that of the herring fillet, there are still a good number of peaks with retention times identical to those of the standard.

The disappearance of negative interferents in the nitrated herring extract can be seen by comparing Figure 1A and 2A. Should these interferents be superimposed on some of the PCC components, the overall result would be an artifically lowered value for the analyte in question. Therefore,

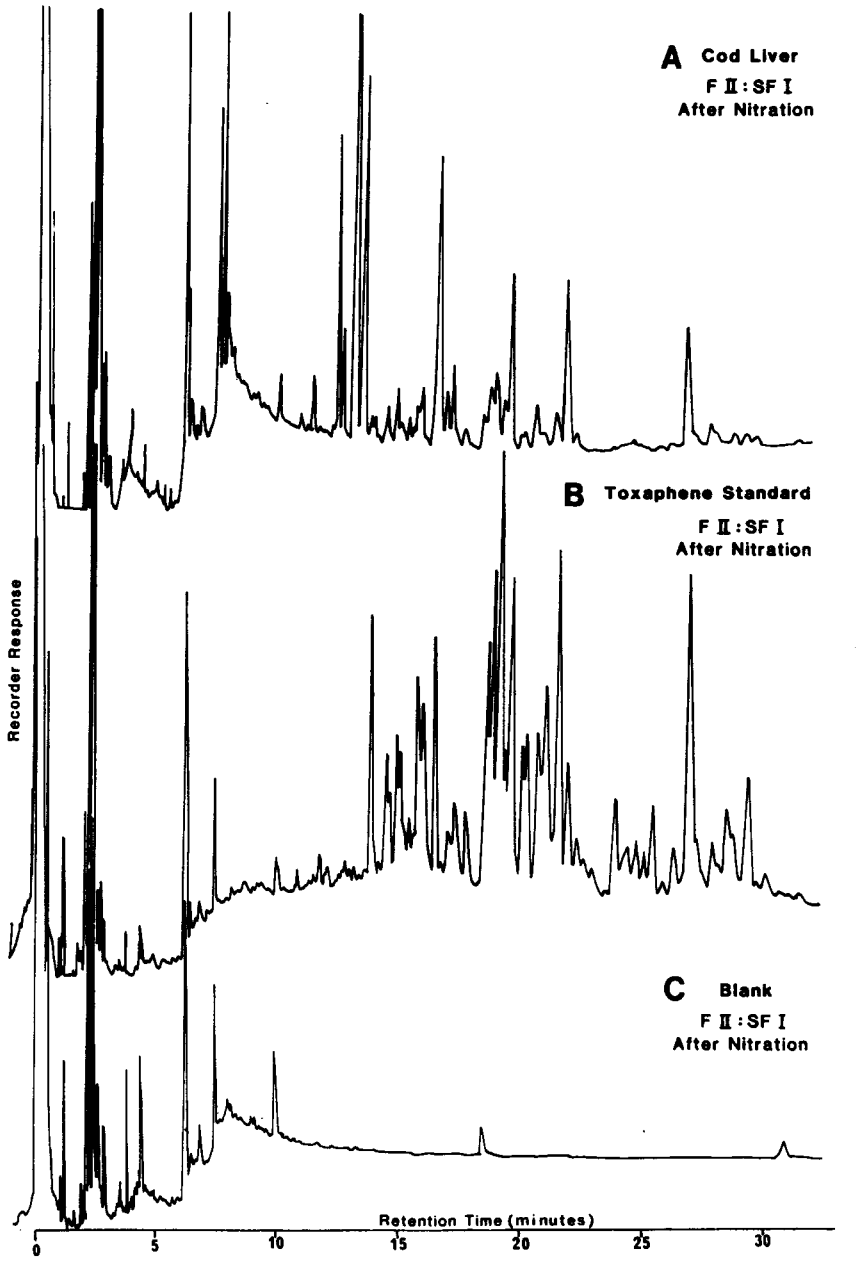

FIGURE 3 Capillary gas chromatograms of (A) cod liver, (B) PCC standard, and (C) procedural blank after nitration and secondary Florisil cleanup procedure.

removal of these interferents is most important and, while GCMS is the only suitable means of confirming the absence of all such interferents, the nitration procedure offers a valuable alternative for those laboratories to which GCMS is not available.

The quantitation of PCC is an even greater problem than the quantitation of polychlorinated biphenyls (PCB). It would be virtually impossible to characterize and quantitate each of the components present in environmental samples; however, several of the larger, chromatographically well-separated peaks can be used to estimate the concentrations of PCC in marine fish. In the current study, 6–10 peaks (peaks 8, 13, 14, 15, 17, 18, 19, 22, 23, 34, Figure 2B) in fractions FII:SFI and FII:SFII were used for comparison with the nitrated toxaphene standard and the concentrations of the two fractions summed. Because of these problems and the lack of proper standards for PCC components, these results should be regarded as semi-quantitative.

Table 2 gives the concentrations of PCC in the various tissues studied. These tissues are representative of pelagic and benthic finfish and of shellfish and give an indication of PCC distribution in the marine environment. These three species also represent a major portion of the Canadian east coast fishery. Organochlorine compounds are known to bioaccumulate in the fatty tissues of fish, therefore cod liver and herring fillet can be compared as representative target tissues of benthic and pelagic species. It is interesting that PCC residues are found in both of these tissues in the Gulf of St. Lawrence, indicating that penetration to the lower strata of the water column has occurred. Since the cod is actually a

TABLE II

Concentrations of PCC in cod liver, herring fillet and scallop meat, male and female gonads

	PCC concentration		
Sample, area	Wet weight basis (μg/g)	Fat weight basis (μg/g)	Fat (%)
Cod liver (Gulf of St. Lawrence)	1.1	2.4	48.0
Herring fillet (Gulf of St. Lawrence)	1.0	12	8.3
Herring fillet (Halifax)	0.4	4.4	9.0
Scallop meat (Georges Bank)	ND*	ND	0.4
Scallop male gonad (Georges Bank)	ND	ND	1.8
Scallop female gonad (Georges Bank)	ND	ND	3.8

*ND = none detected. Limit of detection = 0.10 μg/g for a 5-g sample.

demersal species, following a depth-dependent diurnal cycle, it is possible that this species incurs PCC residues at intermediate depths rather than on the bottom. This is supported by the absence of PCC in the deep-sea scallop tissues.

It has been demonstrated that blue mussels (*Mytilus edulis*) bioaccumulate chlorinated aromatic hydrocarbons.[11,12] Ehrhardt and Heinemann[13] have shown that the same species metabolizes and excretes aliphatic and olefinic hydrocarbons but lacks the ability to deal with cyclic saturated and aromatic hydrocarbons in the same way. We are not aware of any similar work performed on scallops and, because of this, the limited sampling, the lack of seasonal variation studies, and the geographic variability of oceanographic phenomena on Georges Bank we cannot speculate broadly on the meaning of the absence of PCC in this scallop sample.

The gas chromatograms of the PCC residues in herring fillet from the Gulf of St. Lawrence and off Halifax were virtually identical, indicating a uniform input of the pesticide over a wide area. However, we cannot offer any explanation as to why the concentration in herring fillet from the Gulf of St. Lawrence should be greater than that from the vicinity of Halifax. It is interesting to note that the former was taken 2 yr after the latter. The finding of PCC in these fishery products supports the findings of Zitko, who has reported the occurrence of PCC components in tuna from the Canadian east coast.[14]

The occurrence of PCC in the livers of Antarctic cod at a level of 0.068 μg/g fat or 0.022 μg/g wet weight has been reported by Zell *et al.*[1] The level in our cod liver sample was much higher, i.e. 2.4 μg/g and 1.1 μg/g respectively. Ribick *et al.*, reported 25.6 μg/g PCC (wet weight, whole fish) in channel catfish (*Ictalurus punctatus*) from the Arroyo Colorado at a field site known to be contaminated by toxaphene.[17] This is considerably higher than any of the levels in our samples.

Van Hove Holdrinet[15] used the nitration procedure for confirmation of the organochlorine pesticides Mirex and *cis*- and *trans*-chlordane in the presence of PCB. The same author reported that hexachlorobenzene (HCB) survived the nitration procedure but recoveries were not reproducible. Our current work confirms this finding although we have not specifically checked reproducibility of recovery of HCB. Our work also indicates that *trans*-nonachlor passes through the procedure chromatographically unchanged but this observation has not been confirmed by GCMS. Peaks with retention times identical to those of authentic samples of *cis*- and *trans*-nonachlor were found on the gas chromatograms of these tissues, particularly in the case of the cod liver. The chlordane compounds were well-separated from the PCC components

by CGC; therefore the combination of Florisil cleanup plus nitration plus CGC offers an inexpensive way of determining all of these compounds in marine fish and eliminates many of the interfering co-extractives.

Acknowledgements

Thanks are due to Mark Lundy, Invertebrates and Marine Plants Division, Fisheries Research Branch, Department of Fisheries and Oceans, for his assistance in providing the scallop sample, and to Dr. John Loder, Ocean Circulation Division, Atlantic Oceanographic Laboratory, Department of Fisheries and Oceans, Bedford Institute of Oceanography, for his helpful discussions concerning the hydrography of Georges Bank.

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