Research in Fisheries Contaminants and Toxicology at the Biological Station, St. Andrews, N.B., in 1982

V. Zitko, Editor

Biological Station,
St. Andrews, N.B., EOG 2XO

January 1983

Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 1698

Government of Canada
Fisheries and Oceans

Gouvernement du Canada
Pêches et Océans

Canadian Manuscript Report of Fisheries and Aquatic Sciences

These reports contain scientific and technical information that represents an important contribution to existing knowledge but which for some reason may not be appropriate for primary scientific (i.e. *Journal*) publication. They differ from Technical Reports in terms of subject scope and potential audience: Manuscript Reports deal primarily with national or regional problems and distribution is generally restricted to institutions or individuals located in particular regions of Canada. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries management, technology and development, ocean sciences, and aquatic environments relevant to Canada.

Manuscript Reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report will be abstracted by *Aquatic Sciences and Fisheries Abstracts* and will be indexed annually in the Department's index to scientific and technical publications.

Numbers 1-900 in this series were issued as Manuscript Reports (Biological Series) of the Biological Board of Canada, and subsequent to 1937 when the name of the Board was changed by Act of Parliament, as Manuscript Reports (Biological Series) of the Fisheries Research Board of Canada. Numbers 901-1425 were issued as Manuscript Reports of the Fisheries Research Board of Canada. Numbers 1426-1550 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Manuscript Reports. The current series name was changed with report number 1551.

Details on the availability of Manuscript Reports in hard copy may be obtained from the issuing establishment indicated on the front cover.

Rapport manuscrit canadien des sciences halieutiques et aquatiques

Ces rapports contiennent des renseignements scientifiques et techniques qui constituent une contribution importante aux connaissances actuelles mais qui, pour une raison ou pour une autre, ne semblent pas appropriés pour la publication dans un journal scientifique. Ils se distinguent des Rapports techniques par la portée du sujet et le lecteur visé; en effet, ils s'attachent principalement à des problèmes d'ordre national ou régional et la distribution en est généralement limitée aux organismes et aux personnes de régions particulières du Canada. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du Ministère des Pêches et des Océans, notamment gestion des pêches; techniques et développement, sciences océaniques et environnements aquatiques, au Canada.

Les Manuscrits peuvent être considérés comme des publications complètes. Le titre exact paraît au haut du résumé de chaque rapport, qui sera publié dans la revue *Aquatic Sciences and Fisheries Abstracts* et qui figuera dans l'index annuel des publications scientifiques et techniques du Ministère.

Les numéros de 1 à 900 de cette série ont été publiés à titre de manuscrits (Série biologique) de l'Office de biologie du Canada, et après le changement de la désignation de cet organisme par décret du Parlement, en 1937, ont été classés en tant que manuscrits (Série biologique) de l'Office des recherches sur les pêcheries du Canada. Les numéros allant de 901 à 1425 ont été publiés à titre de manuscrits de l'Office des recherches sur les pêcheries du Canada. Les numéros 1426 à 1550 ont été publiés à titre de Rapport manuscrits du Service des pêches et de la mer, Ministère des Pêches et de l'Environnement. Le nom de la série a été changé à partir du rapport numéro 1551.

La page couverture porte le nom de l'établissement auteur où l'on peut se procurer les rapports sous couverture cartonnée.

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 1698

January 1983

RESEARCH IN FISHERIES CONTAMINANTS AND TOXICOLOGY AT THE BIOLOGICAL STATION, ST. ANDREWS, N.B., IN 1982

V. Zitko
Editor

Fisheries Contaminants and Toxicology Section
Fisheries and Environmental Sciences
Fisheries Research Branch
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick EOG 2X0

Cat. No. Fs-4/1698 ISSN 0706-6473

Correct citation for this publication:

Zitko, V., ed. 1983. Research in fisheries contaminants and toxicology at the Biological Station, St. Andrews, N.B., in 1982. Can. MS Rep. Fish. Aquat. Sci. 1698: iii + 13 p.

ABSTRACT

Zitko, V., ed. 1983. Research in fisheries contaminants and toxicology at the Biological Station, St. Andrews, N.B., in 1982. Can. MS Rep. Fish. Aquat. Sci. 1698: iii + 13 p.

The report describes research performed in 1982 by the St. Andrews component of the Fisheries Contaminants and Toxicology Section of the Fisheries and Environmental Sciences Division. The research dealt with the bioavailability of heavy metals in sediments, levels of cadmium in scallops, uptake and excretion of polynuclear aromatic hydrocarbons and potential contamination of lobsters by these compounds, adenylate energy charge as an indicator of sublethal effects, and the determination of organic pollutants by mass spectrometry.

Key words: trace metals, bioavailability, stress indicators, polynuclear aromatic hydrocarbons, mass spectrometry

RÉSUMÉ

Zitko, V., ed. 1983. Research in fisheries contaminants and toxicology at the Biological Station, St. Andrews, N.B., in 1982. Can. MS Rep. Fish. Aquat. Sci. 1698: iii + 13 p.

Ce qui suit est un compte rendu des recherches menées par le groupe de St. Andrews de la Section des contaminants et de la toxicologie des pêches de la Division des sciences halieutiques et de l'environnement. Ces recherches ont porté sur l'accessibilité biologique des métaux lourds dans les sédiments, les niveaux de cadmium chez les pétoncles, l'absorption et l'excrétion des hydrocarbures aromatiques polynucléaires et les possibilités de contamination du homard par ces composés; sur la charge énergétique d'adénylate comme indicateur d'effets sublétaux; et enfin sur la détermination des polluants organiques par spectrométrie de masse.

INTRODUCTION

The long-term objective of the program is to assess hazards posed to fisheries by chemicals. The hazard assessment includes the determination of chemicals in the aquatic environment (water, sediment and biota), the determination of their fate (degradation, migration to sediments, bioavailability), their effects on aquatic biota (lethal and sublethal effects, bioaccumulation), and the development of biochemical and physiological criteria of sublethal stress (biochemical parameters such as the adenylate energy charge, behavioral criteria). In addition, the program involves the development of predictive capabilities based on quantitative structure-activity relationships and studies of the applicability of models in hazard assessment.

In 1982 the emphasis was on the bioavailability of the trace metals copper, cadmium, lead, and zinc from sediments and on their levels in the scallop (Placopecten magellanicus). Work on organic contaminants was focused mainly on polynuclear aromatic hydrocarbons and their potential to contaminate the American lobster (Homarus americanus). In the field of biochemical parameters of sublethal stress, studies of the adenylate energy charge continued and were expanded to several other energy metabolism-related compounds.

A second mass spectrometer was acquired recently to aid in the identification of organic contaminants and considerable time was spent in bringing it on line.

The sections of this report were written by investigators leading the respective projects. The sections were put together with only a minimum of editing, to provide up-to-date information on the results.

INORGANIC ENVIRONMENTAL CHEMISTRY

S. RAY
V. Bailey
M. Woodside

The objectives of the project are to study trace metal-related effects on fishery resources and to determine ways to mitigate them. A monitoring program to determine background levels of several trace metals in scallops from eastern Canadian waters was also undertaken. The trace metals were determined by atomic absorption spectrophotometry.

BIOAVAILABILITY OF CD AND ZN FROM ORE CONCENTRATES

Large amounts of lead and zinc ore concentrates are stored year round at the ore handling wharf, Dalhousie, N. B., for shipment overseas. The harbour bottom has been seriously contaminated over the years due to weathering of the ore piles and wind-blown concentrates being deposited. The contamination of bottom sediment has created serious dredge disposal problems due to potential bioavailability of the trace metals to marine biota. Recently Hoff et al. (1982) reported that Cu and Pb, but not Cd or Zn, are released from mine tailings by sea water, but leaching of trace metals from ore concentrates by sea water and their bioavailability to marine organisms have not been studied. It was thus considered necessary to determine whether these metals could be leached from ore concentrates by sea water and be bioavailable to marine biota.

a) Bioaccumulation

Two zinc and two lead concentrates were available for the study. However, only Heath Steele zinc ore (HZn) and Brunswick Mining and Smelting zinc ore (BZn) concentrates were chosen because of ease in leaching Cd and Zn from them. High metal concentrations in the leachates were likely to facilitate bioaccumulation of the metals by the organisms. Bioaccumulation of Cu and Pb was also monitored because of high Cu and Pb concentrations in the ore (Table 1) and the leachates. Sediment from the St. Croix estuary (New Brunswick, Canada) was used as a diluent in bioaccumulation experiments.

Shrimp Crangon septemspinosa were exposed to St. Croix estuarine sediment containing 1, 2, and 4%, respectively, of the two ores (HZn & BZn) and Dalhousie sediment for a maximum period of 64 d; Nereis virens (polychaete worms) were exposed to Dalhousie sediment only. Animals exposed to 2 and 4% sediment-ore combinations survived 16-32 d and 2-4 d, respectively. Animal and water samples were collected for Cu, Zn, Cd and Pb determination.

The mean concentrations of Cu and Cd in Nereis and Crangon exposed to Dalhousie sediment did not change significantly ($p < 0.05$) from control values while Pb and Zn were significantly higher. Similar results were obtained with Crangon exposed to the sediment-ore combinations. The Zn concentrations in Crangon exposed to the two sediments containing 1% ore and Dalhousie sediment were only 3-4 times higher than in the controls. But the Pb concentrations in animals exposed to Dalhousie sediment were 66 times higher than the controls compared with

Table 1. Concentration of Cu, Zn, Cd and Pb (µg/g dry) in Dalhousie sediment, Heath Steele Zn ore and Brunswick Mining and Smelting zinc ore concentrates.

Material	Cd	Zn	Cu	Pb
Dalhousie sediment	43	2,310	310	5,100
Heath Steele zinc ore concentrate	850	454,000	6,400	39,200
Brunswick Mining and Smelting zinc ore concentrate	840	536,000	3,100	26,200

only 4 times in the animals exposed to the ores. The observed high Pb concentrations in animals exposed to Dalhousie sediment could be related to high Pb concentrations (240 μg Pb/mL) in the overlying water compared with only 21 μg Pb/mL in water overlying the two ore-sediment mixtures at the end of a 64-d exposure period.

Accumulation of Cd by the animals was insignificant ($p < 0.05$) in all cases, although the overlying water contained appreciable concentrations of Cd (2-4 ng/mL). It is suggested that the presence of 600-2000 times higher concentration of Zn (1.2-2.5 μg/mL) may have caused nearly total suppression of Cd accumulation. Decrease in Cd bioaccumulation in the presence of Zn has been reported for a number of marine organisms (Ray et al. 1979, 1980; Jackim et al. 1977).

The bioaccumulation pattern for the trace metals in Crangon and Nereis observed in the present study parallels those reported earlier (Ray et al. 1981) for Dalhousie sediment containing much lower concentrations of the metals. The concentrations (excepting Pb) in the overlying water and in the animals exposed to sediment containing 1% HZn closely resembled those in Dalhousie sediment.

b) Leaching

Preliminary studies on HZn and BZn were followed by a detailed investigation of HZn, using the ore alone, and mixed with St. Croix sediment to simulate field conditions.

Leeching of Cd and Zn from HZn by sea water (28.8 o/oo salinity) and brackish water (2.9 o/oo salinity) was studied by a batch technique. For the ore concentration alone, equilibrium was reached within 6 h; the amount of metal leached by brackish water was always higher than that leached by sea water through the 48-h period. Amounts of Cd and Zn leached from various ore-sediment combinations were always much lower ($p < 0.05$) than from the ore alone.

Amounts of Cd and Zn leached under anaerobic conditions were always lower than under aerobic conditions. Presence of organic matter in the sediment influenced leaching of both Cd and Zn. Use of H_2O_2-treated sediment instead of untreated sediment increased the amounts of Cd and Zn released by 1.1-4 and 1.2-2 times, respectively. The leaching of the metals in the presence of H_2O_2-treated sediments also varied with salinity, the largest increase being observed at lowest salinity.

To conclude, Cu, Zn, Cd and Pb are leached by sea water from the ore concentrates, but the degree of leaching is lowered by the presence of sediment, organic matter, and by increase in salinity. Pb and Zn were bioavailable under the test conditions. However, it would not be appropriate to extrapolate the results obtained in static laboratory tests to the dynamic situation prevailing in Dalhousie harbour.

TRACE METALS IN SCALLOPS

Scallops (Placopecten magellanicus) occur commonly along the eastern coast of North America and are an important commercial fishery in eastern Canada (Bourne 1964). They have been shown to rapidly accumulate high levels of several trace metals including cadmium (Eisler et al. 1972; Nelson et al. 1976) under laboratory conditions. Some organs, especially kidney and digestive gland, have been observed to contain very high concentrations of several metals, including Cd and Pb (Bryan 1973). Greig et al. (1978) determined trace metal contents (Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) in muscle, gonad, and viscera of scallops P. magellanicus from eastern U.S. coastal waters and reported low levels for all trace metals except for Cd (2.7-27 μg/g wet tissue) in the viscera. Recently, high Cd levels have been reported (Gould, pers. comm.) in the declining scallop population of Georges Bank, the source of over 90% of the Canadian scallop fishery. High Cd levels have also been reported in scallops from the Gulf of Maine. High Cd levels in tissues of P. magellanicus from Chaleur Bay in the vicinity of a lead smelter at Belledune have been reported (Ray et al. 1980). Levels of other trace metals were not determined. Scallops from within and around ocean disposal sites were also found to have elevated levels of several trace metals (Pesch et al. 1977).

Due to various reports of occurrence of high Cd levels in P. magellanicus, it was felt to be important to determine trace metal levels in scallops from commercial fishing areas and to establish a baseline level for Cu, Zn, Cd, and Pb.

Scallops were collected from 18 sites along the eastern Canadian seaboard (Chaleur Bay in the Gulf of St. Lawrence to Georges Bank) by staff of Invertebrates and Marine Plants Division. Eight of these sites were selected in Chaleur Bay because of known anthropogenic trace metal input in the area. The animals were collected at various times from May to December 1981. Five scallops of medium size (shell height 90-110 mm) were chosen for analysis.

The scallops were dissected into adductor muscle, mantle, gill, and viscera (or rest of soft tissues) for determination of Cu, Zn, Cd, and Pb in individual tissues. Mean concentrations, standard deviation, and range of Zn, Cu, Pb, and Cd in four tissues of the scallops from the study area are given in Table 2.

Wide variation in trace metal levels were observed in the same tissues of individual animals from different sites in the sampling area. This variation was observed even among individuals in a single population of scallops. However, individuals with high Cd or Pb levels in one tissue tended to have elevated levels of the metal in other tissues as well. Size and age of the animals from only 7 sites of the 12 for which both parameters are known were significantly related ($p < 0.05$). The trace metal concentrations in any particular tissue from individual sites were only occasionally related to either size or age.

Concentrations of the metals in scallop tissues increased in the order muscle < mantle < gill < viscera, with very few exceptions.

Levels of Cu and Zn in the animals' tissues were within the reported range for P. magellanicus and other species of scallops.

Pb concentrations in muscle tissues of animals from 14 out of 18 sites were <1.0 μg/g. The other 4 sites were all in the Bay of Chaleur sources of anthropogenic input. The mean concentrations of Cd in individual tissues were higher than reported for

Table 2. Concentration (mean, standard deviation, range) of trace metals in scallop tissues (μg/g dry) from the study area.

Tissue		Zn	Cu	Pb	Cd
Muscle	Mean	82	2.5	1.1	3.7
	S.D.	18	2.3	1.8	2.5
	Range	48-123	0.3-20.4	0.1-15.7	0.8-26
Mantle	Mean	86	3.1	1.8	8.6
	S.D.	20	2.5	1.8	6.8
	Range	7-150	0.3-15.5	0.2-14.0	0.7-47
Gill	Mean	131	6.9	4.0	23.3
	S.D.	73	4.5	4.1	20.1
	Range	15-574	2.4-48.7	0.5-34.8	2.9-188
Viscera	Mean	161	25.8	5.9	229
	S.D.	85	14.8	6.5	180
	Range	41-600	1.6-78.8	0.4-32.7	21-1780

various species of scallops. Greig et al. (1978) and Zook et al. (1976) reported Cd concentrations in muscle tissues in P. magellanicus in the range 0.06-0.15 and 0.04-0.2 μg/g (wet), respectively, compared to the range 0.8-26 μg/g (dry) in the present study. Mean (range) concentrations of Cd in muscle tissue of animals from Georges Bank and Brown's Bank were 5.0 (2.9-10.5) and 8.6 (5.4-15.4) μg/g compared with the value of 1.1 (0.8-1.6) μg/g in animals from near St. Andrews. Animals from the 4 sites which had higher levels of Pb had high concentrations of Cd as well.

Because of observed high levels of Cd in animals from Brown's and Georges Banks, more animals from the two sites were analyzed. The new data confirmed the results obtained earlier.

High levels of Cd in tissues of scallops from Brown's Bank, Georges Bank, and several other sites far removed from any known source of anthropogenic input are quite unexpected and cannot be explained in the absence of data for trace metals in water and sediment from these areas.

ACID RAIN STUDIES

Large areas in Nova Scotia and southern New Brunswick are underlain by hard granites and metamorphic rock where surface water pH's are significantly below 5.4. Run-off from acidic precipitation has lately added another dimension to the problem, since it may promote considerable mobilization of aluminum (Al) and trace metals like Cu, Zn, Cd, Pb, etc., from the soils in the drainage basin to the lakes and streams (Cronan and Schofield 1979; Hall and Likens 1981; Hermann and Baron 1980).

Al mobilized by acidic water has contributed to fish mortality (Baker and Schofield 1980; Driscoll et al. 1980; Muniz and Leivestad 1980) in several parts of the world and has also been reported in Nova Scotia (Watt, pers. comm.). Our research consisted of three related projects and is discussed below:

1. Development of an analytical technique for determination of Al.

A method for determination of Al, using graphite furnace atomic absorption spectrometry, has been developed and is currently being used in our studies. In the conventional flame technique Al cannot be measured under 5 μg/mL and the sensitivity is 1 μg/mL at 1% absorption. The new technique can determine Al to a level as low as 2 ng/mL without sample pre-concentration.

2. Effect of humic acid on toxicity of Al.

Laboratory studies are under way to determine toxicity of Al to juvenile Atlantic salmon at pH 5.0-7.0. The effect of the presence of 5 and 10 μg/mL humic acid is also being investigated. The results will indicate whether the presence of humic acid has any mitigating effect on the toxicity of Al.

Possible complexation of Al with humic acid in laboratory and field situations is also being investigated. At present, it is not known whether natural humates form complexes with Al as they do with other metals, like Cu and Zn, with consequent reduction of toxicity to fish.

Three macroreticular resins (XAD-2, -4 and -7) of varying polarity have been tested for recovery of Al and humic acid from water under laboratory conditions and have performed satisfactorily. Nearly 100% recovery of Al is obtained with HCl, NH_4OH or NaOH. Humic acid is best retained by XAD-2 and can be eluted from the resin with alcoholic NH_4OH.

Al mixed with humic acid loaded on XAD-2 and XAD-7 columns is separated into two fractions by an acid eluent followed by a base. The first fraction contains only Al while the second contains Al and humic acid, suggesting that a portion of Al may have formed a humate complex on mixing Al with humic acid. A number of humate samples have been collected from Nova Scotia lakes and the technique developed will be applied to determine whether Al is bound to humates under field conditions.

3. Metal profile in lake sediment core.

Four lakes in Nova Scotia were chosen for the

study. The Round and Tupper Lakes are in the headwaters of the Medway River system of the Westfield study area. The other two, Kinsac and Big Indian, are outside the study area and are within 50 km of Halifax. Tupper and Kinsac Lakes are buffered due to geochemistry of the area.

Sediment cores from the lakes were sectioned in 1.5-cm horizontal profiles and Cu, Zn, Cd, Pb, Al, Mn, Fe, and As were determined in all samples (Tupper Lake - 30, Big Indian Lake - 21, Round Lake - 23, and Kinsac Lake - 18). Carbon and nitrogen are also being determined at present. Sample preparation for Pb-210 dating have been completed and the samples have been sent for determination of sedimentation chronology. Data will be analyzed when all results are in and are expected to provide the history of trace metal mobilization due to acidification of the lakes.

DETERMINATION OF Cd IN BIOLOGICAL MATRIX

Effect of nitric acid on the determination of Cd by graphite furnace atomic absorption has been reported only casually. Most dry ashed biological samples are dissolved in concentrated nitric acid for the determination. The effect of 1-6% nitric acid on the determination of Cd in pyrolytically coated and uncoated tubes, in ramp and maximum power heating modes, was investigated with the N.B.S. reference material #1566 (oyster tissue). It was observed that more than 2% concentration of nitric acid in the digest severely depressed the absorption signal, giving as much as 20-30% lower values, depending on acid concentration.

REFERENCES

Baker, J.P., and C.L. Schofield. 1980. Aluminium toxicity to fish as related to acid precipitation and Adirondack surface water quality, p. 292-293. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway, Mar. 11-14, 1980.

Bryan, G.W. 1973. The occurrence and seasonal variation of trace metals in the scallops, Pecten maximus (L.) and Chlamys opercularis (L.). J. Mar. Biol. Assoc. U.K. 53: 145-166.

Bourne, N. 1964. Scallops and the offshore fishery of the Maritimes. Bull. Fish. Res. Board Can. 145: 60 p.

Cronan, C.S., and C.L. Schofield. 1979. Aluminium leaching response to acid precipitation: Effects on high-elevation watersheds in the northeast. Science 204: 304-306.

Driscoll C.T., Jr., J.P. Baker, J.J. Bisogni, Jr., and C.L. Schofield. 1980. Effect of aluminium speciation on fish in dilute acidified waters. Nature 284: 161-164.

Eisler, R., G.E. Zaroogian, and R.J. Hennekey. 1972. Cadmium uptake by marine organisms. J. Fish. Res. Board Can. 29: 1367-1369.

Greig, R.A., D.R. Wenzloff, C.L. MacKenzie, Jr., A.S. Merrill, and V.S. Zdanowicz. 1978. Trace metals in sea scallops, Placopecten magellanicus, from eastern United States. Bull. Environm. Contam. Toxicol. 19: 326-334.

Hall, R.J., and G.E. Likens. 1981. Chemical flux in an acid-stressed stream. Nature 292: 329-331.

Hermann, R., and J. Baron. 1980. Aluminium mobilization in acid stream environments, Great Smokey Mountains National Park, U.S.A. p. 218-219. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway, Mar. 11-14, 1980.

Hoff, J.T., J.A.J. Thompson, and C.S. Wong. 1982. Heavy metal release from mine tailings into seawater - A laboratory study. Mar. Poll. Bull. 13: 283-286.

Jackim, E., G. Morrison, and R. Steele. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. Mar. Biol. 40: 303-308.

Muniz, J.P., and H. Leivestad. 1980. Toxic effects of aluminium on the brown trout, Salmo trutta L., p. 320-321. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway, Mar. 11-14, 1980.

Nelson, D.A., A. Calabrese, B.A. Nelson, J.R. McInnes, and D.R. Wenzloff. 1976. Biological effects of heavy metals in juvenile bay scallops, Argopecten irradians, in short-term exposures. Bull. Environm. Contam. Toxicol. 16: 275-282.

Pesch, G., B. Reynolds, and P. Rogerson. 1977. Trace metals in scallops from within and around two ocean disposal sites. Mar. Pollut. Bull. 8: 224-228.

Ray, S., D.W. McLeese, and M.R. Peterson. 1981. Accumulation of copper, zinc, cadmium and lead from two contaminated sediments by three marine invertebrates - A laboratory study. Bull. Environm. Contam. Toxicol. 26: 315-322.

Ray, S., D.W. McLeese, and D. Pezzack. 1979. Chelation and interelemental effects on the bioaccumulation of heavy metals by marine invertebrates. Proc. Int. Conf. Manage. Control Heavy Met. Environm.: 35-38.

Ray, S., D.W. McLeese, B.A. Waiwood, and D. Pezzack. 1980. The disposition of cadmium and zinc in Pandalus montagui. Arch. Environm. Contam. Toxicol. 9: 675-681.

Ray, S., D.W. McLeese, C.D. Metcalfe, L.E. Burridge, and B.A. Waiwood. 1980. Distribution of cadmium in marine biota in the vicinity of Belledune, p. 11-34. In J.F. Uthe and V. Zitko (Eds.) Can. Tech. Rep. Fish. Aquat. Sci. 963.

Zook, E.G., J.J. Powell, B.M. Hackley, J.A. Emerson, J.R. Brooker, and G.M. Knobl, Jr. 1976. National Marine Fisheries Service preliminary survey of selected seafoods for mercury, lead, cadmium, chromium, and arsenic content. J. Agric. Food Chem. 24: 47-53.

TOXICOLOGY

D. W. McLEESE
L. E. Burridge

The project's overall long-term objective is the assessment of chemical hazards to fisheries. The specific objective is the determination of lethal and sublethal effects of chemicals on aquatic biota, mainly marine invertebrates. An additional objective is development of physiological criteria of sublethal stress (behavioral and feeding responses) in marine organisms.

Polycyclic aromatic hydrocarbons (PAH) are a complex mixture of compounds formed when carbonaceous fuels burn. Creosote, one of the fractions obtained from the distillation of coal tar, is used as a wood preservative. Some PAH, for example benzo(a)pyrene, are carcinogenic. The mixture of PAH present in molluscs, mussels, whelks, and periwinkles resembles that in creosote, indicating contamination originating from creosoted wooden structures such as wharves (Zitko 1975). Dunn and Fee (1979) reported that lobsters (Homarus americanus) held in a commercial tidal pound had high levels of PAH in hepatopancreas and lower levels in tail muscle. Confirming this observation, Uthe et al. (pers. comm.) measured uptake of 12 PAH by lobsters held in a tidal pound with creosoted materials for 3 mo during winter and for 3 mo during summer and also followed excretion of the PAH after lobsters were transferred to an uncontaminated area. PAH uptake was greater in summer than in winter and was greater in hepatopancreas than in tail muscle.

Three projects on PAH were conducted during 1982:

1. Experimental. The uptake and excretion of five PAH (phenanthrene, fluoranthene, pyrene, triphenylene, and perylene) from water and from a contaminated, natural sediment were investigated in four marine invertebrates (soft-shelled clams, mussels, polychaete worms Nereis, and shrimp Crangon) (McLeese 1982). In addition, lobsters were exposed to the five PAH in water. Analyses are completed except for the lobster samples.

Bioconcentration factors (BCF), representing equilibrium concentration factors, are derived from the uptake rate constant (K_1) divided by the excretion rate constant (K_2). Of the five PAH, triphenylene had the largest BCF for each of four invertebrates from exposure to the PAH in water and in the sediment. Phenanthrene had the smallest BCF with clams and mussels, and perylene had the smallest with worms and shrimp.

In terms of species, the BCF for the five PAH from water exposures decreased in the order of clams > mussels > worms > shrimp. From sediment exposure, the order was mussels > clams > worms > shrimp.

The maximum BCF was about 60,000 for triphenylene in clams exposed to PAH in water, and the minimum BCF was about 180 for perylene in shrimp (hepatopancreas) exposed to PAH in sediment.

The large BCF in triphenylene in the four species results from large K_1 and relatively small K_2. The small BCF's for phenanthrene (in mussels and clams) result from moderate K_1 and relatively large K_2. In contrast, the small BCF's for perylene result from low K_1.

Particularly with worms, K_1's for the PAH were larger in sediment than in water exposures. However, the K_2's were larger in the sediment exposures. Consequently, the resultant BCF's for PAH in worms with water and sediment exposure were similar in magnitude.

The maximum K_1 value was about 59 $\mu g/g/h$ for triphenylene by worms in sediment, and the minimum K_1 was about 1 $\mu g/g/h$ for perylene by worms in water and shrimp in sediment exposures.

The maximum K_2 value was about 0.025 $\mu g/g/h$ for fluoranthene and for pyrene by shrimp in water. The minimum K_2 was about 0.0006 $\mu g/g/h$ for triphenylene by clams in the water exposure test.

2. Questionnaires. To obtain information on the potential for lobsters to be exposed to creosote (PAH) during commercial storage, questionnaires were distributed to Fishery Offices throughout the Atlantic Provinces. The questionnaire related to each of the four types of storage units. Replies were received for 69 crate storage units, 74 car units, 110 tank units, and 21 tidal pounds (total 274) in New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland, and Quebec.

Annual landings of market-sized lobsters, i.e. those sold alive and weighing about 1 lb or more (2.2 lb = 1 kg), amount to about 22.5 million lb. Of this total, about 16.7 million lb (74%) are landed in spring and early summer (April-July), 1.4 million lb (6%) in August to October and 4.3 million lb (20%) in November and December.

The data from the questionnaires have not been analyzed fully in relation to suitable weighting for size of units and for season and area of operation. However, preliminary assessment permits the following indications:

Creosoted wood is not used in crate, car or tank units, and apparently little or none was used in 17 or 19 of 21 tidal pounds. Consequently, the major possibility for exposure of lobsters to PAH is from the water if a creosoted source such as a wharf is nearby. A preliminary summary of the information is presented in Table 1.

The preliminary estimates indicate that the total weight of live lobsters that may be exposed to creosote (PAH) annually amounts to about 14.2 million lb in crates, 7.2 million lb in cars, 2.6 million lb in tanks, and about 1.2 million lb in tidal pounds.

3. Sampling lobsters from commercial storage units. During winter 1982, samples of lobsters were obtained from six tidal pounds in Charlotte County, N. B. The lobsters had been stored about 1.5 mo in one pound and about 3 mo in each of the others. In spring 1982, samples of lobsters were obtained from crate, car, and tank storage units (about 1 wk storage) from the Bay of Fundy, a high tidal amplitude area. In addition, J. Uthe obtained samples from similar units from an area with low-to-moderate tidal amplitude (S.W. Nova Scotia). To date, the samples (lobster hepatopancreas and tail muscle) from four of the tidal pounds have been analyzed for 12 PAH. Two PAH, fluoranthene, and the carcinogenic PAH, benzo(a)pyrene, are used as examples to illustrate the results.

According to previous surveys and to the questionnaires, the potential for exposure to

Table 1. Number and capacity of lobster storage units located near sources of creosote (summary from questionnaires).

Storage Type	unit No.	Estimated units near creosote sources No.	%	Estimated annual capacity (million lb)	Estimated poundage exposed to creosote (million lb)	(%)	Usual storage periods
Crates	69	44	63	22.5	14.2	63	<3 d
Cars	74	67	90	8	7.2	32	<1 wk
Tanks	110	40	36	7.4	2.6	12	<1 wk
Tidal pounds	21	5	24	5	1.2	5	2-3 mo

creosote in these pounds was relatively small. However, the samples from three of the four pounds had fluoranthene values in hepatopancreas that exceeded the maximum of the range of control values based on freshly caught lobsters from 18 areas (mean 0.17, range ND-0.47 µg/g wet wt) by 3-6 times. Fluoranthene in tail muscle exceeded the maximum of the range of control values by 1.3-1.5 times (control value mean 0.016, range 0.001-0.075).

In samples from the same three pounds, benzo(a)pyrene values in hepatopancreas and tail muscle exceeded the mean control values but not the ranges (hepatopancreas mean B(a)P0.005, range 0.0001-0.03, tail muscle mean B(a)P0.0006, range ND to 0.003 µg/g wet wt). The indication is for some accumulation of benzo(a)pyrene in lobsters from three of the four pounds but not enough to exceed control values.

The results from the questionnaires (2) and field sampling (3) will provide a basis for assessing the need for further sampling or possibly for the need for a program to monitor PAH levels in commercially held lobsters.

BIVALVE FILTRATION RATES

The accumulation of environmental contaminants by bivalve molluscs, such as mussels and clams, can be from water or from food, either singly or in combination. Contaminants in either water or food could result in variations in filtration rate and the feeding rate which are related because food particles (algal cells) are filtered from the water. The objective is to develop measures of filtration or feeding rates of bivalves for use as indicators of sublethal stress, particularly in relation to bioaccumulation of contaminants.

Observations on filtration rates to obtain control values were done with five groups of similar sized mussels. Algal clearance rates (feeding rates) were measured with the mussels exposed to a concentration of algae ranging from 1.9×10^3 to 16×10^3 cell mL^{-1}. The alga was Dunaliella. Clearance rates varied and resulted in calculated feeding rates ranging from about 15×10^4 to 810×10^4 cells/h/animal. Feeding rate did not show a consistent trend in relation to food concentration. However, when the same groups of mussels were tested on consecutive days, there was a trend for decreased feeding rate with time. Other work indicates that filtration rate of mussels is independent of cell concentration over the range 1.5 to 30×10^3 cells mL^{-1} (Riisgard and Randløv 1981). Accordingly, and unlike our results, feeding rate should increase with increasing cell concentration because feeding rate = filtration (clearance) rate x concentration of cells. More recently, Poulsen et al. (1982) found that the clearance rate of algal cells (hence feeding rate) by mussels did not change with Cd in the water at 0, 10, and 100 ppb.

OCEAN DUMPING

1. Assessment of Saint John Harbour dump site. Marine Research Associates were contracted to investigate the effects of contaminants in dredged sediments from Saint John Harbour on biota in and around the dump site off Black Point. The main objectives were to determine (a) if the benthic biota at the dump site differed appreciably from that in similar nearby areas, and (b) if contaminant loads (heavy metals, PCB, PAH) in the biota differed appreciably from levels in organisms from the control areas. Two sampling sites were chosen on the dump site and two control sites were selected, the first east-northeast, and the second north of the dump site. Benthic organisms were relatively scarce at dump site locations and at one control station. Biomasses of organisms recovered from samples were too small to permit chemical measurement of the contaminants. In the sediments, concentrations of heavy metals, PCB and PAH tended to be higher at dump site than at control stations but the levels ranged from below to only marginally above ODCA-regulated "safe" concentrations.

   The scarcity of organisms was related to the current-scoured substrate rather than to accumulation of contaminants (Yurick 1982).

2. Significance of contaminant load to organisms. M. R. Peterson was contracted to study the significance of contaminant levels within organisms primarily in terms of sublethal effects. The experimental plan is to use one contaminant (dimethyl naphthalene), initially. Several types of marine invertebrates (clams, mussels, worms, shrimp) are to be exposed to levels of the contaminant, ranging from high sublethal to the lethal range, and to measure its accumulation (uptake, excretion, bioaccumulation) within the organism. The project has been started recently and results are not available yet.

REFERENCES

Dunn, B. P., and J. Fee. 1979. Polycyclic aromatic hydrocarbon carcinogens in commercial sea foods. J. Fish. Res. Board Can. 36: 1469-1476.

McLeese, D. W. 1982. Accumulation of polynuclear aromatic hydrocarbons (PAH) from water and sediment by marine invertebrates. In Report on Ocean Dumping R & D Pacific Region (S. C. Byers and G. S. Calderwood, eds.). Department of Fisheries and Oceans 1981-82. Can. Contract Rep. Hydrogr. Ocean Sci. 3: 31 p.

Poulsen, E., H. U. Riisgard and F. Møhlenberg. 1982. Accumulation of cadmium and bioenergetics in the mussel Mytilus edulis. Mar. Biol. 68: 25-29.

Riisgard, H. U., and A. Randløv. 1981. Energy budgets, growth and filtration rates in Mytilus edulis at different algal concentrations. Mar. Biol. 61: 227-234.

Yurick, D. B. 1982. An assessment of the Saint John Harbour dump site. Contractor Report, 24 p.

Zitko, V. 1975. Aromatic hydrocarbons in aquatic fauna. Bull. Environm. Contam. Toxicol. 14: 621-631.

BIOCHEMICAL TOXICOLOGY

K. HAYA
B. Waiwood

Sublethal effects caused by xenobiotics in aquatic organisms can result in decreased reproductive efficiency, increased susceptibility to predators or diseases or behavioral changes, but are difficult to detect or quantitate. Biochemical responses to sublethal levels of pollutants may also be indicative of adverse living conditions. A knowledge of the biochemical responses by aquatic organisms to sublethal concentrations of pollutants is useful in understanding mechanisms of toxic action. These effects may be used as biochemical indicators in:

1) Assessing the overall health of fish communities and populations;

2) Monitoring, as an early warning signal of potential pollution problems;

3) Serving as a tool in the development and assessment of specific water quality criteria;

4) Assessing the hazard potential of xenobiotics.

The objective of the Biochemical Toxicology Program is the development of biochemical parameters as indicators of sublethal effects caused by pollutants. The biochemical parameters investigated as potential indicators of sublethal effects were adenosine triphosphatase (ATPase) activity, chorionase activity and the adenylate energy charge (AEC). The ATPases, especially Na,K-activated-ATPase, are the prime mediators of ion transport across membranes and play a key role in whole body osmoregulation in aquatic animals (Towle 1981). Chorionase is a proteolytic enzyme which dissolves the inner layer of the chorion during the hatching of Atlantic salmon, Salmo salar, eggs (Hagenmaier 1974). AEC is a measure of the metabolic energy state of cells and is a major factor in controlling catabolic and anabolic processes (Atkinson 1977). AEC values may be indicative of the physiological status of animals. A review of recent studies indicated that AEC and ATPase activities are useful toxicological tools for basic research, such as in the determination of mechanisms of toxicant action, intricacies of energy metabolism and osmoregulation. As more baseline information is obtained, these parameters can possibly be developed as indicators of sublethal effects of chemicals in aquatic animals (Haya and Waiwood 1983).

Current studies of this program will be discussed under the following headings:

1) Toxicity of organochlorines and the metabolic state of polychaetes;

2) AEC of field samples of aquatic organisms;

3) Acid rain related studies;

4) Copper toxicity and the molt stage of lobsters.

TOXICITY OF ORGANOCHLORINES AND THE METABOLIC STATE OF POLYCHAETES

In previous studies organochlorine pesticides were found to be 500 times more toxic to shrimp, Crangon septemspinosa, than to the polychaete, Nereis virens, (McLeese et al. 1982). Several of the possible explanations are that:

1) there are differences in intermediary metabolic processes between the two species;

2) there are differences in uptake and excretion of organochlorines by the two animals;

3) Nereis is capable of metabolizing the organochlorines by mixed function oxidases more efficiently.

An interesting capability of polychaetes is their ability to survive extended periods of anoxia (at least 10 d) by utilizing anaerobic pathways of energy metabolism. The ability to switch to anaerobic metabolism may serve as a protective mechanism against chemicals in the water. To test this hypothesis polychaetes were exposed to endosulfan (11 ppm nominal) under anoxic or normoxic static conditions. The worms were exposed for 96 h, then transferred to unspiked water for 96 h and were sampled periodically. Since AEC decreases in polychaetes during anaerobiosis (Schottler 1979), AEC was determined for whole body perchloric acid extracts to ascertain the effect of anoxia as well as endosulfan on the metabolic energy state of the polychaetes.

Under our experimental conditions, anoxia did not cause a significant reduction in AEC (normoxic controls vs anoxic controls). However, a significant decrease in AEC was observed in the polychaetes after exposure to endosulfan for 72 h and 96 h under anoxic and normoxic conditions,

respectively, compared to zero hour values ($p < 0.05$, ANOVA between treatments, Table 1). AEC of the normoxic endosulfan exposed group was lower than both control groups and AEC of the anoxic endosulfan exposed group was less than the normoxic endosulfan exposed group. After 96 h ATP levels of the endosulfan exposed groups were significantly lower than the controls. ADP and AMP levels were higher in the endosulfan treated groups than in the control groups. There was no significant difference in total adenylate concentrations among all treatments. Thus, changes in AEC resulted from a change in the relative levels of the adenine nucleotides rather than a change in the size of the adenine nucleotide pool. All the parameters measured except AEC of the anoxic endosulfan treated group did not differ significantly from controls 24 h after transfer to unspiked sea water. AEC's of the anoxic endosulfan exposed group were not significantly different from controls after 48 h of depuration.

These results indicated that anoxia alone did not appear to affect metabolic energy state of Nereis virens under the conditions of this experiment. However, a sublethal level of endosulfan decreased AEC in Nereis virens and the decrease was greater under anoxic conditions. The values of AEC decreased below that indicative of optimal physiological status for microorganisms (below 0.8) in the endosulfan treated groups. Thus, endosulfan probably interferes with some energy producing metabolic pathway which appears to be more susceptible during anoxia. Some other factor besides endosulfan may have caused the decrease in AEC as excretion of endosulfan is expected to take much longer than the time it took for AEC to return to control values.

To determine the effect of anoxia on the uptake and excretion of organochlorines by polychaetes, Nereis virens were exposed to 14C-DDT for 96 h under anoxic and normoxic conditions. The polychaetes were then transferred to unspiked sea water and depuration of 14C-DDT was followed for 96 h. The average measured concentration of 14C-DDT in the sea water of the normoxic group was 2.66 and 4.93 ppb during 0-48 and 48-96 h of the exposure phase, respectively. The sea water of the anoxic group had an average measured concentration of 14C-DDT of 3.47 and 3.19 ppb during the same periods. Polychaetes were sampled periodically and the radioactivity of whole body homogenates was determined by liquid scintillation techniques.

After 96 h of exposure the mean level of radioactivity in the "normoxic" polychaetes was 15,115 dpm and in the "anoxic" polychaetes was 28,272 dpm. After 96 h of depuration the level of radioactivity was 16,175 and 16,355 dpm in the normoxic and anoxic groups, respectively. An ANOVA within each group by time indicated that no significant excretion of radioactivity occurred in the normoxic group. In the "anoxic" polychaetes, after an initial rapid excretion of radioactivity, the rate of excretion decreased when the level of radioactivity approached that found in the normoxic group. Preliminary results indicate that similar differences in uptake and excretion of radioactivity are found if the levels are calculated on a μg DDT/g lipid.

Since the level of radioactivity in the polychaetes exposed to 14C-DDT and anoxia was higher than those exposed under normoxic conditions, it appears that under anoxic conditions polychaetes absorbed more 14C-DDT. Thus, switching to anaerobiosis does not provide tolerance to DDT by decreasing the uptake of DDT. Also, there does not seem to be any large increase in the rate of excretion of DDT under anoxic conditions, and the mechanism of tolerance to organochlorine pesticides by polychaetes remains to be determined.

MONITORING OF AEC

Variations in biochemical and physiological parameters with season, environmental factors such as temperature and salinity, water quality, physiological status (age, reproductive state, sex), and between different species occur frequently. Before any potential index of sublethal effects caused by chemicals can be used to assess the condition of field samples, the fluctuations in the index caused by these factors must be determined. To obtain information on possible variations in AEC, samples of sea raven, flounder, clams and mussels were obtained and the AEC and creatine phosphate in the fish or arginine phosphate in the invertebrates were determined. Freshly caught animals were sampled monthly and the biochemical parameters were measured in extracts of the gills, muscle and liver of the fish and whole body homogenates of the invertebrates on a bimonthly basis.

The AEC or the levels of adenine nucleotides remained stable throughout the period (Oct. 1981-June 1982). Variations were observed in the

Table 1. Adenine nucleotides (μmol/g wet wt) and AEC of Nereis virens after 96 h of exposure to endosulfan under anoxic and normoxic conditions (values are the mean of n individuals).

	Controls		Endosulfan treated	
	Normoxic	Anoxic	Normoxic	Anoxic
n	6	6	4	6
ATP	1.816	1.786	1.478[a]	1.238[a]
ADP	0.390	0.348	0.503[a]	0.622[a]
AMP	0.105	0.106	0.189[a]	0.241[a]
ATP + ADP + AMP	2.311	2.239	2.171	2.101
AEC	0.870	0.875	0.795[a]	0.737[a]

[a] $p < 0.05$, ANOVA

creatine phosphate levels in all three tissues; however, there was no consistent or regular pattern with time.

Variations in AEC and creatine phosphate were observed in all three flounder tissues (Table 2). Generally AEC was lowest in the summer in gills and liver. AEC values increased and peaked during January-March, then decreased until July. The same pattern is observed for creatine phosphate levels in the liver, but in the gills peak levels appear during April-May. Although an ANOVA indicated significant differences with time for AEC and creatine phosphate levels in muscle, there does not seem to be a distinct pattern and the AEC values appear to be stable. Correlation of the observed variations with physiological status such as feeding habits and reproductive state or environmental factors is still to be determined.

Clams from three different areas in Passamaquoddy Bay, N.B., and mussels from one of these areas were collected during March 1981-March 1982. AEC and arginine phosphate levels varied inconsistently between the clams from the different areas. Clams from all three areas showed variation in AEC and arginine phosphate with time. There was no pattern to the arginine phosphate variation, but AEC values were lowest in August and highest in February. The AEC values ranged from 0.577-0.796, 0.642-0.760 and 0.566-0.755 in clams from Pottery Creek, Bar Road and Chamcook Wharf, respectively. AEC's of mussels were lowest in May (0.530) and highest also in February (0.648), but there was no discernible pattern in AEC or arginine phosphate levels in mussels.

Completion of analyses of the biochemical parameters for the remaining samples in this project may help in identifying trends in the observed variations with season and other environmental factors.

ACID RAIN STUDIES

In many watersheds, low pH associated with acid precipitation has resulted in the depletion of many aquatic species that inhabited these waters. Mortality or delayed hatching occurs during incubation of salmon eggs at low pH. A proteolytic enzyme, chorionase, dissolves the inner layer of the chorion during the initial phase of the hatching process. Our previous studies demonstrated a 52% decrease in chorionase activity in eggs kept at pH 4.5 for varying time periods compared to those kept at pH 6.5 (Haya and Waiwood 1981).

In a followup study, salmon eggs were held at pH 4.5 and pH 6.5 from fertilization and the chorionase activity determined periodically. The data were similar to the previous years' studies in that salmon eggs held at pH 4.5 had lower chorionase activity (154 proteolytic units/egg, n=47) than that of eggs held at pH 6.5 (425 proteolytic units/egg, n=47) (Waiwood and Haya 1982). Although there was a large scatter in the data, there was a trend which indicated that the amount of chorionase was less and that development of chorionase occurred at a slower rate in those eggs raised at pH 4.5 than at pH 6.5.

Currently, a study is in progress to determine if there is an effect on energy metabolism that can be related to decreased chorionase development or delayed hatching. The experimental setup was the same as previous experiments and alevins were sampled periodically from hatching through to swim-up stage. Sensitive methods are being developed for analysis of the low level of adenine nucleotides in alevins. The cumulative mortalities of eggs and alevins were higher among those raised at pH 4.5 than at 6.5 (68% and 10%, respectively). The greatest number of deaths occurred during the early part of the exposure and was probably due to the inability of the eggs to adapt to pH 4.5; however, there was no difference in the degree days of incubation for the first successful hatching to occur in both pH regimes.

Low pH interferes with the smoltification of salmon parr (Saunders et al. 1982). An experiment to determine the effects of acid pH on energy metabolism during the parr-smolt transformation process was initiated. Salmon parr were held at pH 4.5 and 6.5 from February until the smoltification process was completed by the controls (pH 6.5). Muscle, gills and liver were obtained weekly from samples of salmon which were anaesthetized prior to dissection. AEC, creatine phosphate, glycogen and

Table 2. AEC and creatine phosphate levels (μmol/g wet wt) in liver, muscle and gill of flounder during July 1981-July 1982 (values represent the mean of six flounders). CP = creatine phosphate; ANOVA ($p < 0.05$) indicated significant variation in all cases by time.

Month	Liver		Muscle		Gill	
	AEC	CP	AEC	CP	AEC	CP
July (81)	0.470	0.518	0.927	11.315	0.733	0.351
Sept (81)	0.643	0.231	0.931	9.921	0.747	0.356
Nov (81)	0.657	0.330	0.930	13.105	0.793	0.383
Jan (82)	0.826	1.472	0.928	16.366	0.823	0.342
Mar (82)	0.777	2.408	0.908	10.602	0.824	0.404
Apr (82)	0.681	1.218	0.914	10.459	0.756	0.634
May (82)	0.671	1.320	0.905	15.352	0.741	0.581
July (82)	0.595	0.311	0.935	12.014	0.691	0.314

glucose are being determined in the liver and muscle samples by spectrophotometric enzymatic methods; however, analysis of these parameters in the gills will require more sensitive techniques.

Preliminary analysis of the data (2-way ANOVA by time and treatment, $p < 0.05$) indicates that parr held in pH 6.5 water increased in length and weight but parr held in pH 4.5 water did not. Significant variations are indicated for glucose levels and AEC of liver between the treatments (Table 3). The variations in AEC occur randomly and do not appear to have any biochemical significance. The glucose levels were higher in livers of the pH 4.5 group than in the pH 6.5 group after 7 d and remained higher throughout the experiment. There were no significant differences in the glycogen or creatine phosphate content of livers between the two treatment groups. The creatine phosphate level did not change significantly with time; however, glycogen levels varied randomly with time.

Analyses of muscle tissue for the biochemical parameters are complete for nine of the sampling periods. There is no significant difference between the AEC of the two treatment groups, although total adenylate levels appear to be less in the pH 4.5 group. The variations in the creatine phosphate levels were not consistent with time. The glucose levels were higher in the muscles of the pH 4.5 group than in the pH 6.5 group from the third day of exposure and remained slightly elevated. The glycogen content of muscles in the pH 4.5 group was less than in the pH 6.5 group from the third day of exposure to the last sampling period.

These studies indicate that, among the parameters measured, the main effects of exposure to low pH of salmon parr during the parr-smolt transformation are on growth and the utilization and storage of glucose and glycogen. The effects on glucose and glycogen stores occur readily and no compensatory mechanism appears to develop.

COPPER TOXICITY AND THE MOLT STAGE OF LOBSTER

In crustaceans, biochemical fluctuations occur during the molt cycle. There are accumulations of organic and inorganic reserves which are stored in the hepatopancreas during intermolt (Aiken 1980). As crustaceans enter premolt, serum protein and ionic content increase and with ecdysis changes in water content affect osmolality, ionic content, hemolymph proteins and pH. These all pose potential problems for osmoregulatory processes.

The purpose of this collaborative study with the Invertebrate Physiology Section was to determine if any changes in ATPase activity in gills and hepatopancreas and of AEC in tail muscle of juvenile lobsters occur during several molt stages. A similar experiment was conducted after 96 h of exposure to sublethal concentrations of copper. There were no differences in Na,K-ATPase activity of the gills or hepatopancreas or AEC of tail muscle associated with molt stage. ATP and arginine phosphate levels were higher in the tail muscle during the immediate premolt and postmolt stages, respectively. Exposure of juvenile lobsters to copper (24 ppb, measured) did not affect ATPase activity of gills or hepatopancreas or AEC of tail muscle. All lobsters attempting to molt (6) in the Cu treated group died during the first stage of molt while all control lobsters that underwent molting (2) were successful. Although the data are limited, it appears as if juvenile lobsters are most sensitive to copper during the initiation of molting.

REFERENCES

Aiken, D. E. 1980. Molting and growth, p. 91-163. In B. F. Phillips and T. S. Cobb (eds.) The Biology and Management of Lobsters, Vol. 1. Academic Press, N.Y.

Atkinson, D. E. 1977. Cellular energy metabolism and its regulation. Academic Press, N.Y., p. 85-107.

Hagenmaier, H. E. 1974. The hatching process in fish embryos. IV. The enzymological properties of a highly purified enzyme (chorionase) from the rainbow trout, Salmo gairdneri Rich. Comp. Biochem. Physiol. 49B: 313-324.

Haya, K., and B. A. Waiwood. 1981. Acid pH and chorionase activity of Atlantic salmon (Salmo salar) eggs. Bull. Environm. Contam. Toxicol. 27: 7-12.

Table 3. Total means (µmol/g wet wt) of biochemical parameters measured during the smoltification of salmon parr at pH 4.5 and pH 6.5.

Biochemical parameter	Liver		Muscle	
	pH 4.5	pH 6.5	pH 4.5	pH 6.5
ATP + ADP + AMP	2.54[a]	2.16	6.78[a]	7.62
AEC (unitless)	0.73[a]	0.70	0.92	0.94
Creatine phosphate	0.26	0.24	6.97[a]	8.81
Glycogen	156.9	158.5	14.2[a]	23.2
Glucose	14.69[a]	7.09	1.54[a]	1.02

[a]Significant difference between treatments, $p < 0.05$, ANOVA.

Haya, K., and B. A. Waiwood. 1983. Adenylate energy charge and ATPase activity: potential biochemical indicators of sublethal effects caused by pollutants in aquatic animals. In J. O. Nriagu (ed.) Aquatic Toxicology. John Wiley and Sons, Inc., N.Y. (in press).

McLeese, D. W., L. E. Burridge, and J. Van Dinter. 1982. Toxicities of five organochlorine compounds in water and sediment to Nereis virens. Bull. Environm. Contam. Toxicol. 28: 216-220.

Saunders, R. L., E. B. Henderson, and P. R. Harmon. 1982. Influence of low environmental pH on smolting of Atlantic salmon. Proc. of the Workshop on Acid Rain held at St. Andrews, N.B., February 1982.

Schottler, U. 1979. On the anaerobic metabolism of three species of Nereis (Annelida). Mar. Ecol. Prog. Ser. 1: 249-254.

Towle, D. W. 1981. Role of Na^+,K^+-ATPase in ionic regulation by marine and estuarine animals. Mar. Biol. Lett. 2: 107-122.

Waiwood, B. A., and K. Haya. 1982. Development of chorionase activity at low pH. Proc. of the Workshop on Acid Rain held at St. Andrews, N.B., February 1982.

ORGANIC ENVIRONMENTAL CHEMISTRY

V. ZITKO
M. Babineau
H. Akagi

The objective of the project is to identify and measure assorted organic chemicals and their transformation products in aquatic biota, sediments, and water. Particular emphasis is on persistent and frequently very toxic organochlorine compounds, but many other chemicals and classes of chemicals are investigated as well. The main technique used is mass spectrometry. Since this technique can provide very large amounts of data from a sample, attention is also given to methods for data processing and evaluation. The number of chemicals entering or potentially entering the aquatic environment is very large; consequently, this project is also concerned with the development of predictive relationships between structure and environmental properties of chemicals.

MASS SPECTROMETRY

The Finnigan Model 4500 mass spectrometer has been brought into routine operation. Its capability was expanded by inclusion of the 'PPINICI' (pulsed positive ion/negative ion chemical ionization) accessory, capillary column injector, a second interface for data acquisition by INCOS from the old Model 1015 mass spectrometer, and a modem. Some of these additions required changes in the operating software. Quite a few of the original programs have been replaced by NRC Halifax and our own modifications. Consequently, implementing new software could not be accomplished simply by copying Finnigan 'master disks', but required fairly complicated file-by-file transfers.

For better appreciation of its capabilities, the following is a brief description of the INCOS software:

The operating system is IDOS (INCOS disk operating system). IDOS is almost completely invisible to the routine user who only encounters another system, MSDS (mass spectrometer data system). MSDS runs under IDOS control and consists of numerous individual programs for calibration, scan control (mass range and scan rate), data acquisition, and manipulation. The data can be acquired in continuous m/z ranges or under MID (multiple ion detection) conditions, when the spectrometer can scan up to 25 mass ranges or masses at individual scan rates. This is important for the determination of specific compounds in trace concentrations since the sensitivity increases with decreasing scan rate (more ions have a chance to reach the detector). Data manipulation includes the conventional gas chromatography-resembling format displaying the RIC (reconstructed ion chromatogram) graph and plots several selected masses (optional), a projection of the three-dimensional mass/intensity/scan matrix, a display of individual mass spectra, etc. The system also carries a library of mass spectra, currently containing some 31,000 entries. The search program provided is based on a very fast algorithm, but it is rather inconvenient for just retrieving spectra of individual compounds. The commercial 'MSSS', a component of the chemical information system (CIS) is far superior and continues to be used for this purpose.

MSDS maintains several auxiliary 'LISTS' such as the scan list with the numbers of scans of interest, usually RIC peaks, the name list containing the names of files to be processed, the library list with the library entries of interest from the 'MASTER' library or from a user-assembled library, and a quantitation list for automatic quantitation.

In addition, MSDS keeps several user-defined integer variables and performs addition, subtraction and some logical operations on these and on other variables determined by the status of the system. The MSDS lists and variables are important in the third programming level, the 'PROCEDURES'. These are user-defined strings of MSDS and IDOS commands which perform specific tasks. For example, a procedure may find RIC peaks in a file, print their spectra, perform library search for each peak, print the results, and play a song on completion. Parameters may be written permanently into procedures, or different sets of parameters may be called from 'METHODS', strings of commands similar to 'PROCEDURES'. IDOS contains a variety of utility programs, an Editor, a FORTRAN Compiler, an Assembler, and a Relocatable Loader. On the whole, the data system is operated by an excellent and extremely efficient, although quite poorly documented, software package. The price to pay is the effort needed to learn to manipulate the system effectively.

So far the system has been used to acquire electron impact spectra of hydrocarbon standards including a PAH standard from NBS, Mirex, PCB (Aroclor 1254), chlordane, A-, B-, and G-BHC, and chemical ionization spectra (positive and negative ions) of toxaphene. In all cases the chromatography was performed on a 50-M fused silica capillary column. The hydrocarbon standards established a relationship between compounds and their retention times.

PCB, chlordane, and toxaphene were assembled into respective libraries and will be used in

computer search for these in 'real world' samples. Samples of a highly PAH-contaminated sediment, Nereis, Crangon, and mussels exposed to the sediment in the laboratory have been analyzed. The data are being compared to those obtained on a packed column by the 1015D mass spectrometer.

In chemical ionization (methane), the system has very good sensitivity for toxaphene, particularly in the negative ions mode, since these are about 100 times more abundant than positive ions. The fragmentation patterns are characterized by both positive and negative (M-Cl) and negative (M+1) and (M) ions. The (M) ions are particularly abundant for polychlorobornenes as opposed to polychlorobornanes, presumably because of the stabilization of the negative molecular ions by the double bond. Even the capillary column does not fully resolve all toxaphene components. This is indicated by the Cl isotope distribution patterns which result, in many cases, from the superposition of several clusters. A complete elucidation of the spectra will require considerable time since a sample of toxaphene yields about 80 good quality negative ions spectra and approximately 40 positive ions spectra, talking in terms of 'major' components.

The presence of mirex was confirmed in a sample of eel from the St. Lawrence River (request by Fish Inspection, Quebec Region). The sample also contains additional organochlorine compounds, mostly the routine contaminants, but a relatively high level of octachlorostyrene is notable.

A-BHC and PAH were confirmed in several samples for Environmental Management Laboratories in Moncton. Benzene sulfonamide was confirmed in the 'cod capsule' (see below).

A factor analysis program was written for the Model 6100 data system and its performance is being tested. The program will determine the number of components in a mixture (for example the number of components in an 'RIC' peak) and will attempt to extract mass spectra of pure components. The limited workspace on the 6100 data system required careful programming and led to the development of an algorithm for storing symmetric matrices in linear arrays (Zitko 1983a).

Summary formulae of ions are determined on the INCOS by a program based on accurate masses (COMP). The current version of the program (NRC Halifax modification) is very good. Unfortunately, accurate mass determination (high resolution type, several decimal places) is essential for its good performance. In most instances our masses are not that accurate. Consequently, we continue to rely more on the isotope distribution and use ELANAL, described previously and implemented originally on the Tektronix 4051. To make ELANAL's use more convenient, the program was translated into HP3000 BASIC. It was also split into ELANAL proper, an input program, editor, and a print program. Ions of interest can now be entered into an input file. Since ELANAL proper does not require any terminal input, it can be run off-hours (it may take a very long time). The results may be edited manually or according to various user-selected criteria such as limits on rings and double bonds, number of substituents, etc., and only the final edited version may be printed.

Thanks to our 'in-house' expertise, the operation of the 6100 data system is relatively continuous, but problems have been encountered with the ion gauge control and scan module of the 1015D mass spectrometer, causing prolonged downtimes.

The Tektronix 4051 computer can now be used as a terminal to acquire and process data from the 1015D mass spectrometer via the new interface, but only at 2400 baud (the normal operating rate is 9600 baud). For most purposes the slower rate causes no inconvenience, but graphics programs, particularly 'MAP' take a much longer time. By 'flip of a switch' the 4051 can also be used to communicate with the HP3000. A link between INCOS and HP3000 is possible via modem and the 'phone' program. Unfortunately, the program assumes another INCOS at the other end and we have not been able to transfer files so far.

STRUCTURE-ACTIVITY STUDIES

In evaluation of the accumulation of contaminants, a second compartment may have to be added to the usual one-compartment model. The need for the expansion becomes obvious when the excretion data cannot be described satisfactorily by a single exponential term.

Fitting the two-compartment model to experimental points is considerably more difficult and requires very good quality data. A set of three programs has been written for this purpose. One program calculates concentrations (amounts) in the compartments as functions of time and user-specified uptake and excretion rate constants. This program helps to give a 'feeling' for the effect of rate constant values on the time profile of concentrations (amounts) in the compartments. The main program finds best fitting values of the rate constants for user-provided data. The standard simplex algorithm is used in the search. The algorithm is quite insensitive to initial values but is by no means fail-safe and may not converge from a wrong starting point. To facilitate the reasonable choice of starting parameters and to emphasize potential problems with the search, a program plotting a projection of the sum of squared deviations (SUSQ) surface was written as well (Zitko 1982a).

Lethality curves (graphs of time to 50% mortality vs toxicant concentration) are used to summarize the results of acute toxicity tests. Lethality curves characterize the response of the test animals to the toxicant and are extrapolated frequently to find the lethal threshold (incipient lethal level). A program to express lethality curves in terms of three parameters and to determine the lethal threshold has been written and an earlier 'programmable calculator version' has been documented to facilitate its portability (Zitko 1982b).

Prediction of concentration of organic chemicals in various environmental compartments aids preliminary hazard estimation and risk assessment. This approach has been used, for example, to rank the hazard of several pesticides in forest spraying against spruce budworm (Zitko and McLeese 1980). At the initiative of NIWR (National Institute for Water Research, Burlington, Ontario), a joint project dealing with the evaluation of three models of environmental distribution and behavior of organic chemicals has been started. In the first phase, two of the

models are being implemented on the HP3000 by the System Manager (S. Bellis). First of these, the 'NRC' model has been translated by Mr. Bellis and is being tested and, hopefully, improved. The second one, the U.S. EPA 'EXAMS' presents a problem by its size and will require considerably more effort. In addition, the 'FUGACITY' model is being translated from TRS-80 BASIC. The programs will be later provided to other participants (NRC, NH&W, NIWR) for testing and evaluation. It is anticipated that the selected model will become a routine tool for the development of DFO's input to pesticide registration, experimental permit applications, and new chemicals evaluation.

A review of organic contaminants in aquatic fauna was prepared (Zitko 1983b). It deals with analytical techniques in general, with procedures for individual contaminants, and with the assessment of the results.

ACID RAIN

Programs for recording, plotting, and evaluating acid/base titration data have been documented (Zitko 1982c) and brought into routine use.

COD CAPSULE

A brown plastic capsule (4.5 x 10.5 x 3.0 cm) about 3/4 full with a liquid and some solids has been found in the stomach of a cod (90 cm, 7 kg) off Cape Sable Island during the last week of July 1982. It was forwarded to Fisheries Research Branch and reached eventually Fisheries Contaminants and Toxicology St. Andrews. Preliminary examination showed that the capsule floats in water and that the contents do not freeze even at -30°C, a strong indication of the presence of an organic rather than an aqueous solution. The freezing experiments made the capsule brittle and a small crack developed a few days later. The crack was enlarged and the contents of the capsule were retrieved, revealing the presence of a broken glass container. The glass container consisted of a glass tubing (length ca. 5 cm, O.D. ca. 0.9 cm, slightly conical shape) and probably a bulb whose shape is difficult to reconstruct. Other solids in the mixture sedimented rapidly as well, leaving a clear, neutral and miscible with water supernatant. The liquid started to boil at about 79°C (1 atm) and the temperature rose gradually to about 90°C, when a small second fraction of the distillate was collected. Infrared spectrum of both fractions indicated ethyl alcohol. The distillation residue consisted of white crystalline solids. Similar crystals were also obtained from the supernatant on standing. The crystals had a relatively sharp melting point at 147-149°C and were well soluble in ethanol. The ethanolic solution had absorption maxima at 271, 263, 258 nm, and a very strong maximum at 219 nm, indicating a substituted benzene. Infrared spectrum (Br) had strong absorption maxima at 1170 and 1130 cm^{-1} and at 3240 and 3350 cm^{-1}. On the basis of this information, the compound was identified as benzene sulfonamide. The identification was confirmed by mass spectrometry of the sample crystals as well as by an authentic benzene sulfonamide.

Solids settled from the original capsule contents were extracted exhaustively with ethanol, leaving behind an ethanol-insoluble residue. Infrared spectrum of the residue had absorption maxima at 670, 910, 1050, and 3450 cm^{-1}. These and the overall appearance of the spectrum indicated an inorganic compound. On heating in air to red heat the compound turned yellowish and generated white fumes. The heat treatment removed the absorption at 670 and 1050 cm^{-1}. A fairly strong band remained at 920 cm^{-1} and minor absorption bands appeared at 880, 950, and 980 cm^{-1}. The salt is sparingly soluble in water and the solution gives a precipitate with silver nitrate. It is well soluble in hydrochloric acid.

The examination of both distillate fractions by gas chromatography-mass spectrometry revealed the presence of additional relatively minor components including (tentative identification only) 1,1-diethoxyethane, 1,1-diethoxy-2-chloroethane, trichloroacetaldehyde, at least two phthalates, and several as yet unidentified compounds.

Two manufacturers of benzene sulfonamide, US FDA, NH&W, and several knowledgeable industrial chemists have been contacted, but the purpose of the capsule remains unknown. The identified compounds are not particularly toxic, but could possibly harm a child taking such a capsule apart.

REFERENCES

Zitko, V. 1982a. Two-compartment model of uptake and excretion of chemicals. Can. Tech. Rep. Fish. Aquat. Sci. 1076: 27 p.

Zitko, V. 1982b. LETCUR, the lethality curve program. Can. Tech. Rep. Fish. Aquat. Sci. 1134: 10 p.

Zitko, V. 1982c. Computer programs for evaluation of acid/base titrations. Can. Tech. Rep. Fish. Aquat. Sci. 1102: 20 p.

Zitko, V. 1983a. Symmetric matrices in one-dimensional arrays. TEKniques (in press).

Zitko, V. 1983b. Organic contaminants in fish and shellfish. In Recent advances in the chromatographic analysis of organic compounds in foods. J. Lawrence (ed.) Marcel Dekker (in press).

Zitko, V., and D. W. McLeese. 1980. Evaluation of hazards of pesticides used in forest spraying to the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 985: 21 p.

ACKNOWLEDGMENTS

The authors and the editor thank the Word Processing Staff for typing, Ms. R. Garnett for editing, and Ms. M. M. Irwin and Ms. R. Garnett for proofreading. Dr. D. J. Scarratt commented on the manuscript.