Compte rendu des communications du septième atelier annuel sur la toxicité aquatique : du 5 au 7 novembre 1980 Montréal (Québec)

Proceedings of the Seventh Annual Aquatic Toxicity Workshop: November 5-7, 1980 Montreal, Quebec

Éditeurs

Editors

N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert et/and M. Speyer

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COMITÉS ORGANISATEURS DU SEPTIÈME ATELIER ANNUEL SUR LA TOXICITÉ AQUATIQUE	ORGANIZING COMMITTEES OF THE SEVENTH ANNUAL AQUATIC TOXICITY WORKSHOP

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RÉSUMÉ DE COMPTE RENDU

Le compte rendu du Septième Atelier Annuel sur la Toxicité Aquatique comporte; 20 articles, 33 résumés, une liste des programmes de recherches sur la toxicité menés au Canada, les délibérations des quatre activités de travail en atelier ainsi que les procès verbaux de la première réunion du comité de coordination pour les Ateliers sur la Toxicité Aquatique.

Les travaux ont permis de traiter de cinq thèmes soient:

1) Dimension pluridisciplinaire de la toxicologie
2) toxicologie marine
3) mécanismes de toxicité
4) toxicologie, outil de gestion environnemental et
5) courts exposés divers sur la toxicité aquatique

ainsi que de quatre sujets de discussion en atelier

1) utilité des essais biologiques pour prédire les risques sur la santé
2) cheminement des toxiques dans l'environnement
3) prise de décision suite à un désastre en milieu marin et
4) approche bioanalytique dans l'évaluation des répercussions environnementales d'un rejet minier.

ABSTRACT OF PROCEEDINGS

The proceedings of the Seventh Aquatic Toxicity Workshop consists of 20 papers, 33 summaries, a list of aquatic toxicity research program in Canada, summaries of the four workshop activities as well as the minutes of the 1st Meeting of the Steering Committee for the Aquatic Toxicity Workshops.

Five themes were dealt with at the workshop:

1) Multidisciplinary dimension of toxicology
2) Toxic mechanisms
3) Marine Toxicology
4) Toxicology, a tool for environmental management and
5) Diverse aquatic toxicity short exposés

Also four topics were dealt with in workshop activities,

1) Usefulness of bioassays in predicting health hazards
2) Pathways of Toxic Substances in the environment
3) Decision making subsequent to a marine environmental disaster and
4) Bioassays in assessing the environmental effects of a mine effluent.

PRÉFACE

ENVIRONNEMENT CANADA (Région du Québec) a été l'hôte du 7ième Atelier Annuel sur la Toxicité Aquatique préparé en collaboration avec des représentants de l'Association Industrielle Laval, du Centre de Recherche Noranda, des Conseillers Beak Limitée, d'Eco Recherches C.I.L., du Ministère de l'Environnement du Québec, de l'Université Concordia, de l'Université McGill et de l'Université du Québec I.N.R.S.-Eau.

L'Atelier a eu pour but d'offrir un forum pour:

a) discuter des points d'intérêt relatifs à la toxicologie aquatique;
b) décrire de nouveaux concepts, approches ou méthodologies;
c) faire l'analyse critique de l'état actuel des connaissances;
d) présenter les dernières données provenant d'étude ou de recherche sur les substances toxiques; et
e) établir des contacts parmi les participants venant des universités, de l'industrie et des gouvernements.

Les comptes rendus des ateliers antérieurs peuvent encore être disponibles. A ce sujet, veuillez communiquer avec le président en assurant la continuité

ATELIER SUR LA TOXICITE AQUATIQUE
DIRECTION DE LA GESTION DE L'HABITAT DU POISSON
240 rue Sparks, 7ième étage
Ottawa, Ontario. K1A 0E6

PREFACE

ENVIRONMENT CANADA (Quebec Region) has hosted the 7th Annual Aquatic Toxicity Workshop which was prepared in collaboration with representatives from Beak Consultants Limited, Concordia University, Eco Research C.I.L., I.N.R.S.-Eau University of Quebec, Laval Industrial Association, McGill University, Noranda Research Center and the Quebec Ministry of the Environment.

The Workshop has provided a forum for:

a) discussions of issues relevant to aquatic toxicology;
b) description of new concepts, approaches and methodology;
c) critical review of "the state of the art";
d) presentation of new results of monitoring and research on toxic substances; and
e) contact among participants from universities, industries and governments.

Proceedings of post meetings may still be available. Please address your requests to the continuity chairman,

AQUATIC TOXICITY WORKSHOP
FISH HABITAT MANAGEMENT BRANCH
240 Sparks Str., 7th Floor West
Ottawa, Ontario
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COMMENTAIRES DE L'EDITEUR - Le compte rendu du Septième Atelier Annuel sur la Toxicité Aquatique couvre les 35 exposés présentés ainsi que les quatre activités de travail en atelier.

Le désir de produire cette publication dans les plus brefs délais ainsi que des restrictions financières ont imposé une date limite pour la réception des textes finals. Les résumés seulement ont été publiés pour ceux qui ne nous ont pas soumis de textes convenables avant le 12 janvier 1981.

EDITOR'S COMMENTS - The proceedings of the Seventh Annual Toxicity Workshop cover the 35 presentations and the four workshop activities held in Montreal on November the 5, 6 and 7, 1980.

The desire to publish at an early date and financial restraints forced a deadline for the reception of the final text. Those who were unable to submit a proper text of their presentation by January the 12, 1981 have had only their abstract published.

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THEME IV

THEME V

THEME I

Dimension pluridisciplinaire de la toxicologie.

Conférencier invité.

Multidisciplinary dimension of toxicology.

Invited speaker.

Dr. FRANK RIGLER
McGILL UNIVERSITY

COMMENTAIRES DE L'EDITEUR - Un exposé enrichissant sur la dimension pluridisciplinaire de la toxicologie a été présenté par le Dr. Frank Rigler. Ses remarques ont créé une atmosphère appropriée pour le déroulement de cette session. Cependant, puisque l'orateur croit que les idées avançées n'ont pas été suffisamment développées pour mériter d'être publiées, aucun manuscrit n'a été soumis.

EDITOR'S COMMENTS - An enriching talk on the multidisciplinary dimension of toxicology was given by Dr. Frank Rigler. His remarks set an appropriate mood for that morning session. However, since the speaker believes that the ideas advanced have not been developed to the point where they are worthy of publication, no manuscript was submitted.

BIOESSAIS ECOTOXICOLOGIQUES AVEC LES ALGUES POUR ETUDIER L'IMPACT DE CREATION DES RESERVOIRS

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RESUME

Pour simuler l'impact de la création d'un réservoir d'eau potable en milieu non oligotrophe (rivière Bulstrode, Victoriaville, Québec), des bacs expérimentaux ont été installés *in situ*. Des bioessais effectués au 64ième jour avec des algues (*Selenastrum capricornutum* et *Chlorella vulgaris*) ont montré que les eaux issues des bacs avec sols décapés étaient moins favorables pour une productivité biologique que celles issues des bacs avec sols intacts. Les fonds de sable limoneux décapé pouvaient alors occasionner un effet écotoxicologique par rapport aux bacs témoins n'ayant pas de sols. Ces phénomènes évidents au niveau des algues ressortaient peu au niveau des poissons.

Durant la mise en eau d'un réservoir hydroélectrique en milieu oligotrophe (rivière Desaulniers, territoire de la baie James, Québec), la matière organique dissoute provenant en majorité des terres avoisinantes s'avérait peu propice à la croissance des algues *Selenastrum capricornutum* et la biodisponibilité des nutriments inorganiques (principalement P) pour celles-ci apparaissait affaiblie malgré un apport de ces nutriments avec l'inondation. Le contraire survenait lorsqu'il y avait un arrêt et un recul de la mise en cour, ce qui stoppait l'apport de matière organique allochtone. Ces constatations nous amènent à formuler l'hypothèse que cette dernière exerçait un effet écotoxicologique en captant les nutriments inorganiques.

Les divers impacts précédents ont été précisés de manière plus rapide et plus évidente chez les algues avec des paramètres biochimiques tels que la protéosynthèse et la photosynthèse qu'avec le critère de la croissance cellulaire.

SUMMARY

In order to measure the impact of a drinking water reservoir on a non-oligotrophic environment (Bulstrode River, Victoriaville, Québec), experimental tanks were installed *in situ*. Bioassays using algae (*Selenastrum capricornutum* and *Chlorella vulgaris*) conducted over a period of 64 days, showed that water issuing from tanks using sediments with topsoil removed was less favourable for biological productivity than water from those tanks using intact soil samples. Tanks with beds comprised of clayish sand lacking topsoil occasionally produced toxicological responses compared to control tanks not having any sediments. These observations, evident at the level of algae, were not as obvious at the level of fish.

During the filling of an hydroelectric power reservoir in an oligotrophic environment (Desaulniers River, James Bay Region, Québec), the dissolved organic material derived principally from neighbouring land proved to be less favourable for the growth of the alga, *Selenastrum capricornutum*, and the bioavailability of inorganic nutrients (mainly P) for the species appeared weak despite the additional nutrients resulting from the flooding. The contrary was the case when filling ceased and the influx of organic material was arrested. These findings lead us to suggest that the organic material produced toxicological effects by removing inorganic nutrients.

The different impacts described were defined clearly and rapidly in algae using biochemical parameters such as proteosynthesis and photosynthesis along with cellular growth data.

INTRODUCTION

L'utilisation des algues en écotoxicologie aquatique présente les avantages suivants:

(i) elle ne nécessite qu'un petit volume à tester et n'exige point d'installations coûteuses, contrairement aux bioessais de toxicité réalisés avec des poissons;

(ii) elle révèle souvent davantage la présence d'une toxicité que l'emploi d'autres organismes cibles tels que les daphnies (Couture & Visser 1978 a);

(iii) elle apparaît moins dépendante d'une diversité fonctionnelle et/ou génétique que les tests effectués avec des bactéries;

(iv) elle traduit les effets primaires d'un toxique dans l'écosystème aquatique vu que les algues y constituent les producteurs de base.

Bien qu'elle s'avère moins rapide que la plupart des bioessais faits en écotoxicologie aquatique, elle ajoute aux quatre avantages précédents celui d'une sensibilité à certaines répercussions qui se détectent plus directement et facilement chez les algues qu'à d'autres niveaux. Le présent article vise à montrer cette sensibilité pour deux facteurs reliés à la création de réservoirs: les effets d'un décapage des sols et ceux de l'exhaussement des eaux.

MATERIEL ET METHODES

a) Bioessais de croissance des algues

Selon les procédures préconisées par l'EPA (1971), les eaux à tester étaient stérilisées et ensuite filtrées (0,45 µm) avant de recevoir un inoculum de *Selenastrum capricornutum* ou *Chlorella vulgaris* (5000 algues/ml). L'incubation en triplicata des cultures durait 21 jours à 24°C ± 2° avec agitation continue et photopériode quotidienne de 16 heures (5400 lux). Le dénombrement cellulaire final était fait avec un compteur à particules (Beckman TA). Les intervalles de confiance des valeurs obtenues en triplicata ne dépassaient généralement pas 10 à 15% de leur moyenne.

b) Mesure de la photosynthèse

A cette fin, on appliquait la technique de Parsons et Strickland (1972) pour quantifier l'assimilation de CO_2 (à partir de bicarbonate radioactif, $NaH^{14}CO_3$) chez des cultures de *Selenastrum capricornutum* ensemencées (5000 algues/ml) dans des eaux filtrées. Après un délai de 2 heures et une acidification-aération pour éliminer le radio-carbone non fixé, la quantité de 14C incorporé dans les algues retenues par filtration était déterminée en milieu aquasol à l'aide d'un compteur à scintillation (Picker Liquimat 2000).

c) Mesure du taux de protéosynthèse

Un acide aminé radioactif, la L leucine radiomarquée à tous ses carbones (activité spécifique: 342 mc/mM), était ajouté (5µc/ml) à des cultures de *Chlorella vulgaris* ayant incubé 5 jours après ensemencement (5000 algues/ml) dans des eaux préalablement stérilisées et filtrées.

Tel que vérifié, cet acide aminé s'incorporait assez rapidement (60 minutes) et spécifiquement (90% de la radioactivité fixée) dans la fraction protéique des chlorelles. Après ce radiomarquage sélectif, on séparait les algues par une brève centrifugation et on les homogénéisait ensuite à 0°C en tampon Tris à pH 7.0. Une précipitation des protéines au TCA (trichloroacétate) suivait au moyen de la méthode de Mans et Novelli (1971). Finalement, la concentration des protéines et celle du radiocarbone qui s'y trouvait incorporé étaient précisées grâce à un dosage spectrophotocolorimétrique (Lowry et al., 1951) et à un comptage à scintillation en aquasol effectué en parallèle.

RESULTATS ET DISCUSSION

I. EFFETS D'UN DECAPAGE DES SOLS

a) Contexte

Un projet de création d'un réservoir d'eau potable à partir de la rivière Bulstrode près de Victoriaville au Québec posait entre autres la question suivante: quels sont les effets de l'inondation des sols laissés tels quels par rapport à ceux de l'inondation des sols décapés, vis-à-vis de la qualité future de l'eau et de son potentiel pour la vie aquatique? En vue de répondre à cette question et concomitamment à d'autres, 14 bacs expérimentaux (5 m diamètre x 1,2 m hauteur) ont été installés *in situ* par paires avec des fonds différents selon le protocole suivant (Campbell et collègues, 1976):

	Limon	Argile	Sable
1) Fond de limon argileux intact	45%	38%	17%
2) Fond de limon argileux décapé*	53%	32%	17%
3) Fond de limon sablonneux intact	41%	34%	25%
4) Fond de limon sablonneux décapé*	22%	56%	22%
5) Fond de sable limoneux intact	30%	23%	47%
6) Fond de sable limoneux décapé*	26%	24%	50%
7) Fond témoin (sans sol)	-	-	-

* Le décapage recommandé mettait à nu le sol minéral stable situé 30 cm sous la surface.

Chacun des bacs renfermait 17500 litres d'eau provenant de la rivière Bulstrode; leur débit de renouvellement simulait celui du futur réservoir, soit 0,36 m^3/24 heures. Dans chaque bac, on ajouta 40 poissons *Notropis cornutus* (espèce piscicole la plus abondante dans la Bulstrode) immatures âgés de 2 ans (scalimétrie) et ayant une longueur à la fourche de 5-6 cm et un poids global voisin de 92 g.

b) Croissance des algues

Au 64ième jour de l'expérimentation, des échantillons d'eau furent prélevés dans chacun des bacs afin de les tester en triplicata pour la croissance de *Selenastrum capricornutum* et *Chlorella vulgaris*. Ceci a fourni les résultats des tableaux 1 et 2.

Ils apportent trois indications:

i) la croissance des 2 espèces d'algues est stimulée par la présence des sols, sauf par celle du sable limoneux décapé qui lui est défavorable (lequel effet pouvait déjà être explicité au 35ième jour de l'expérimentation);

(1 à 5) > Témoin > (6)

ii) les sols intacts lui sont plus propices que les sols décapés;

(1, 3, 5) > (2, 4, 6)

iii) l'influence des sols s'exerce à son niveau selon l'ordre décroissant suivant:

limon argileux > limon sablonneux > sable limoneux
(1, 2) (3, 4) (5, 6)

c) Protéosynthèse des algues

Lorsqu'on fait des bioessais avec les algues, il faut un délai assez long (21 jours dans le cas présent) avant d'obtenir une réponse évidente à partir de leur croissance, ce qui s'avère un désavantage reconnu. Y a t'il possibilité de préciser une réponse analogue avec un délai plus court en choisissant d'autres paramètres que leur multiplication cellulaire? C'est ce que nous avons cherché à vérifier en optant pour un paramètre anabolique, à savoir la protéosynthèse qui prépare la multiplication des algues avec un à plusieurs cycles cellulaires d'avance (Cameron, 1966). A cette fin, parallèlement au bioessai de croissance de *Chlorella vulgaris* réalisé avec les eaux des différents bacs, une incubation similaire de cette algue a été limitée à 5 jours et s'est terminée par une incorporation d'un précurseur radiomarqué de protéines. On obtint alors les résultats anaboliques exposés au tableau 3.

Les trois indications que procurent les tableaux 1 et 2 ressortent encore mieux dans le tableau 3 qui les confirme. Le degré de protéosynthèse constitue dès lors un paramètre plus rapide et tout aussi sensible que la croissance

cellulaire pour les bioessais avec les algues. Certes, ces deux avantages peuvent également être offerts par d'autres méthodes telles que l'assimilation photosynthétique de radiocarbone, la mesure bioluminescente de l'ATP (Adénosine-Tri-Phosphate) et la fluorescence *in vivo* de la chlorophylle (Berland et al, 1972; Samuelson et Oquist, 1977) mais l'interprétation de leurs résultats fait souvent appel à plusieurs variables. En étant plus directement reliée à la croissance cellulaire, la protéosynthèse nous semble un paramètre particulièrement pertinent (Van Coillie et Rousseau, 1974). De plus, les aspects techniques et financiers de l'étude de ce paramètre ne sont guère plus exigeants, nonobstant les apparences, que ceux de plusieurs méthodes couramment employées pour les algues.

d) Comparaison avec la croissance des poissons

Les différents sols ennoyés tels quels ou après décapage n'avaient pas d'effets létaux à court terme (96 heures) sur les poissons *Notropis cornutus*. Il était toutefois opportun de vérifier s'ils exerçaient une influence à long terme sur ceux-ci. Pour cela, on attendit jusqu'au 64ième jour de l'expérimentation *in situ* avant de reprendre à la seine dans chaque bac les poissons qui y avaient été placés et qui y avaient été nourris tous les 2 jours en dispersant 10 g de foie de boeuf/bac. Après ce délai, les taux de mortalité étaient difficiles à préciser compte

tenu des conditions de bioessai *in situ* comme, par exemple, la possibilité de refuge des poissons entre les interstices des sols des bacs lors de leur recapture à la seine pour leur dénombrement final. Néanmoins, on a pu estimer, à partir des pourcentages de recouvrement par rapport à ceux obtenus chez les témoins, que les taux de mortalité se situaient bien en dessous du seuil reconnu de significativité (50%). Il fallait toutefois encore déterminer si, durant les 64 jours du bioessai, les différents sols des bacs avaient occasionné d'autres effets chez les poissons. Rappelons (voir b et c) qu'ils induisaient alors via l'eau des effets nets sur la croissance des algues. Retrouve t'on des influences analogues chez les poissons? Ceci a été étudié au niveau de leur accroissement en poids et longueur; afin d'obtenir des indications sur la qualité future des poissons dans le réservoir, leurs teneurs en calories et en lipides furent également déterminées avec une bombe microcalorimétrique d'une part (Paine, 1971) et un "micro Soxhlet" d'autre part (Giese, 1966). Les résultats de cette étude figurent aux tableaux 4 et 5.

Ces résultats révèlent que:

i) la croissance des poissons en poids et longueur est plus prononcée en présence des sols et leur coefficient de condition apparaît alors aussi plus élevé;
(1 à 6) > Témoin

ii) leurs teneurs calorimétriques et lipidiques sont également favorisées par les sols, excepté le sable limoneux décapé qui les abaisse;
(1 à 5) > Témoin > (6)

iii) les effets des sols intacts sur leur accroissement et leurs teneurs calorimétriques ou lipidiques ne semblent guère supérieurs ou inférieurs à ceux des sols décapés;

iv) les influences des différents sols sur leur croissance en poids et longueur décroissent comme suit:
(1, 4) > (6) > (2, 5) > (3)

v) elles présentent un ordre quelque peu changé pour leur coefficient de condition:
(4, 6) > (1, 5) > (2) > (3)

vi) elles décroissent de façon différente pour leurs teneurs calorimétriques et lipidiques:
(3) > (1, 5) > (4) > (2) > (6)

Si l'on examine ces 6 constatations avec celles fournies par les bioessais avec les algues (voir b et c), les points suivants peuvent être mis en évidence:

- la présence des sols avantage les algues comme les poissons, sauf celle du sable limoneux décapé (6) qui exerce un effet défavorable écotoxicologique chez les deux types d'organismes;
- les sols intacts ont un effet plus bénéfique sur la productivité biologique que les sols décapés mais ceci

ressort surtout au niveau des algues;

- les influences des différents types de sol apparaîssent cohérentes chez les algues tandis qu'il est difficile de les intégrer pour les poissons (hormis le cas du limon sablonneux intact qui, chez ces derniers, favorise plus les teneurs calorimétriques et lipidiques et moins leur accroissement).

Ce qui précède soutient que l'utilisation des algues apporte plus d'indications intégrables que l'emploi des poissons pour préciser l'impact d'inondation et de décapage des sols lors de la création d'un réservoir.

II. EFFETS D'UN EXHAUSSEMENT DES EAUX

a) Contexte

Dans le cadre de l'aménagement du réservoir hydroélectrique LG2 à la rivière La Grande au territoire de la Baie James au Québec, la rivière Desaulniers fut coupée par une digue; à partir du 26 mai 1977, il s'ensuivit un exhaussement progressif de ses eaux et la formation d'une retenue aquatique destinée à être incluse dans le réservoir LG2. Des échantillonnages d'eau furent alors effectués à différents moments et à divers sites afin d'évaluer sa qualité et son potentiel pour la vie aquatique. Parmi les nombreux résultats qu'apportèrent les études faites à ce sujet

(Boucher et *al*, 1978; Couture et Visser 1978 b; Sylvestre, 1979), nous ne présentons ici que ceux acquis à partir des eaux prélevées au site G2-129 (2 km en amont de la digue) les 20 juin, 11 juillet, 19 septembre et 12 décembre 1977 car ils permettent d'expliciter les effets d'une variation de l'exhaussement des eaux. De fait, ce dernier a eu, durant cette période, une fluctuation qui était maximale au site précité:

Dates	Niveaux d'eau	fluctuation
20 juin :	4,0 m*	
11 juillet :	5,4 m	élévation
21 août :	3,4 m	diminution
19 septembre :	6,0 m	élévation
12 décembre :	9,2 m	

* 138 m par rapport au niveau de la mer

b) Croissance des algues

Etant donné qu'un des principaux effets de l'exhaussement des eaux est l'apport de matière organique à partir des sols inondés, la croissance des algues dans les eaux prélevées a été étudiée en fonction de la matière organique dissoute présente. Pour cela, cette dernière a été extraite des eaux par ultrafiltration (Amicon UM05) et lyophilisation: sa concentration moyenne étant voisine de 25 ppm, on considéra cette teneur comme représentative de la

concentration naturelle et/ou adopta le protocole suivant (Visser et Couture, 1978):

0X:	Absence de matière organique dissoute	: 0 ppm MO
1X:	Présence de matière organique dissoute	: 25 ppm MO
3X:	Matière organique dissoute présente en concentration amplifiée 3 fois	: 75 ppm MO
9X:	Matière organique dissoute présente en concentration emplifiée 9 fois	: 225 ppm MO

Les bioessais de croissance de *Selenastrum capricornutum* réalisés selon ce protocole avec les eaux du site G2-129 prises à 4 dates différentes donnèrent les résultats stipulés au tableau 6.

En examinant ces résultats en relation avec les niveaux d'eau aux dates de prélèvement (voir a), on remarque que:

i) en absence de la matière organique dissoute (o x), le potentiel des eaux pour la croissance des algues diminuait lorsque le remplissage du réservoir avait décru et augmentait lorsque celui-ci avait repris; ceci soutient que, pendant l'exhaussement du réservoir, un apport autre que la matière organique y favorisait la productivité biologique, lequel apport consistait sans doute en nutriments inorganiques (phosphates, nitrates, oligoéléments);

ii) les eaux contenant la matière organique dissoute (1 x) avaient un potentiel pour la croissance des algues qui régressait durant le remplissage du réservoir et qui s'amplifiait durant une interruption de

ce processus;

iii) si, pour expliciter l'influence de la matière organique dissoute présente, on augmentait sa concentration (3 x et 9 x), le potentiel des eaux pour la productivité d'algues s'affaiblissait nettement pour celles prélevées lors de l'exhaussement du réservoir et devenait beaucoup plus élevé pour celles prises pendant un arrêt et un recul de la mise en eau.

c) Photosynthèse des algues

En déterminant l'assimilation photosynthétique du radiocarbone chez *S. capricornutum*, on a obtenu les mêmes indications avec un délai de réponse plus court (9 jours au lieu de 21 jours) et des écarts plus prononcés entre les valeurs. Les résultats de cette étude sont exposés au tableau 7.

d) Facteurs impliqués

Lors du remplissage du réservoir, l'inondation des terres périphériques entraîne dans ce dernier une matière organique "allochtone" par rapport à la matière organique "autochtone" originale de la rivière. Nos résultats permettent de penser que la première exerce un effet écotoxicologique sur la croissance des algues et que la seconde est favorable à celle-ci.

En plus de l'apport organique, il y a un enrichissement en nutriments inorganiques (phosphates, nitrates, oligoéléments) durant la mise en eau, lequel stimule les algues comme le montrent les bioessais réalisés sans la matière organique dissoute.

Les deux types d'apport présentent donc durant le remplissage un antagonisme dans leurs effets sur la productivité d'algues. Pour préciser ce phénomène, des bioessais complémentaires ont été réalisés avec les eaux prises au site G2-129; leur matière organique dissoute ne fut pas extraite et elles furent enrichies d'un volume égal en milieu minéral nutritif (EPA, 1978) sans azote ou sans phosphore afin de déterminer des coefficients de stimulation au phosphore ou à l'azote. Pour les eaux prélevées pendant le remplissage du réservoir, il y avait des coefficients de stimulation élevés pour le phosphore tandis que le contraire survenait pour celles prises lors de l'interruption du remplissage; les premières s'avéraient d'ailleurs pauvres en phosphore disponible contrairement aux secondes (Couture et Visser 1978 b, Sylvestre 1979). Mentionnons enfin que de telles différences étaient nettement moins perceptibles pour l'azote.

Ces diverses indications mènent à l'hypothèse suivante pour un mécanisme écotoxicologique: la matière organique dissoute allochtone, qui contient environ 0,2% de phosphore (Visser

Couture 1978) interfère t'elle avec le phosphore biodisponible pour les algues?

Une telle interférence pourrait aussi expliquer, au moins partiellement, que le phosphore et l'azote, tel que vérifié par des stimulations de croissance d'algues à l'aide d'un complément de phosphore et/ou d'azote (Campbell et collègues, 1976), étaient moins biodisponibles dans les eaux issues de milieux avec des sols décapés du futur réservoir de Victoriaville que dans celles issues de milieux avec des sols intacts de ce dernier et dans celles provenant de bacs sans sols.

CONCLUSIONS

1) Pour la création d'un réservoir d'eaux non oligotrophes telles que celles de la rivière Bulstrode, le décapage des sols destinés à être submergés apparaît moins favorable à la productivité biologique que leur inondation. Entre autres, un sol de sable limoneux décapé peut exercer un effet écotoxicologique sur cette productivité. Ces phénomènes sont détectables par simulation *in situ* avec plus d'évidence à l'aide de tests faits avec des algues (*Selenastrum capricornutum* ou *Chlorella vulgaris*) qu'à laide de bioessais effectués avec des poissons (*Notropis cornutus*).

2) Pendant le remplissage d'un réservoir d'eaux oligotrophes telles que celles de la rivière Desaulniers, l'inondation des sols périphériques entraîne de la matière organique dissoute défavorable à la productivité d'algues (*Selenastrum capricornutum*). Concomitamment, la biodisponibilité des nutriments inorganiques (surtout P) stimulant celle-ci diminue alors que l'inondation en fournit.

3) Pour préciser les impacts précédents, l'utilisation de paramètres anaboliques comme la protéosynthèse ou la photosynthèse lors des bioessais réalisés avec les algues a donné plus rapidement des résultats plus départagés que l'emploi du critère de la croissance cellulaire.

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ADDENDUM

L'article de G. Joubert dans le présent compte rendu du 7ième symposium de toxicité aquatique du Canada montre que les tests faits avec *Selenastrum capricornutum* se révèlent plus sensibles aux composés toxiques que ceux réalisés avec d'autres organismes, notamment des poissons (truites). Nos résultats corroborent également cette constatation au point de vue écotoxicologique.

TABLEAU 1 - CROISSANCE DE *SELENASTRUM CAPRICORNUTUM* DANS DES EAUX ISSUES DE MILIEUX ENNOYES AVEC SOLS INTACTS ET DECAPES.

Fond du bac de provenance de l'eau		Cellules/µl		Coefficient variation
1) Limon argileux intact	a		440	
	b		322	
		$\overline{X}$ =	381	22
2) Limon argileux décapé	a		156	
	b		133	
		$\overline{X}$ =	144	11
3) Limon sablonneux intact	a		306	
	b		210	
		$\overline{X}$ =	258	26
4) Limon sablonneux décapé	a		110	
	b		148	
		$\overline{X}$ =	129	21
5) Sable limoneux intact	a		69	
	b		231	
		$\overline{X}$ =	150	76
6) Sable limoneux décapé	a		69	
	b		29	
		$\overline{X}$ =	49	58
7) Témoin (sans sol)	a		125	
	b		58	
		$\overline{X}$ =	91	52

$\overline{X}$: moyenne des valeurs acquises pour chaque paire de bacs

TABLEAU 2 - CROISSANCE DE *CHLORELLA VULGARIS* DANS DES EAUX ISSUES DE MILIEUX ENNOYES AVEC SOLS INTACTS ET DECAPES

Fond du bac de provenance de l'eau				Cellules/µl
1)	Limon argileux	intact	a et b	628
2)	Limon argileux	décapé	a et b	330
3)	Limon sablonneux	intact	a et b	541
4)	Limon sablonneux	décapé	a et b	276
5)	Sable limoneux	intact	a et b	347
6)	Sable limoneux	décapé	a et b	89
7)	Témoin (sans sol)			183

TABLEAU 3 - PROTEOSYNTHESE DE *CHLORELLA VULGARIS* DANS DES EAUX ISSUES DE MILIEUX ENNOYES AVEC SOLS INTACTS ET DECAPES

Fond du bac de provenance de l'eau			Synthèse des protéines
1) Limon argileux	intact	a et b	1186*
2) Limon argileux	décapé	a et b	550
3) Limon sablonneux	intact	a et b	1048
4) Limon sablonneux	décapé	a et b	410
5) Sable limoneux	intact	a et b	701
6) Sable limoneux	décapé	a et b	126
7) Témoin (sans sol)		a et b	339

* dpm (désintégrations par minute)/µc ^{14}C/h incorporation/mg protéines/g poids sec; moyennes avec intervalles de confiance (P 0,95; n = 8) n'excédant pas 17% de leur valeur

TABLEAU 4 - CROISSANCE DE *NOTROPIS CORNUTUS* DANS DES MILIEUX ENNOYES AVEC SOLS INTACTS ET DECAPES

Fond du bac			P*	L_F*	K**
A) INITIALEMENT					
-			2,3 g	5,5 cm	1,38
B) APRES 64 JOURS					
1) Limon argileux	intact	a et b	4,9 g	6,7 cm	1,63
2) Limon argileux	décapé	a et b	3,9 g	6,3 cm	1,56
3) Limon sablonneux	intact	a et b	2,6 g	5,7 cm	1,41
4) Limon sablonneux	décapé	a et b	4,8 g	6,6 cm	1,67
5) Sable limoneux	intact	a et b	3,9 g	6,2 cm	1,64
6) Sable limoneux	décapé	a et b	4,2 g	6,3 cm	1,68
7) Témoin (sans sol)		a et b	2,7 g	5,9 cm	1,32

* Moyennes avec intervalles de confiance (P 0,95; n = 32 à 72) inférieurs à 12% de leur valeur

** Coefficient de condition = $(P / L_F^3) \times 100$

TABLEAU 5 - TENEURS CALORIMETRIQUES ET LIPIDIQUES DE *NOTROPIS CORNUTUS* DANS DES MILIEUX ENNOYES AVEC SOLS INTACTS ET DECAPES

Fond du bac			Calories *	Lipides *
A) INITIALEMENT			5545 cal/g	4,5%
-				
B) APRES 64 JOURS				
1) Limon argileux	intact	a et b	5445 cal/g	4,5%
2) Limon argileux	décapé	a et b	5426 cal/g	-
3) Limon sablonneux	intact	a et b	5542 cal/g	7,5%
4) Limon sablonneux	décapé	a et b	5439 cal/g	3,7%
5) Sable limoneux	intact	a et b	5452 cal/g	4,8%
6) Sable limoneux	décapé	a et b	5392 cal/g	2,8%
7) Témoin (sans sol)		a et b	5424 cal/g	3,6%

* Moyennes avec intervalles de confiance (P 0,95; n = 32 à 72) ne dépassant pas 11% de leur valeur

TABLEAU 6 - CROISSANCE DES *SELENASTRUM CAPRICORNUTUM* DANS DES EAUX ISSUES D'UN SITE TOUCHE PAR UN EXHAUSSEMENT AQUATIQUE AVEC INTERRUPTION PASSAGERE

Dates de prélèvement	algues/µl			
	MO* (0X)	MO* (1X)	MO* (3X)	MO* (9X)
20 juin 1977	34	41	46	45
11 juillet 1977	32	35	-	-
19 septembre 1977	19	40	58	120
12 décembre 1977	36	29	26	28

* Matière organique dissoute enlevée (0X), rajoutée (IX) ou amplifiée en concentrations (3X = 75ppm et 9X = 225ppm)

TABLEAU 7 - PHOTOSYNTHESE DE *SELENASTRUM CAPRICORNUTUM* DANS DES EAUX ISSUES D'UN SITE TOUCHE PAR UN EXHAUSSEMENT AQUATIQUE AVEC INTERRUPTION PASSAGERE.

Dates prélèvements	dpm	14C/j	poids sec	
	MO* (OX)	MO* (1X)	MO* (3X)	MO* (9X)
20 juin 1977	260	740	--	2900
11 juillet 1977	730	690	--	1200
19 septembre 1977	110	1510	1760	5100
12 décembre 1977	880	610	590	470

* Matière organique dissoute enlevée (OX) rajoutée (IX : 25 ppm) ou amplifiée en concentrations (3X : 75 ppm et 9X : 225 ppm)

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QUE CHOISIT LA PERCHAUDE (PERCA FLAVESCENS) L'HIVER, QUAND ELLE A LE CHOIX ENTRE DES EAUX NORMALES COUVERTES DE GLACE ET UNE ZONE DE DÉVERSEMENT D'EFFLUENTS CHAUDS?

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RÉSUMÉ - Afin de protéger les populations indigènesde poissons, il est nécessaire, l'hiver, de refroidir les effluents chauds; et si c'est bien nécessaire, dans quelle mesure et pour combien de temps faut-il refroidir les effluents produits? Nous avons tenté d'apporter une réponse à ces questions au cours d'une série d'expériences en laboratoire et sur le terrain.

La première a été menée en laboratoire avec la perchaude (Perca flavescens), "Température hivernale assurant la maturation et la ponte chez la perchaude (Perca flavescens)" par B.R. Jones, K.E.F. Hokanson et J.H. McCormick, 1977. Nous avons découvert alors qu'il fallait une période de refroidissement en hiver pour assurer la ponte printannière. Celle-ci était meilleure quand les perchaudes étaient gardées dans l'eau à 4° C pendant 185 jours, mais sa qualité s'appauvrissait progressivement à 6, 8 et 10° C et quand la période d'exposition à l'eau à 4° C était raccourcie ou prolongée au-delà des 185 jours.

Dans un deuxième temps, nous avons tenté de découvrir en laboratoire les préférences saisonnières de température de la perchaude, (effets des saisons sur les réactions selon la température de la perchaude, Perca flavescens), article publié dans l'EPA Ecological Research Series, EPA 600/3-77-088, par R.W. McCauley, 1977. Nous avons découvert alors qu'en hiver, la perchaude adulte cherche une eau entre 12 et 14° C quand elle est acclimatée à 5° C et qu'à la fin de l'hiver, elle préfère l'eau à 24°C.

Dans la nature, l'hiver, l'eau ne dépasse pas 4°C, mais que fera la perchaude quand elle a le choix entre des eaux aux températures normales d'hiver et un effluent plus chaud? Les travaux de laboratoire décrits précédemment indiquent que les perchaudes peuvent choisir ces effluents. Cette idée a paru assez inportante pour justifier des travaux sur le terrain.

Une série d'études radio-télémétriques a été effectuée. Nous avons eu recours à des techniques de localisation périodique de sujets bagués et à des mesures de la température de l'eau à des points déterminés. Il a fallu suivre individuellement certains sujets afin d'avoir une idée de l'ensemble des conditions thermiques auxquelles les populations sont soumises. Les données cherchées ont été obtenues en reproduisant les déplacements des sujets sur des cartes à thermo-isoplèthes en fonction du panache thermique des effluents et en accumulant les informations transmises par des émetteurs fixés aux sujets, mesurant la température de l'eau.

La poursuite radio a montré que la perchaude ne réagit pas uniquement à la température, mais qu'elle est influencée par une série d'autres facteurs du milieu, ce qui n'est pas le cas en laboratoire, où des réponses propres à certains facteurs son recherchées. Sur le terrain, la perchaude réagit à toute une gamme de stimulis qui l'incitent à choisir une direction nette ou un habitat donné; la température exerce un effet parmi tous les autres facteurs, et ceux-ci varient selon les saisons ou l'heure du jour. Les résultats sont présentés dans un article intitulé "Distribution spatiale et choix des températures de poissons au voisinage du panache thermique des effluents d'une centrale électrique à l'automne, en hiver et au printemps", par M.G. Ross et D.B. Siniff, Environmental Protection Agency, rapport n° 600/3-80-009.

WHERE DO YELLOW PERCH (PERCA FLAVESCENS) GO IN WINTER WHEN GIVEN THE OPTION BETWEEN NORMAL ICE COVERED WATER OR A HEATED DISCHARGE.

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SUMMARY - It is necessary, in order to protect indigenous fishes, to require cooling of heated discharges during the winter; and if necessary, to what degree should such cooling be required and for what period of time? These are the questions we attempted to answer in a series of laboratory and field tests.

The first in this series was a laboratory test with yellow perch (Perca flavescens) "Winter temperature requirements for maturation and spawing of yellow perch (Perca flavescens Mitchell)" by B.R. Jones, K.E.F. Hokanson, J.H. McCormick, 1977. We learned in this study that a winter chill period was required for successful spring spawing. Spawing was most successful among yellow perch held over winter at 4°C for 185 days and was progressively poorer at 6,8 & 10°C and as the chill period at 4°C was shortened or extended beyond 185 days.

The second step was another laboratory study with yellow perch to determine their preferred temperature on a seasonal basis, "Seasonal effects on temperature performance in yellow perch, Perca flavescens", U.S. EPA Ecological Research Series, EPA 600/3-77-088 by R.W. McCauley, 1977. In this study it was learned that adult yellow perch in winter seek water at temperatures from 12 to 14°C when acclimated to 5°C and that their final winter preferendum is 24°C.

Under natural winter conditions, 4°C is the warmest water available but given the choice between normal winter temperatures and a thermal discharge where will yellow perch be found? The information gained from the laboratory tests just described suggests that they may choose the thermal discharge. This was such an important concept that field testing was deemed justified.

To answer this question, a series of radio telemetry studies were undertaken. Techniques for periodic determination of location of radio-tagged fish and determination of water temperature at these locations were used. Thermal exposures of individual fish were necessary to estimate their cummulative thermal experience. Thermal exposures were determined by matching fish locations to thermo-isopleths on maps of the thermal plume from the discharge and by thermo indicative signals from externally attached radio tags.

Radio tracking revealed that yellow perch do not respond to temperature independence of other field present factors as they do in a laboratory gradient tank where single factor responses are carefully sought. In the field, yellow perch respond to an array of stimuli that result in net directional or habitat selection response, where temperature exerts its influence along with all of the other factors and as they shift with the season or with time of day. This work was reported in "Spatial distribution and temperature selection of fish near the thermal outfall of a power plant during fall, winter, and spring", M. J. Ross and D.B. Siniff, U.S. Environmental Protection Agency 600/3-80-009.

To Go To Florida for the Winter or Not to Go to Florida, That is the Question: Where Do Yellow Perch (*Perca flavescens*) Go in Winter When Given the Option Between Normal Ice Covered Waters or a Heated Discharge

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Introduction

As part of a series of studies to determine the thermal requirements for yellow perch we progressed through a sequence of steps that led us to an appreciation for the dangers of making environmental decisions based on insufficient knowledge. Using carefully designed and well conducted studies that provided what we believe are valid information we learned that logical conclusions based on good research can lead to erroneous decisions. Because this was so eye-opening for me I thought I would like to share my experience with you, believing that you will also find it at least interesting (maybe somewhat amusing in retrospect) but also that if tempted you may be more cautious in similar situations.

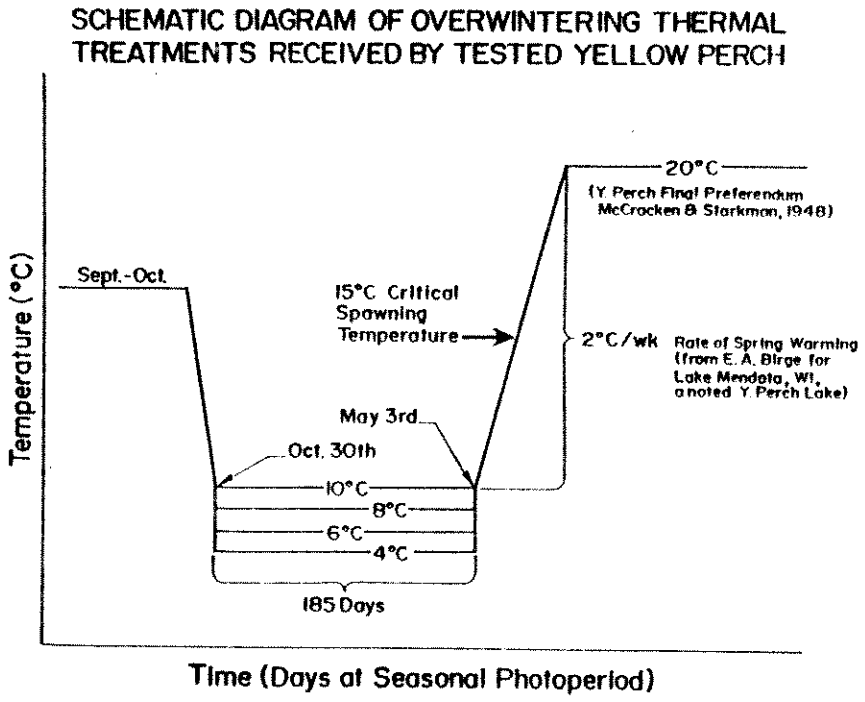
As we began to study thermal requirements of freshwater fishes we were cognizant that the findings of our work would eventually have an influence on thermal discharge standards. That is, I believe, to be expected and as it should be; however, the findings should not result in control beyond requirements. Such an unrealistic influence can result in unnecessary costs to the public we serve and eventually to a lack of trust and effectiveness at some later date.

With this in mind we decided to gather information on the biological need to provide cooling of thermal discharges during the winter months. Heated water fish kills were not anticipated due to failure to cool at this time of year, but we though it prudent to study the biological response of fishes to elevated temperatures during that portion of their seasonal cycle before we stated that cooling at that time was unnecessary.

To study this we chose yellow perch as the tested species. Perch was chosen because of convenience of size and its importance in our northern latitude as a sport fish and as a forage base; and because of its taxonomic relationship to other percids also of high importance. We then asked what processes of significance are taking place in this species during the winter months. We decided that the precursors to spawning were functioning at that time; so we decided to look at spawning success subsequent to thermally altered overwintering conditions.

We brought the fish in from the field in the fall and placed them in 152 x 26 x 28 cm deep troughs where they were fed a meat-meal-gelatin diet and where the temperature was gradually reduced over the next few weeks to four constant temperatures, 10, 8, 6, and 4°C. The fish were then held over winter at these temperatures. The photoperiod during this time was adjusted to follow the natural seasonal cycle. The duration of the winter minimum temperature exposures - "the winter chill period" - was also varied from 123 to 241 days for separate lots of test fish. Various winter chill periods and depth of chilling were studied to determine if a winter chill was necessary; and if so, to what degree and for how long must such a chill be extant to insure subsequent successful spring spawning. Both of these questions are important to the discharger as well as to the environment. For one, these questions are important for economic reasons and for the other, to maintain a self-sustaining population of perch.

We found that in fact a "winter chill period" was necessary and that its duration should be in the vicinity of 185 days. Cooling during that time



Slide: 1. Over-wintering thermal regimes used in establishing yellow perch winter chill requirements.

should provide for over wintering of perch at temperatures of 6°C or cooler. At 8°C some reproduction may be expected but at 10°C reproductive failure is probable. We found our perch were reproductively most successful after over wintering at 4°C. (It is interesting to observe that 4°C is the warmest water available to a yellow perch during ice-covered winter conditions in the normal range of the species.)

Table 1. Potential for Production

% Viable Eggs			
100-75	75-50	50-25	25-0
°C/days	°C/days	°C/days	
4° 185	4° 213	4° 164	
	6° 164	6° 185	
		6° 213	All
		8° 210	other
		8° 241	treatments

From Jones, Hokanson, & McCormick, 1977

Table 2. Effects on Total Number of Eggs and Percent Viable Eggs per Female

°C	Duration (days)	Total eggs (X1,000)	Viable eggs (%)
4	123, 143, 164,	5	17
	185,213	11	74
6	123, 143, 164,	10	27
	185, 213	11	41
8	157, 201, 241	7	31
10	157, 201, 241	1	4

From Jones, Hokanson & McCormick, 1977

With this information in hand we progressed to another question. If a thermal discharge is introduced into a body of water possessing an important perch or perch dependent fishery, will the perch be attracted to the heated water mass and if so, to what temperature water might they be attracted? This work was undertaken with the winter preferendum of mature perch being of particular interest. To accomplish this we asked Dr. Robert W. McCauley of Wilfred Laurier University, Waterloo, Ontario for his assistance.

Dr. McCauley's work was reported in EPA Report 600/3-77-088 "Seasonal Effects on Temperature Preference in Yellow Perch, Perca flavescens". In this study using adult yellow perch acclimated to 5, 10, 15, and 20°C. Dr. McCauley obtained the data found in Table 3. Note particularly that even in winter when acclimated to 5°C adult yellow perch preferred and sought

Table 3. Mean Preferred Temperatures of Samples of Yellow Perch Held at Four Constant Acclimation Temperatures Throughout the Year

Year	Season	Acclimation temperature, (C)				Final preferendum, (C)
		5	10	15	20	
1974	Winter	13.0	15.5	18.4	24.2	25
	Spring	12.3	18.8	20.2	20.2	21
	Summer	13.8	13.5	17.6	16.1	17
1975	Winter	12.0	14.2	18.0	23.2	30
	Spring	13.1	18.1	21.0	19.5	21.1
	Summer	13.5	14.1	18.1	17.0	18

From McCauley, 1977

temperatures from 12-14°C, considerably above the 4-6°C previously established as optimum for successful spawning gametogenesis. Note also that if 12-14°C is sought, acclimation to that range soon will follow until eventually the final preferendum is reached, 17-25°C.

Now is where the problem develops. In attempting to relate the findings of these two well conducted studies producing apparently valid data to control practices for heated effluents one could - and I did - become concerned for how yellow perch would respond to the availability of heated water introduced into their environment in the winter. This I suppose could be stated more poetically as "To go to Florida for the winter or not to go to Florida, that is the question". The yellow perch is a species whose evolutionary development has taken place where the warmest water available to it during the winter months under ice cover was 4°C (the species is most commonly in that portion of the North American continent where lakes are ice-covered in winter). Now with the introduction of anthropogenic heating of their environment they have a choice between natural winter temperature of 4°C and below or water heated several degrees warmer; frequently discharge plumes may be well above the 10°C. Ten degrees you will remember was found to inhibit successful reproduction. The data also suggests that given this choice, all other things being equal, they will choose temperatures, when available, of 12-14°C even if acclimated to 5°C. But, if once these warmer waters have been inhabited for a time they will seek even warmer temperature as their acclimation increases (see Final Preference column, Table 3).

If this can actually be expected in the natural environment standards for heated discharges would have to be proposed to prevent discharges at temperatures greater than 6°C, (with the encumbent costs of operation and maintenance to the industry and eventually to the consumer). Such a biological response could be expected to lead to a large accumulation of yellow perch in the area of the thermal plume. Accumulation of yellow perch in the heated water area could be expected to result from chance encounters by individual fish with the thermal gradient, followed by movement up the gradient until residence is taken up in the area with temperatures nearest their final thermal preferendum. A predicted consequence of such a response would be reproductive failure in possibly a major portion of the affected population.

For reproductive failure to happen, however, the perch would have to dwell within the heated water long enough to accumulate a thermal dose sufficient to prevent subsequent reproductive success: and to concentrate the fish in sufficient numbers so that year-classes are adversely effected.

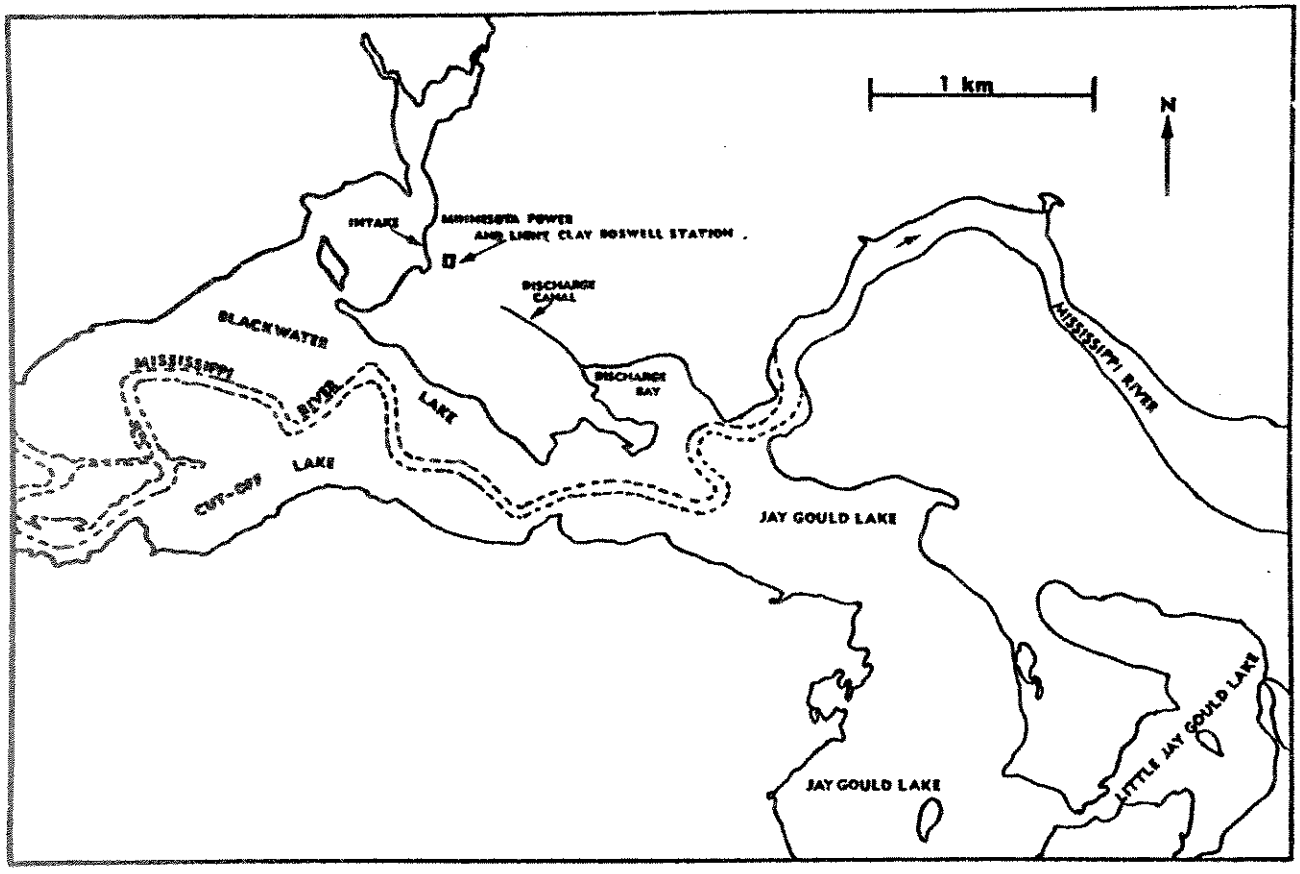
The expense of winter cooling to $\leq$ 6°C or the loss of reproductive success of a species of major importance was of such significance that we proposed another set of studies, this time in the field, to determine what would be the response of yellow perch in their natural habitat to an "on-line" power plant discharge during the over-wintering period.

Conventional methods of determining distribution of fishes were not adequate to answer these questions since we required information on temperature experience of individual fish over time, and population concentrating effects of the thermal gradient on those fish that enter into its influence. Would the presence of a heated plume during the cool-cold

portion of the year tend to cause them to ascend the gradient to the thermal "trap" of the heated plume; and once in that "trap" how long would they remain under its influence? These were the questions that had yet to be answered.

To obtain answers to these questions we enlisted the services of Dr. Donald B. Siniff, Jimmy D. Winter, and M. Jon Ross of the University of Minnesota Ecology and Behavioral Biology Department who have developed reputations for their work in radio tracking of free-roaming fishes. By employing their technology it was possible to follow the movements of individual fish and to know their location not only at one point in time, as in a netting procedure, but to follow their movements to determine cumulative thermal experiences over time.

To facilitate this work a transmitter was developed and pressed into service that not only allowed positioning of the fish but also signaled the temperature of the water at that location. Plume temperatures at the positions where the fish were located were used to estimate temperature experience of radio-tagged fish prior to the use of this new transmitter.



Slide: 2. Simplified area map

Slide: 3. Yellow perch with radio

Slide: 4. Antenna on boat, and

Slide: 5. Coordinates on map (fish location by triangulation, using map and coordinates from fixed location antennas.

The radio tracking work was conducted over two fall-winter-spring intervals. Tracking information gathered the first season provided important fish thermo-distribution data but also revealed some unexpected findings that directed the further development of the study. We found that winter movements were somewhat random throughout the discharge bay (sitings predominating along the periphery) but in spring prior to and during the spawning period there was a movement into the bay from outside the heated area followed by a post spawning egress to a downstream site where several of the radio-tracked fish appeared to establish a new home range.

The assumption was then made that come fall as seasonal temperatures fell below preferendum these fish would ascend the thermal gradient and once again be found in the discharge bay. In an attempt to establish this as fact, fall trapping was conducted at that downstream site. Thirty-five of fifty-three perch trapped and radio-tagged below the discharge bay moved upstream through the mixing zone. Of those fish, only two turned into the discharge bay during the functional life of the transmitter and then only briefly. Most took up winter residence in ice-covered Jay Gould Lake across from the discharge bay; however, some continued to move upstream beyond the discharge bay.

With this rather unexpected discovery we felt relieved that a long term over-wintering of a significant concentration of adult yellow perch in a heated discharge was not as eminent a problem as at first contemplated. But, if there is an exodus each spring what is the source of these emigrants? Apparently it is the rest of the contiguous environment and attraction is only brief during the spawning season.

Then, too, what is the thermal experience of those perch that do inhabit the discharge bay in winter? Trapping and tracking revealed that the perch were not present in the warmest available water; the discharge current avoidance, as previously reported by Kelso, 1976, is probably sufficient explanation of that. Most were apparently located in the peripheral areas where vegetation was more abundant. The perch were not sedentary but moved around, in, out, and about in the discharge bay. During these movements they were found to accumulate a mean temperature experience of about 5.4°C but, if only movement within the bay was used to calculate the mean thermal experience, 6.3°C was obtained. These findings further alleviated my concern for yellow perch exposed to an alternative choice between inhabiting normally 4°C maximum temperatures in winter or that of the heated discharge.

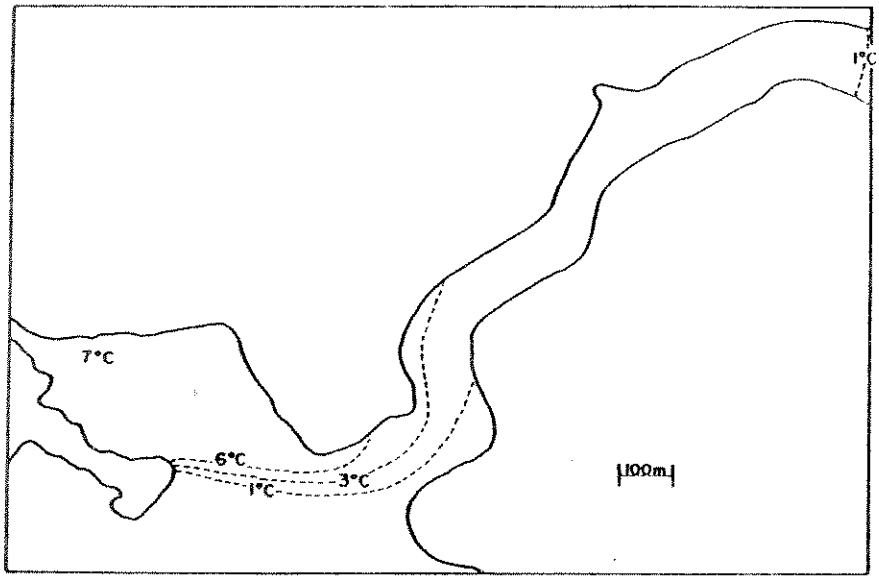
Now, however, we are confronted with curiosity: where did we go wrong in our original expectations? First we had to realize that heat in the natural environment is not the only variable, as in carefully controlled single variable laboratory experiments. Heat is only one of many variables and each has, so to speak, a vector-like force on the resultant movements and distribution of naturally exposed fishes. Apparently though we know that temperature is an important vector it may not always be a dominant force

particularly when not at some life-threatening level or when other more eminently critical factors are working.

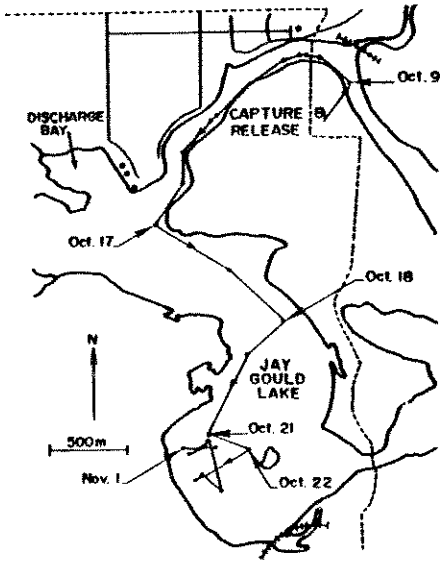
Our interpretation of the situation at our study site is that cover is the driving force working in that environment. During the study period, the heated effluents primary affect on distribution of the yellow perch appears to be exerted in the form of removal of ice cover during the winter months. As late fall progresses and winter sets in vegetation in the relatively shallow river becomes less available as cover, and the perch move to find better cover. When they move upstream they eventually reach the junction of the river with the discharge bay on the west and Jay Gould Lake on the east. The bay was open due to the heated discharge, Jay Gould was or would soon become ice-covered and the cover of the darkened water of the lake was chosen in preference to that of the open water bay (Storr and Schlenker, 1973). Not until spring was the ice cover attraction of the lake lost and it was also then that the perch were preferentially attracted to the discharge bay. This we believe was also due to the heated discharge since now the winter warmer open water has permitted more abundant growth of vegetation in the heated bay than in surrounding thermally unaltered areas.

Contributing to this belief were observations made by Hokanson and Kleiner at another of our research facilities, the Monticello Ecological Research Station at Monticello, Minnesota, located on the Mississippi River upstream from Minneapolis-St. Paul, Minnesota, on the site of a nuclear power station.

Slide: 6. Winter photo of ice free thermal discharge bay

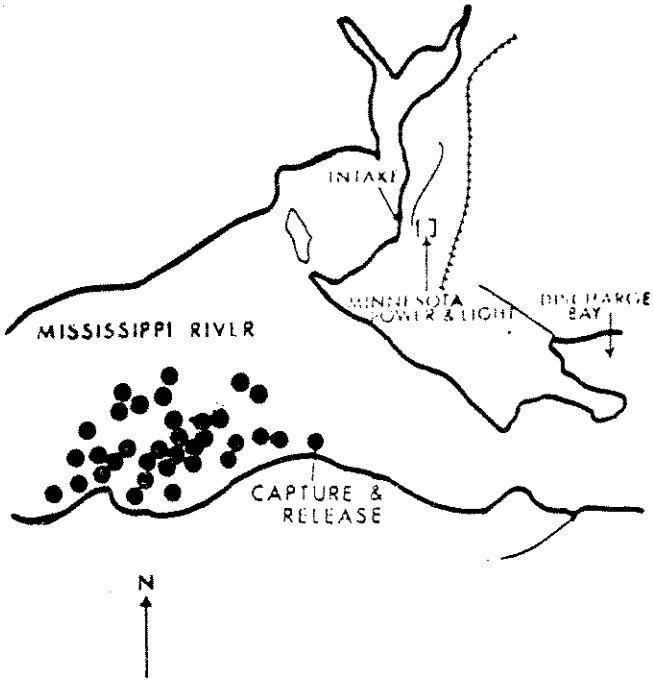


Slide: 7. Thermo isopleths within study area



From Ross & Siniff, 1980

Slide: 8. Map of movements of a fish captured and released at a point in Mississippi River below thermal discharge.



Daily upstrema perch locations for Perch #1808
October 3, 1975 to November 19, 1975.

Slide: 9. Map of movements of a fish captured and released at a point in Mississippi River above thermal discharge.

Slide: 10. Monticello Ecological Field Station in the summer

Slide: 11. Monticello Ecological Field Station in the winter.

Here utilizing water heat from the condensing steam for power generation via heat exchangers, thermal effects studies were conducted in large scale semi-natural flowing water systems. During the winter of 1975 a study of the movements of six adult white suckers and five saugers was conducted. The heated water was introduced at the upstream end where there was a considerable area of open water. Cooling downstream resulted in ice cover in that end of the channel. Daytime residence of the fish was in the ice covered pools in the lower end of the channel, but under the cover of darkness the fish moved as much as 305 m to the upstream end where temperatures were nearer their preferendum by about 7.5°C. At dawn they would again retreat into the pools in the darkness of the ice-covered lower end of the 490 m channels (Qt. Rept. Nat. Water Qual. Lab. March, 1975).

With all this behind us what is our position concerning controls on heated discharges into environments containing important populations of yellow perch (and for that matter percids in general)? Well, first of all I am not making definite predictions of catastrophe if discharges exceed 6 C during the over-wintering period. If asked my opinion I suggest that there may well be no adverse effects under these conditions and there may be some benefits due to extended growing season. A caveat on such a statement must, however, be that factors serving as vectors on distribution must not be substantively different from those present in our study area. To substantiate this a confirmatory tracking program should be initiated particularly where percids are of considerable importance.

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RETOMBÉES ET DISTRIBUTION DE Zn, Cd, Cu, Pb ET As ET CERTAINS EFFETS SUR DES ÉCOSYSTÈMES AQUATIQUES ENVIRONNANT UNE FONDERIE DE MÉTAUX COMMUNS SITUÉE SUR LE BOUCLIER PRÉCAMBRIEN CANADIEN

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RÉSUMÉ - Sauf dans les régions urbaines, les retombées atmosphériques de métaux à l'état de traces sont ordinairement très faibles, ce qui complique considérablement l'étude de leurs effets sur les écosystèmes aquatiques; il faut des relevés couvrant de longues périodes. Au voisinage des fonderies, les métaux se déposent beaucoup plus rapidement, l'accumulation dans les écosystèmes aquatiques est accélérée, ce qui diminue considérablement le temps requis pour les observations. Nous avons étudié des écosystèmes lacustres entourant une fonderie de métaux communs située sur le bouclier précambrien dans le nord-ouest du Manitoba, Canada. Nous avons trouvé que la concentration au sol de Zn, Cd, Cu, Pb et As était inversement proportionnelle à la distance. En mg/m^2, les retombées annuelles mesurées de ces éléments entre 4.3 et 71 km de la fonderie s'établissent comme suit: Zn, 1780-9.3; Cd, 4.5-0.1; Cu, 173-1.3; Pb, 42-1.7 et As, 6.2-0.04. L'estimation du dépôt des métaux sur la neige formée en congères sur les lacs à la fin de l'hiver était en moyenne 1.8 fois plus élevée que celle obtenue à partir des pluviomètres en service au même endroit et pendant une durée comparable. Des quantités considérables de ces éléments, de même que de Fe et de Mn, ont été trouvées dans les congères, mais seule la concentration de Zn, de Cd et de Pb s'est accrue de façon mesurable dans les eaux sous-jacentes au cours de la fonte des neiges. À ce moment-là, la concentration de Zn et de Cd était beaucoup plus élevée que la normale. Tous les éléments se concentraient surtout dans les sédiments des systèmes aquatiques. À première vue, il ne semble pas y avoir de rapport entre la concentration des métaux dans les plantes, le plancton et les tissus de poissons et celle mesurée dans les sédiments ou dans l'eau; tout ce qu'on pouvait conclure était qu'il y a des lacs très contaminés

et des lacs non contaminés. Par contre, la concentration de calcium dans l'eau des lacs étudiés, variant entre 10 et 30 mg/L, modifie considérablement l'absorption du Cd en particulier, à tel point que les tissus de plancton, de végétaux et de poissons d'un petit lac contaminé, mais contenant peu de Ca, contenaient autant de cadmium que des échantillons obtenus dans des lacs très contaminés, mais dont la concentration de Ca était supérieure de 50 %. Nous avons comparé les populations du meunier noir d'un lac contaminé et d'un lac relativement intact. Nous avons pu montrer que la population du lac contaminé était soumise à un stress résultant des importantes concentrations de métaux, si l'on en juge par le frai difficile, le faible recrutement, la diminution de la durée de vie et le moindre diamètre des oeufs de la population contaminée. Ces résultats nous renseignent sur le sort des lacs éloignés et contenant peu de calcium et qui sont exposés aux retombées atmosphériques contenant des produits acides et des métaux.

FALLOUT, DISTRIBUTION AND SOME EFFECTS OF Zn, Cd, Du, Pb AND AS IN AQUATIC ECOSYSTEMS NEAR A BASE METAL SMELTER ON CANADA'S PRECAMBRIAN SHIELD

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SUMMARY - Atmospheric fallout of trace metals in non-urban areas typically occurs at very low rates making study of its effects on aquatic ecosystems difficult except over many years. The area around metal smelters offers accelerated metal deposition rates and consequently accumulation in aquatic ecosystems thereby reducing dramatically the time required for observable effects to occur. We have studied lake ecosystems in the vicinity of a base metal smelter on the Precambrian Shield in northwestern Manitoba, Canada. Deposition of Zn, Cd, Cu, Pb and As was found to be negatively correlated with distance from the smelter. The measured annual fallout of these elements in mg/m^2 over a distance of 4.3 to 71 km from the smelter was a follows: Zn, 1780-9.3; Cd, 4.5-0.1; Cu, 173-1.3; Pb, 42-1.7 and As, 6.2-0.04. Samples of snowpacks on lakes in late winter yielded estimates of deposition on the average about 1.8 times greater than precipitation collectors operated at the same place for a comparable time period. Although considerable quantities of these elements, as well as Fe and Mn, were found in snowpacks in late winter, only Zn, Cd and Pb were increased measurably in waters under ice during snowmelting. At this time Zn and Cd concentrations were much elevated over normal water concentrations. The distribution of all the elements in aquatic systems was weighted heavily in favour of sediments. Plant, plankton and fish tissue metal concentrations appeared to be unrelated to sediment or water metal concentrations other than a general trend of heavily-contaminated versus uncontaminated lakes. However, the water Ca concentrations of the study lakes, varying from 10 - 39 mg/L, strongly affected uptake of Cd in particular, such that a little contaminated lake with low Ca concentrations showed Cd uptake in a plant, plankton and fish tissues comparable to that seen in heavily contaminated lakes with 50 % higher Ca concentrations. White sucker populations were

compared in a contaminated and relatively uncontaminated lake. The population in the contaminated lake was shown to be under stress from high metal concentrations as evidenced by poor spawning success, low recruitment, reduced longevity and smaller egg size when compared to the unaffected population. These findings have a bearing on the fate of remote lakes of low Ca concentration which receive atmospheric fallout containing acid and metals.

Fallout, distribution and some effects of Zn, Cd, Cu, Pb and As in aquatic ecosystems near a base metal smelter on Canada's Precambrian Shield[1]

by

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[1] This paper was presented as a poster at the International Conference on the Ecological Impact of Acid Precipitation, at Sandefjord, Norway, March 11-14, 1980 as well as at the 7th Annual Aquatic Toxicity Workshop at Montreal, Nov. 5-7, 1980. A short summary paper with the same title will appear in J. Chem. Research along with proceedings of the Norwegian conference.

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ABSTRACT

Franzin, W.G. and G.A. McFarlane. 1980. Fallout, distribution and some effects of Zn, Cd, Cu, Pb and As in aquatic ecosystems near a base metal smelter on Canada's Precambrian Shield.

Atmospheric fallout of trace metals in non-urban areas typically occurs at very low rates making study of its effects on aquatic ecosystems difficult except over many years. The area around metal smelters offers accelerated metal deposition rates and consequently accumulation in aquatic ecosystems thereby reducing dramatically the time required for observable effects to occur. We have studied lake ecosystems in the vicinity of a base metal smelter on the Precambrian Shield in northwestern Manitoba, Canada. Deposition of Zn, Cd, Cu, Pb and As was found to be negatively correlated with distance from the smelter. The measured annual fallout of these elements in mg/m^2 over a distance of 4.3 to 71 km from the smelter was as follows: Zn, 1780-9.3; Cd, 4.5-0.1; Cu, 173-1.3; Pb, 42-1.7 and As, 6.2 - 0.04. Samples of snowpacks on lakes in late winter yielded estimates of deposition on the average about 1.8 times greater than precipitation collectors operated at the same place for a comparable time period. Although considerable quantities of these elements, as well as Fe and Mn, were found in snowpacks in late winter, only Zn, Cd and Pb were increased measurably in waters under ice during snowmelting. At this time Zn and Cd concentrations were much elevated over normal water concentrations. The distribution of all the elements in aquatic systems was weighted heavily in favour of sediments. Plant, plankton and fish tissue metal concentrations appeared to be unrelated to sediment or water metal concentrations other than a general trend of heavily-contaminated versus uncontaminated lakes. However, the water Ca concentrations of the study lakes, varying from 10 - 39 mg/L, strongly affected uptake of Cd in particular, such that a little contaminated lake with low Ca concentrations showed Cd uptake in a plant, plankton and fish tissues comparable to that seen in heavily contaminated lakes with 50% higher Ca concentrations. White sucker populations were compared in a contaminated and relatively uncontaminated lake. The population in the contaminated lake was shown to be under stress from high metal concentrations as evidenced by poor spawning success, low recruitment, reduced longevity and smaller egg size when compared to the unaffected population. These findings have a bearing on the fate of remote lakes of low Ca concentration which receive atmospheric fallout containing acid and metals.

The primary metal smelting industry is recognized as a major source of anthropogenic airborne sulphur, acid and metals, yet comprehensive studies of the output of smelter emissions and their environmental effects, particularly on aquatic ecosystems, are few. A base metal smelter at Flin Flon, Manitoba, Canada, on the edge of the lake-dotted precambrian shield afforded us an opportunity to examine a situation of accelerated input of atmospheric metal fallout into lakes and some effects of the metals on selected lake ecosystems. Previous work (Van Loon & Beamish, 1977) had indicated probable contamination of many lakes in the area by airborne metals and potential for ecological disturbance.

We estimated the input of metals to Flin Flon area lakes by way of bulk precipitation collectors operated over a year and by collections of snowpack samples from lake surfaces near the end of the winter snow accumulation period (Franzin *et al.*, 1979). Further, we examined the input of metals in the lake snowpacks into lakes in early spring to determine the presence of peak surges in lake water metal concentrations relative to lake water column metal concentrations monitored at regular intervals throughout the year (Franzin & McFarlane, 1980a). Since we had many lakes receiving varied amounts of fallout at our disposal, we were able to examine the effects of different levels of contamination on the uptake of smelter-related metals by selected plant (Franzin & McFarlane, 1980b) and fish species (McFarlane & Franzin, 1980) in several lakes. Finally we examined the effects of metal contamination on populations of white suckers *(Catostomus commersoni)* in a heavily contaminated versus a relatively uncontaminated lake (McFarlane & Franzin, 1978).

METHODS

Study Area

The Flin Flon Area (Figure 1) is situated on the Manitoba-Saskatchewan border at approximately 55°N, 102°W. The city is the site of a base metal smelter, in operation since 1930, which currently processes approximately 7300 t/D of ore from area mines. Detailed descriptions of the study area may be found in McFarlane & Franzin (1978), Franzin *et al* (1979) and Franzin & McFarlane (1980a).

Sampling Methods

Bulk precipitation samples were collected over one year at intervals of two months in 13 open-mouthed collectors (Figure 1, Letters A-M) constructed of ∿1M lengths of aluminum stovepipe lined with polyethylene bags. Snowpack samples were collected with a plexiglass tube corer in Frebruary, 1977 from all the lakes shown in Figure 1. Details of these procedures and sample analyses are described in Franzin *et al.* (1979).

The passage of snow melt-water into ice-covered lakes in early spring 1977 was monitored by sampling carefully the waters immediately under the ice of Hamell and Thompson Lakes (Figure 1) as described in Franzin & McFarlane (1980a).

Our studies of sediment, plants, plankton and fish tissue metal concentrations centred on the six lakes named in Figure 1. Sediment cores were collected by SCUBA diver. Plant species were screened for a potential

indicator species prior to collection of the selected species, *Myriophyllum exalbescens*, from all six lakes. Details of collection and analyses of sediments and plant tissues are described in Franzin & McFarlane 1980b).

White sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*) samples were collected by gillnet from five of the six study lakes within one week in August, 1976. Population samples were obtained to represent a number of individuals of five size/age classes from each species in each lake. Several tissues of each species from the most contaminated lake (Hamell) were analyzed for Fe, Mn, Pb, Cu, Cd, Zn and Hg in order to determine important tissue metal interactions with growth and age prior to analysis of samples from the other lakes. Details of procedures and analyses may be found in McFarlane & Franzin (1980).

The effects of metals on fish populations were examined by investigating the relative success of white sucker populations in Hamell (contaminated) and Thompson (control) lakes. Details of fish capture methods may be found in McFarlane & Franzin (1978).

RESULTS

Atmospheric Fallout of Metals

A) Bulk precipitation collections: The deposition of Zn, Cu, Cd, Pb and As declined with increasing distance from the smelter, indicating with reasonable certainty that these metals originated from the smelter stack. The thirteen precipitation samplers (Letters A-M, Figure 1) ranged from 4.3 to 71 km from the smelter. Annual depositions in mg/m^2 of the five smelter metals over this distance were: Zn, 1780 to 9.3; Cu, 173 to 1.3; Cd, 4.5 to 0.1: Pb, 42 to 1.7 and As, 6.2 to 0.04. We estimated from this data, the total annual depositions of these metals within circular areas around the smelter by integration of deposition curves of the type $y = ax^b$ to arbitrary limits (Table 1A). These calculations were based on data from essentially nine data points, six of which were in two directions only, SE and SW from the smelter.

B) Winter snowpack samples: We sampled the snowpacks on all of the lakes shown in Figure 1 in late winter of 1976-777, approximately 85 days after freeze-up in order to obtain estimates of metal deposition directly onto lakes. Fifty-three lake snowpack collections were obtained in all eight major compass directions relative to the smelter at distances of from 4.0 to 71 km from the stack. The ranges of depositions in mg/m^2 of the five smelter metals were: Zn, 3735 to 1.8; Cu, 113 to 0.1; Cd, 7.0 to 0.01; Pb, 81 to 0.2 and As, 10 to 0.02. Using again the model $y = ax^b$, the metal deposition curves were integrated to obtain estimates of the total depositions of the metals over circular areas around the smelter for the 85-day period (Table 1B). Although metal depositions were calculated for circular areas of radius 'X', in reality the deposition zones were skewed strongly to the SE during the winter of 1977 due to prevailing NW winds. The actual deposition of metals in these directions was therefore probably higher at any distance from the smelter than that indicated by deposition curves. The relative importance of the Flin Flon smelter as a source of atmsopheric metals in shown in Table II. It is readily apparent that this smelter is a significance source of atmospheric Zn, Cu and Cd, Pb deposition while high was comparable to that of a large urban centre (e.g. Copenhagen).

We were able to compare directly our two methods of assessing metals deposition at five different locations during the winter perior. Bulk precipitation collections provided estimates of deposition that were on the average only 55% of estimates derived from coring snowpacks. This degree of difference was comparable to the expected inefficiency of open-mouthed precipitation collectors.

Spring Peak Metal Concentrations in Lakes

We examined the input of snow melt-water into Hamell and Thompson lakes (Figure 1) in the spring of 1977. The snowmelt occurred over a period of about one week in early spring, approximately three weeks prior to break-up of the ice. Thin layers of waters under the ice were sampled with a vacuum-operated tubing apparatus described by Franzin & McFarlane (1980a) which allowed continuous collection of water at different depths.

April, 1977 profiles of total Zn in waters immediately below the ice of Thompson Lake (Figure 2) show a typical spring pattern of metals input into lakes in the Flin Flon area. Coincident with elevated concentrations of Zn, Cd and Pb in the surface waters of the lakes (Franzin & McFarlane, 1980a) was a decline in specific conductance (Figure 3). We attributed both observations to an influx of snow melt-water from the lake surface, through cracks and holes in the ice, into the water column below. We observed no depression of pH in waters under ice but this was not unexpected since the surface snows were not markedly acidic. Our samples of melt-water runoff from the lakes' basins indicated that it was unlikely that runoff was a major source of elevated metal concentrations in the lakes since the runoff was small in volume at the time as well as relatively lower in metal concentrations than melted snow from the lakes' surfaces. Similarly, freeze-out of metals during ice formation or melting of the ice were eliminated as potential sources for the elevated metal concentrations since no changes in metal concentrations were observed prior to the snowmelt and ice was lower in metal concentration than the waters adjacent.

Variable proportions of the total concentrations of the different metals in snow were judged to be in "soluble" form as determined by filtration of precipitation samples through 0.45μ membrane filters. The portions of total Zn, Cd and Pb in precipitation samples which passed through this type of filtration were sufficient to explain the higher concentrations of these metals in waters under the ice of both lakes (Franzin & McFarlane, 1980a).

The spring surges in metal concentrations we have observed in these lakes represent potential chemical stress to biota in the lakes. Peak Zn concentrations were as much as 30 times normal concentrations in Thompson lake and 2 to 3 times the high normal background Zn concentrations in Hamell lake. Although these surges are relatively short in duration they occur during final gamete maturation for spring spawning fish such as northern pike, walleye and white suckers. High concentrations of these metals, in laboratory tests, have been shown to deleteriously affect reproduction in some fish species (Benoit *et al* 1976; Bengtsson, 1974; Brungs, 1969).

Smelter Metals in Lake Ecosystems

The physical and chemical environments of the six lakes in which the distribution of smelter metals were studied (Figure 1) are shown in Table III. Concentrations of Zn, Cd and Cu were monitored in the water columns of Hamell and Thompson lakes only for over a year while water samples,

sediment cores, a single plant species, surface plankton samples and population samples of two fish species were collected only once, during August, 1976, from all six lakes. Table IV shows the concentrations of smelter metals in physical and biological (other than fish) compartments of the six lake ecosystems. Deposition data in this table were calculated from deposition curves (Franzin *et al* 1979) rather than measured. Our year-long study of depth profiles of Zn, Cd and Cu concentrations in Hamell and Thompson Lakes (unpublished data) revealed that 3m water samples collected in mid-August approximated yearly average metal concentrations for these lakes. Therefore we have assumed that our August water samples from the other four lakes are reasonable estimates of their relative levels of contamination. It appeared from these data that metal concentrations in the physical compartments of lake ecosystems (water, sediment) were functions of metal deposition and lake area or morphometry possibly tempered to a degree by differences in water and sediment chemistry. Biota metal concentrations, on the other hand, did not reflect accurately the concentrations of metals in the physical system. Rather it appeared that biota metal concentrations all were modified similarly by some factor(s) which caused or allowed some biota to accumulate fairly high metal concentrations in relatively uncontaminated lakes and vice versa e.g. compare Nesootao and Hook lakes. This factor(s) may be the Ca concentration of the lake waters and will be discussed further in a later section.

Metal Uptake by Fish

Metal uptake by two species of fish, northern pike and white sucker, was assessed in five of the study lakes (Figure 1, Lake 6 excepted). Approximately 20 individuals of each species were selected from catches to provide several individuals each in about five size/age classes. We considered age to be the most important characteristic of the samples since this could be interpreted directly as exposure time. Screening of tissue analyses from the Hamell Lake samples revealed that only Cd in white sucker livers and Cd, Cu and Hg in northern pike livers showed appreciable increments in concentration with increasing fish age. Only these analyses were undertaken in the other four lakes.

Covariance analysis of length at age was conducted on each group of species samples to ensure that the fish population samples were similarly exposed and sufficiently similar in growth parameters for further comparisons (Figure 4). No differences were found among the white sucker samples but the Thompson Lake northern pike samples showed significantly slower growth than the samples from the other four lakes. Since this was our "control" population the interpretation of metal accumulation data was not seriously affected. Analysis of variance among sample ages showed no significant differences in the mean ages of the samples of either species.

Regressions of liver metal concentrations at age are shown in Figures 5 and 6. Covariance analysis was used to evaluate the data for differences among the samples in each group. Rates of increasing Cd concentration in liver with advancing age differed significantly in both species. Rates of increasing Cu concentration in livers of the three northern pike samples for which the trends were indicated differed significantly. No significant difference was found among the northern pike samples for rates of increasing liver Hg concentration with age but pike liver Hg concentration increased with advancing age in all five populations.

All of the curves of increasing liver metal concentrations with increasing age followed the normal distribution in our samples. Giesy & Wiener (1977) suggested that metal uptake that follows a normal distribution is indicative of some form of control by the organism. Since the liver is a major organ of detoxification and fish are known to possess the metal-sequestering protein metallothionein (Friberg *et al* 1971) these metals may be detoxified by sequestration in the liver. This could account for our observations of increasing concentrations of Cd in livers of white suckers and Cd, Cu and Hg in livers of pike with increasing age particularly if the sequestered product was stored in the liver.

Our data indicated liver metal concentrations that were contrary to expected concentrations relative to the degree of contamination of the five lakes. The curves of liver metal concentration at age in fish from these lakes fall into two groups, particularly for Cd and Hg. One group (Cliff, Hamell, Nesootao) shows high slopes of increase while the other (Hook, Thompson) shows low or negative slopes. We found no correlation of liver metal concentration with any of the measured environmental variables. However it appeared that liver metal concentration was affected by two unrelated factors - the amount of deposition (=contamination) and the Ca concentration of the lake waters. For example Nesootao lake was relatively uncontaminated, had a Ca concentration of 10 mg/l and a high rate of fish liver metal uptake whereas Hook Lake was highly contaminated by deposition and high sediment metals, had a Ca concentration of 39 mg/l and low fish liver metal uptake. The effect of different levels of contamination at similar Ca concentrations is indicated by comparison of Cliff and Hamell Lakes with Thompson Lake. These data while too limited for statistical validation suggest that the Ca concentration of lake waters may have a modifying role in fish liver metal uptake. Christopher Wren (University of Guelph, personal communication) has made a similar observation in his studies of lakes and marshes in the Parry Sound area of Ontario. He found that Hg concentrations of liver and muscle of several fish species as well as whole clam homogenates from environments of similar Hg concentration varied as a function of the Ca concentration of the waters which ranged from 2 to 28 mg/l. Our observations of like trends in metal concentrations in one plant species and surface plankton samples (previous section, Table IV) in the Flin Flon lakes suggest that this phenomenon may be a general one. Laboratory studies (Carroll *et al* 1979; Kinkade and Erdman, 1975) have shown that Ca ion is a major source of protection against uptake and toxicity of Cd in fishes rather than the Ca CO_3 system. The mechanism for this effect is poorly understood but may involve competition between many metals and Ca for cellular binding sites (Zitko & Carson, 1976) or an "accidental uptake mechanism" by a Ca regulating mechanism (Wright, 1980).

Effects of Metals on Fish Populations

Populations of white sucker, a relatively unexploited fish species were studied in Hamell (contaminated) and Thompson (uncontaminated) lakes to determine if high concentrations of Zn, Cu and Cd induced effects on fishes at the population level (McFarlane & Franzin, 1978). Both lakes (Figure 1) are in the vicinity of the smelter but Hamell Lake has received and continues to receive much heavier atmospheric deposition than Thompson Lake (Table IV). The lakes are similar chemically (Table III) (other than in smelter metals) and in area but Thompson Lake is nearly twice the mean depth and volume of Hamell Lake. Fish were obtained during two successive summers by trapnet and gill netting. During these periods growth rates,

mortality rates, longevity, fecundity, spawning success and catch rates of the two populations were monitored. Hamell Lake white suckers grew significantly faster than those from Thompson Lake after age 2 but did not live as long, reaching a maximum age in Hamell Lake of about 9 years compared to 13 years in Thompson Lake. Similarly the mean ages of the samples were significantly different at 4.27 and 6.22y respectively. Mortality rates of the adult fish of the two populations, directly comparable only for 8 and 9 year-olds, were similar at about 0.8. However catch per unit effort of adult fish indicated a much smaller population in Hamell Lake. The fecundity of Hamell Lake white suckers was significantly greater than for Thompson Lake fish (Figure 7) but at the same time the eggs of Hamell Lake fish were significantly smaller. Spawning success was observed to be reduced in the Hamell Lake population with difficulties occurring in timing between the sexes and in the release of eggs (Figure 8). As a result of this, catch per unit effort of young-of-year fish in Hamell Lake was very much less than Thompson Lake, indicating prenatal or larval mortality. The concentrations of Zn and Cd in Hamell Lake were within the range of concentrations known to have effects on spawning success in laboratory studies (Benoit *et al* 1976: Brungs, 1969; Bengtsson, 1974: Eaton, 1973) with spring peak concentrations in waters under ice exceeding acutely toxic limits for many species. White suckers, being spring spawners, were exposed to these very high concentrations just prior to their spawning period. Water quality, in terms of life support parameters was adequate throughout the year. Our observations, we believe, indicate that the white sucker population in Hamell Lake has shown a distinct response to elevated concentrations of metals, particularly Zn, and Cd. Nicholson (1957) in his famous studies of blow-fly populations demonstrated that stress that increases the mortality rate of individuals in a specific age-class is made up for by compensatory adjustments in reproductive rates and mortality rates of other age-classes resulting in increases in the proportion of individuals in the age-class just previous to the class most severely/stressed. Reduced spawning success and increased larval mortality indicate that the eggs and young are the age group most severely stressed. Therefore the expected compensatory adjustment, and the one we observed in Hamell Lake white suckers, would be increased fecundity.

SUMMARY

The effects of accelerated inputs of several metals in atmospheric fallout into lake ecosystems near a base metal smelter provide a base from which to predict long term effects on remote lakes of low level metal deposition from long range transport. The effects one chooses to measure are dictated by the constraints of measurement by instrumentation in the case of metal uptake by the physical and biological components of the environment, and by the degree of sophistication of the tests and time for assessment of biological effects. Since biological effects may not be noticed in brief surveys of impacted lakes it may be necessary to consider possible effects predicted from areas of intense research and severe industrial impact. At Flin Flon, we have observed in lakes close to the smelter, the effects of gross additions of comparatively toxic metals and in a short term study were able to show moderate to severe stress on resident fishes. The possible role of Ca ion as an ameliorator of metal toxicity and uptake has an important place in research on long term inputs of metals (and acid) into remote lakes, especially those on poorly buffered granitic basins.

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Table I. Total metals deposited within circular areas of radius x around the Flin Flon smelter as determined by integration of curves $y=ax^b$.

Metal	a	b	x (km)	Metal deposition (y) at distance x (mg/m^2)	Metal deposition in area - x^2 (tonnes)
a) August, 1976 - July, 1977					
Zn	15371	-1.7285	264	1	1616
Cd	23	-1.3716	284	0.01	8.0
Pb	155	-1.1284	87	1	55
As	60	-1.6714	46	0.1	4.0
Cu	509	-1.5196	60	1	48
b) November 2, 1976 - February 2, 1977					
Zn	7269	-1.6354	217	1	850
Cd	15	-1.5349	113	0.01	2
Pb	72	-1.1299	44	1	14
As	1	-1.0600	68	0.1	0.4
Cu	190	-1.5048	33	1	14

Table II. Metal deposition at Flin Flon and elsewhere.

Location	Zn	Cd	Pb	As	Cu	Reference
	($mg.m^{2-1}.y^{-1}$)					
Flin Flon, Channing (4.3 km from smelter)	1780	4.5	42	6.2	57	Franzin et al. (1979)
Wollongong, NSW Australia, (2.3 km from smelter)	130	2.0	36	-	80	Beavington (1977)*
Copenhagen, Denmark (average of rural, suburban and urban estimates)	82	-	50	-	7.3	Andersen et al. (1978)
Chadron, Nebraska, USA (non-industrial town of 5,000)	4.0	0.12	1.9	-	1.8	Streumpler (1976)
New Hampshire, USA (remote Montaine site)	-	0.87	19.6	-	-	Schlesinger et al. (1974)

* similar sampling apparatus used.

Table III. Physical descriptions and selected water chemistry parameters of the study lakes.

Lake	Hamell	Cliff	Hook	Nesootao	Thompson	Lake 6
Surface area (ha)	203	246	90	40	220	19
Distance from smelter (km)	4.2	4.0	9.5	12.5	20.2	5.2
Maximum depth (m)	6	30*	12*	10*	12	5
pH	7.8	8.1	8.4	8.2	7.9	8.0
SO_4 (mg/L)	13.6	14.4	15.6	12.2	5.6	11.8
Cl (mg/L)	3.2	2.0	3.2	0.8	1.4	1.4
Ca (mg/L)	14.9	16.2	38.8	10.0	14.7	15.0
Mg (mg/L)	2.98	3.97	7.73	2.37	4.30	3.64
Na (mg/L)	1.30	1.78	2.35	1.67	1.93	0.97
K (mg/L)	1.48	1.51	1.48	1.31	1.34	0.91

All chemical parameters measured August, 1976.
* Estimated.

Table IV. Distributions of smelter metals in deposition and various components of the ecosystems of six lakes near the smelter at Flin Flon, August 1976.

Lake	Metal						
	Zn	Cu	Cd	Pb	As	Mn	Fe
			Deposition $mg \cdot m^{-2} \cdot y^{-1}$				
Cliff	1400	62	3	32	6	-	-
Hamell	1287	58	3	31	6	8	241
Lake 6	889	42	2	24	4	-	-
Hook	313	27	2	27	3	4	74
Nesootao	195	11	0.7	9	0.9	-	-
Thompson	87	5	0.4	5	0.4	5	36
			Water $\mu g \cdot L^{-1}$				
Lake 6	223	10	0.3			11	-
Hamell	220	14	0.7			26	69
Cliff	124	11	0.4			5	-
Nesootao	80	7	0.3			14	-
Hook	45	4	0.6			11	-
Thompson	15	20	0.1			41	-
			Sediment $\mu g \cdot g^{-1}$ dry wt.*				
Lake 6	3766	902	28	575	206	889	13455
Hamell	1986	442	16	274	72	175	8145
Hook	987	242	6	131	39	342	17775
Cliff	803	174	5	72	22	291	22323
Thompson	133	188	0.8	225	5	348	16352
Nesootao	110	29	0.8	24	9	113	4643
			Surface plankton $\mu g \cdot g^{-1}$ dry wt.				
Hamell	2485	468	24	157		978	
Cliff	804	84	10	20		195	
Lake 6	384	53	9	9.3		73	
Nesootao	308	54	64	14.5		587	
Hook	199	21	4.8	8.7		128	
Thompson	163	144	1.9	8.8		729	
			Myriophyllum exalbescens $\mu g \cdot g^{-1}$ dry wt.				
Lake 6	12250	346	31	242	263	6265	8065
Hamell	1790	54	7	44	40	3150	1810
Nesootao	1470	75	9	24	32	4675	6040
Cliff	1375	90	10	20	48	1004	7980
Hook	848	21	4	15	13	2670	1485
Thompson	185	80	1	4	4	874	1505

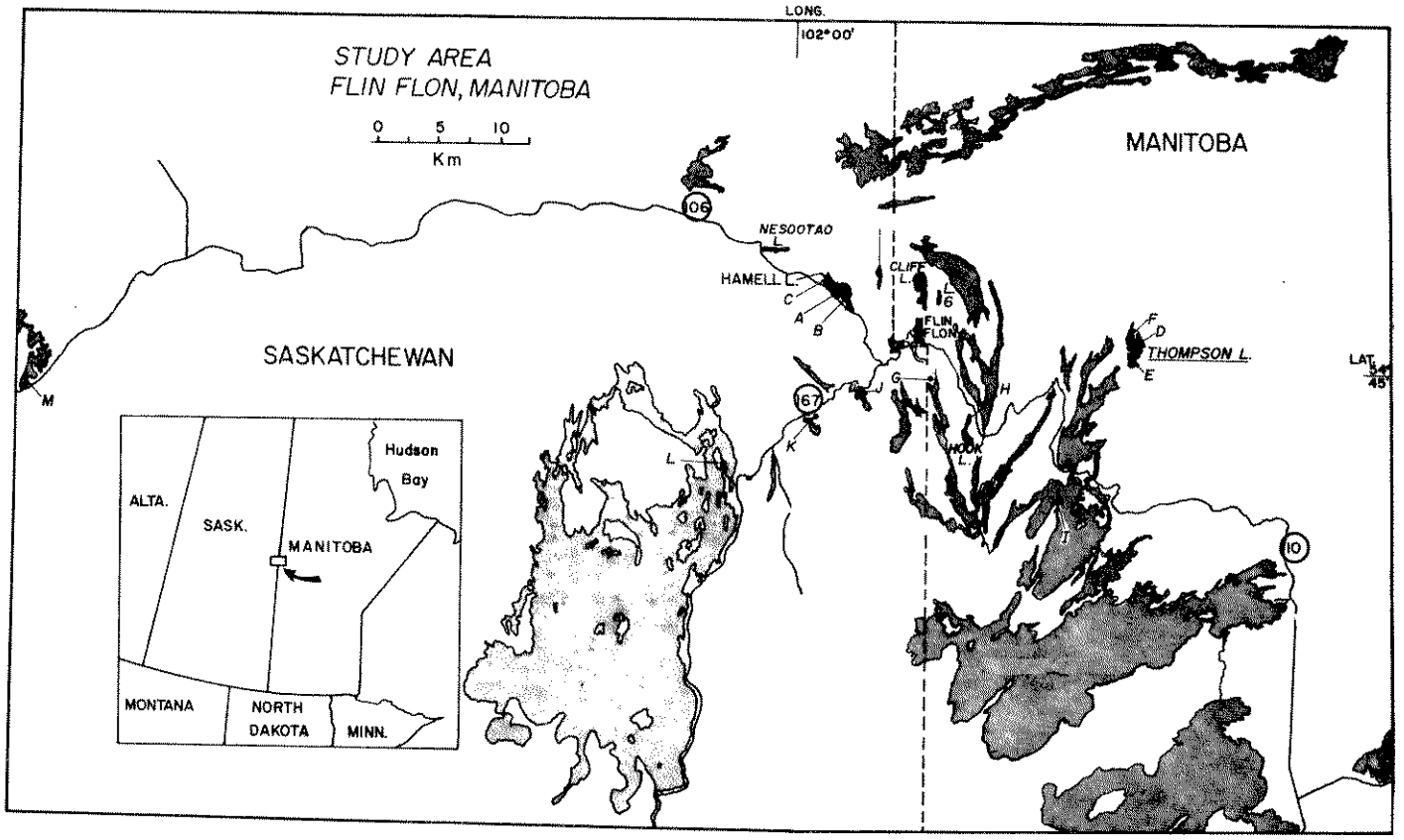

Fig. 1 Study area in the vicinity of Flin Flon, Manitoba. Snowpack samples were collected from all lakes shown while darkly-shaded and named lakes were studied more intensively. Capital letters indicate where precipitation collectors were located.

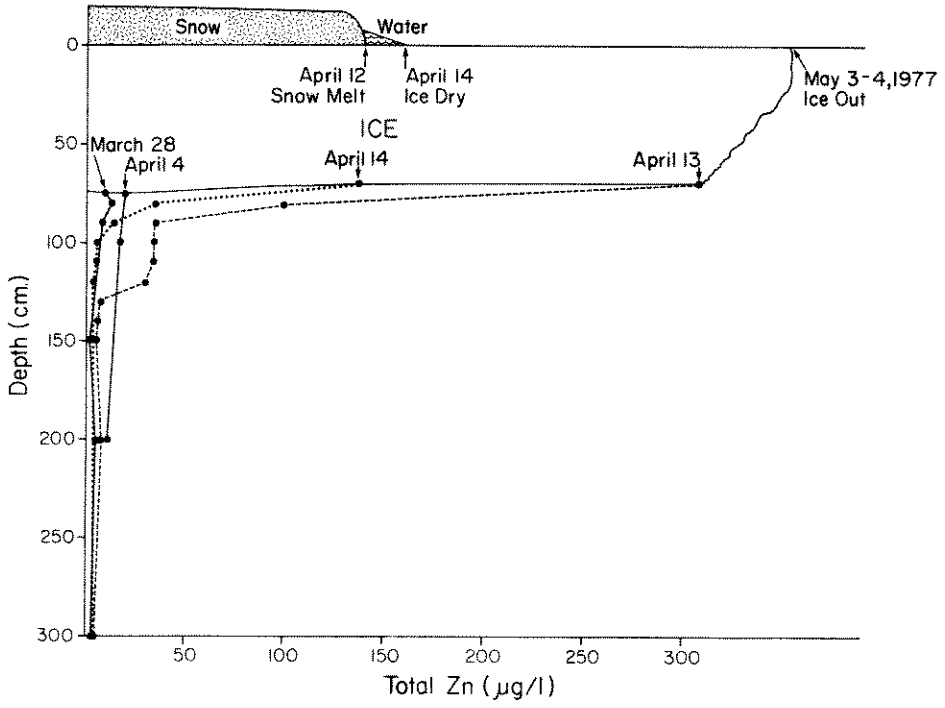

Fig. 2. Profiles of total Zn in Thompson Lake in early spring, 1977.

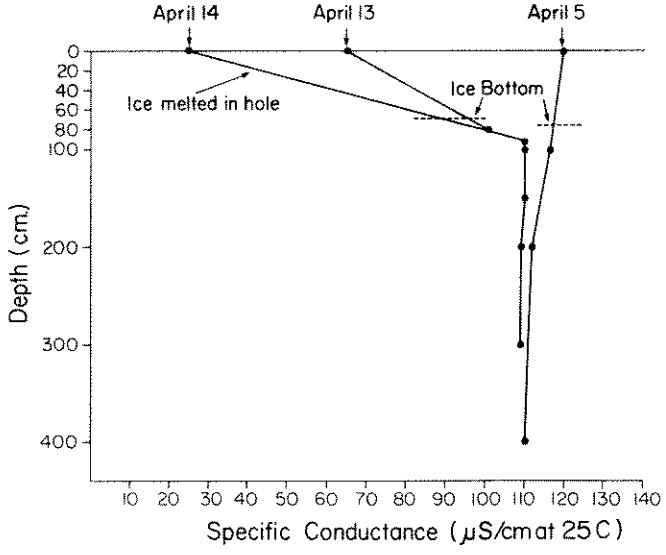

Fig. 3. Profiles of specific conductance in Thompson Lake in early spring, 1977.

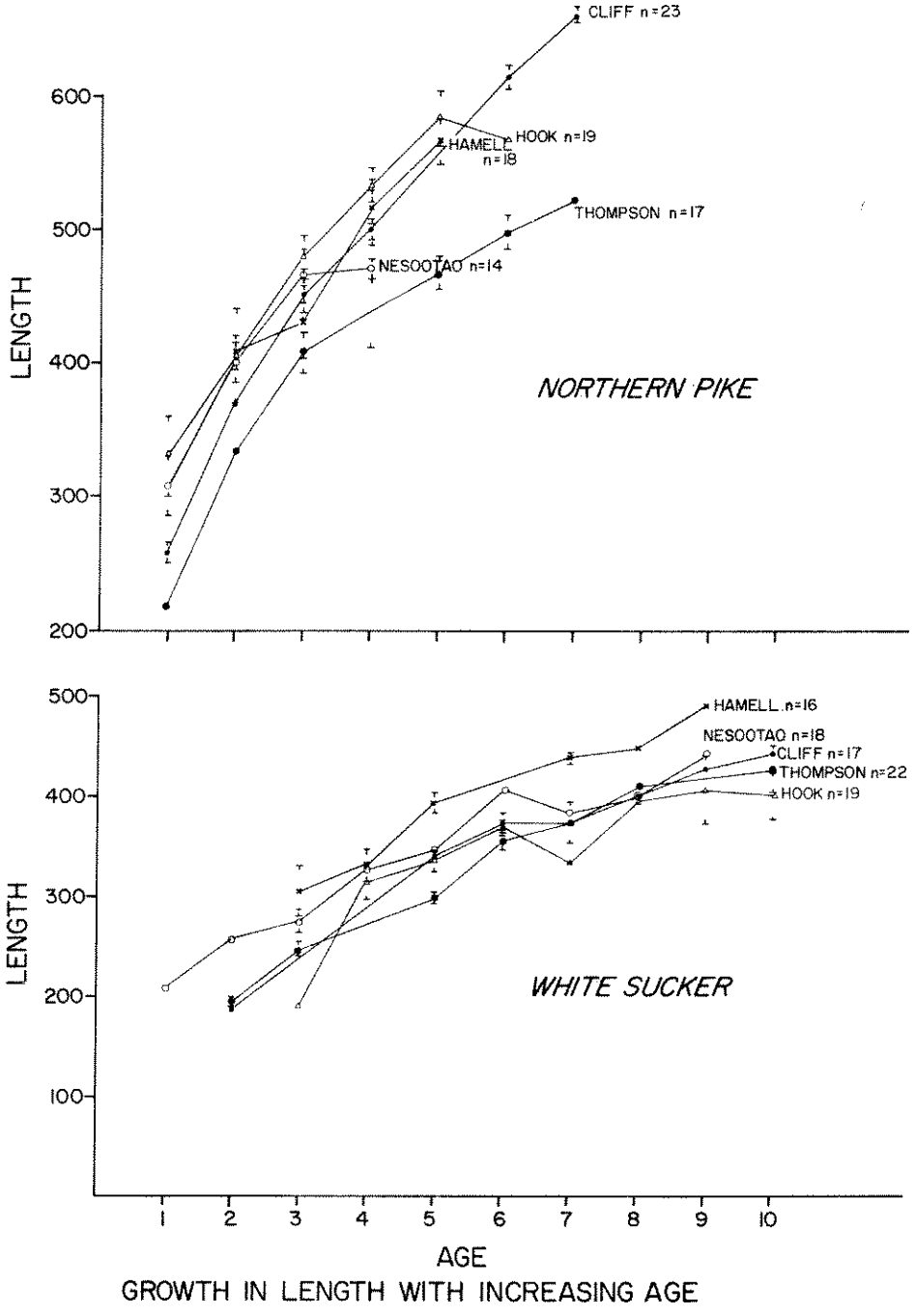

Fig. 4 Growth curves of white suckers and northern pike from five study lakes near Flin Flon.

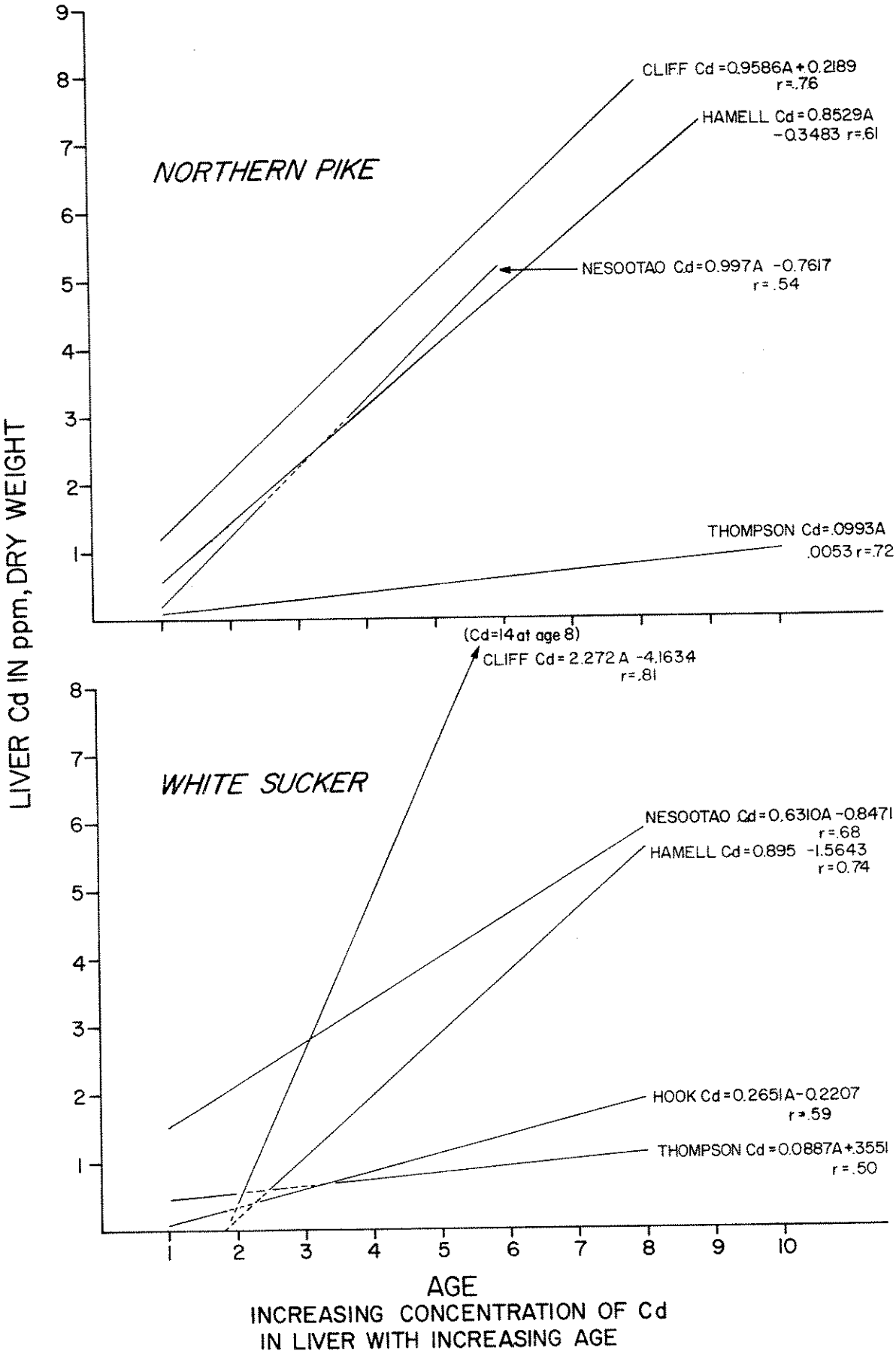

Fig. 5. Regressions of liver Cd concentration at age in northern pike and white sucker from the five study lakes.

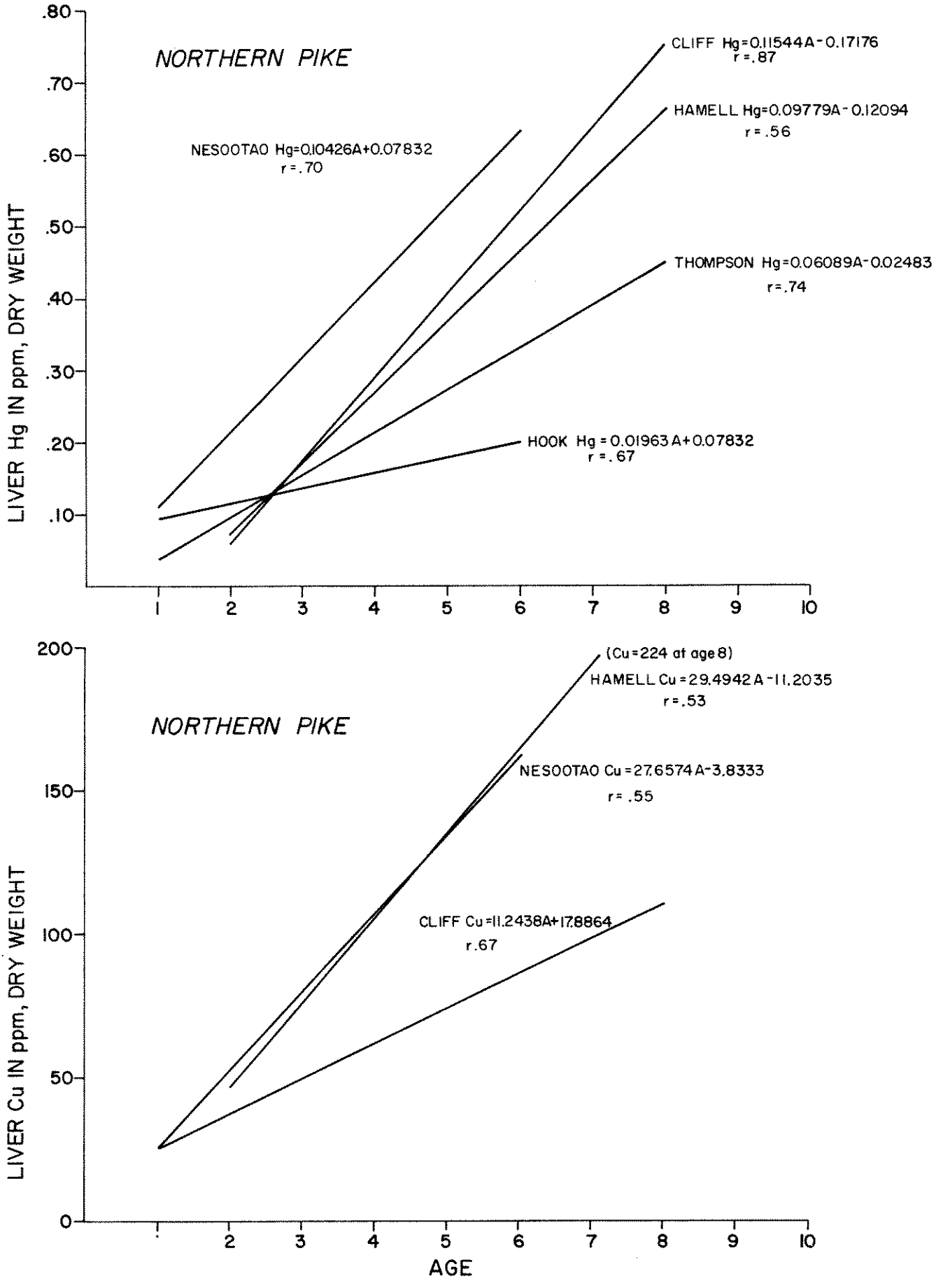

Fig. 6. Regressions of liver Hg and Cu concentrations at age in northern pike from three of the five study lakes.

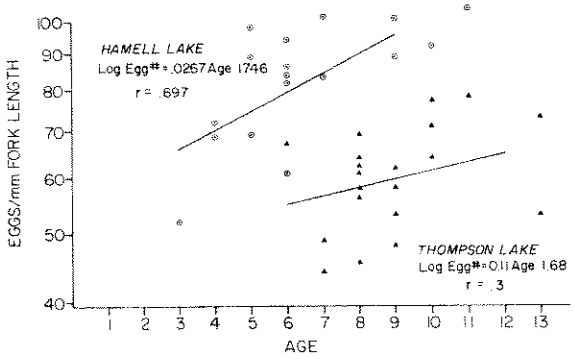

Fig. 7 Comparison of fecundity of Hamell Lake and Thompson Lake white suckers.

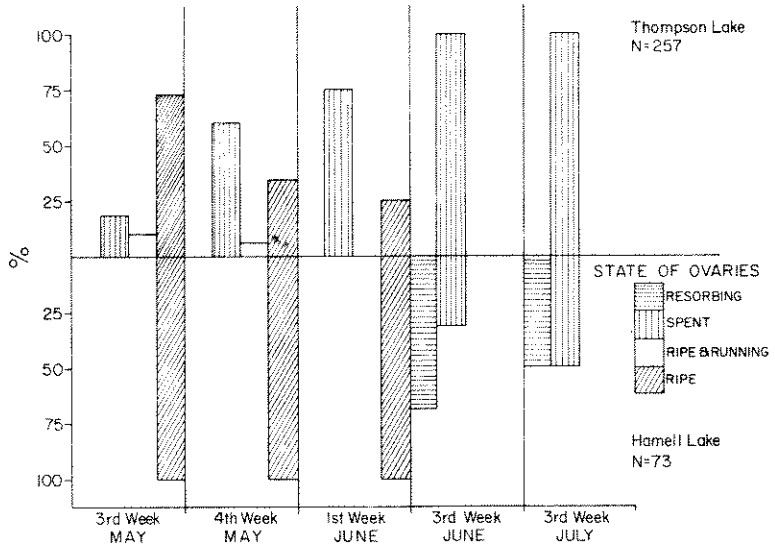

Fig. 8 Comparison of reproductive status of spawning female white suckers in Hamell and Thompson, May to July 1976, determined by state of ovaries.

DISPOSITIF VIDÉO-INFORMATIQUE POUR QUANTIFIER LES DÉPLACEMENTS À LA NAGE LORS D'ÉTUDES DE TOXICOLOGIE

Don C. Miller
U.S. EPA - Narragansett R.I

RÉSUMÉ - La quantification des réactions observées constitue l'un des principaux obstacles à la mise au point et à l'emploi courant de tests de comportement lors des études de toxicologie en milieu aquatique. La technique employée doit être assez exacte et assez précise, mais aussi assez rapide pour permettre la répétition des tests afin de pouvoir distinguer entre les changements provoqués par les polluants et la variabilité naturelle. Un dispositif vidéo-informatique en direct, interactif et à programme de présentation (le Bugsystem) a été mis au point et permet d'obtenir une certaine quantification du comportement lors d'études toxicologiques dans nos laboratoires. Ce dispositif comprend un circuit fermé de télévision, un mini-ordinateur (Data General Eclipse S/200) et une composante spéciale, un processeur vidéo-numérique permettant la conversion des images en une forme traitable par l'ordinateur. Les paramètres descriptibles de la nage comprennent le tracé et les vitesses de déplacements linéaires et angulaires, le sens des déplacements, l'orientation, ainsi que les rapports entre organismes pour des groupes de cinq à quinze organismes suivis à la fois.

Notre rapport décrit brièvement le fonctionnement du Bugsystem et délimite le champ de ses possibilités et ses limites pour des applications à la toxicologie en milieu aquatique. Nous montrerons ce qui peut être tiré de l'étude du comportement dans le cadre d'essais toxicologiques sur des protopléons de balane exposés au cuivre. Les résultats seront brièvement comparés à ceux d'études récentes sur les mysidées, les copépodes et les larves de crabe. La nage désordonnée est particulièrement modifiée par l'intoxication au cuivre, beaucoup plus que les comportements à la lumière. Les conséquences possibles des modifications d'activité observées sont étudiées par des tests prédateurs-proies.

A NEW VIDEO-COMPUTER SYSTEM TO QUANTIFY SWIMMING BEHAVIOR FOR TOXICOLOGICAL STUDIES

Don C. Miller
U.S. EPA - Narragansett R.I

SUMMARY - One obstacle to the development and routine use of behavioral tests in aquatic toxicology is the task of quantifying the observed responses. The quantification technique should not only be fairly accurate and precise, but also be rapid enough to permit sufficient test replications in order to distinguish pollutant-induced changes from natural response variability. An interactive, interpretive, on-line computer television system (vis. the Bugsystem) has been developed as a quantification tool for behavioral toxicological studies at our laboratory. The system utilizes a standard closed circuit television system, a minicomputer (Data General Eclipse S/200) and one specially designed component, a video to digital processor, to convert the video data to a computer compatable form. Parameters of swimming which can be described include patterns and rates of linear and angular momentum, direction of travel, orientation, plus inter-organism relationships for groups of 5 to 15 organisms recorded simutaneously.

This paper will briefly outline the operation of the Bugsystem and illustrate some of its capabilities and limitations for aquatic toxicology. The contribution of behavioral effects information from a toxicology protocol will be described for a study with barnacle nauplii and copper. These results will be briefly compared with results of current studies with mysid shrimp, copepods and crab larvae. Undirected swimming activity is particularly sensitive to copper; photobehavior less so. Possible consequences of observed shifts in activity are being explored in predator-prey tests.

A New Video-Computer System to Quantify Swimming Behavior for Toxicological Studies

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Narragansett, R.I., U.S.A.

ADDENDUM TO ABSTRACT

One obstacle to the development and routine use of behavioral tests in aquatic toxicology is the task of quantifying the observed responses. The quantification technique should not only be fairly accurate and precise, but also be rapid enough to permit sufficient test replications in order to distinguish pollutant-induced changes from natural response variability. An interactive, interpretive, on-line computer television system (vis. the Bugsystem) has been developed as a quantification tool for behavioral toxicological studies at our laboratory. The system utilizes a standard closed circuit television system, a minicomputer (data General Eclipse S/200) and one specially designed component, a video to digital processor, to convert the video data to a computer compatible form. Parameters of swimming which can be described include patterns and rates of linear and angular momentum, direction of travel, orientation, plus inter-organism relationships for groups of 5 to 15 organisms recorded simultaneously.

We have used the Bugsystem to monitor behavior during 96 h assays with barnacle larvae exposed to copper and cadmium. The parameters measured include survival, rate of development from stage II to stage III nauplii, post-molt morphology, spontaneous swimming speed and photobehavior.

Copper (40, 80 ppb) and cadmium (50 ppb) initially (24 h) cause hyperactivity, followed by hypoactivity (20 ppb Cu, 50 ppb Cd). The magnitude and temporal aspects of this response is dose dependent. Copper also alters larval phototaxis. In 24-48 hrs, intermediate concentrations (40 ppb) will reduce + phototaxis, while at higher concentrations (80, 160 ppb) there is a reversal of the phototactic sign to negative.

Significant changes in swimming speed occurred at concentrations of copper comparable to those which delay development and, for copper, were just slightly above the 10 ppb level which impacts post-molt morphology. Reduction of + phototaxis is a relatively sensitive response and may be an ecologically significant test as it demonstrates an important sublethal effect of copper. Further, each of these deviations of swimming speed from control levels may render the larvae more susceptible to predation; reduction in swimming ability would also jeopardize a zooplankter's ability to remain in the water column.

The Bugsystem has proven to be a powerful and versatile tool to quantitatively document motile behaviors. It may be employed as a research tool to further elucidate ways that pollutants impact behavioral response systems. It can also be utilized for routine testing. System costs may be as low as $20,000 for laboratories with minicomputer facilities.

ÉVALUATION À L'AIDE DE LIMNO-CORAUX DE L'IMPACT D'UN PYRÉTHROÏDE, LA PERMÉTHRINE, SUR UN ÉCOSYSTÈME LACUSTRE
II. EFFETS SUR LE ZOOPLANKTON

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RÉSUMÉ - L'action sur un écosystème aquatique de la perméthrine, un pesticide, a été évaluée sur des limno-coraux à l'intérieur d'enceintes flexibles de grand volume (125 m^3), dans un bassin d'un petit lac du sud de l'Ontario. Trois limno-coraux ont été traités avec 50 µg/L de perméthrine (qualité technique), trois autres ont été traités avec 5 µg/L de perméthrine, et trois autres ont servi de groupe témoin. La composition taxonomique et la densité des populations zooplanctoniques de tous les coraux ont été évaluées toutes les semaines entre août 1979 et avril 1980. Les auteurs ont obtenu une très bonne corrélation quand les tests ont été répétés.

De 40 à 400 animaux x 10^3 m^{-3}, la population de crustacés a périclité dès l'application de la perméthrine aux deux concentrations ci-dessus. Les populations coraliennes décimées par la concentration la moins forte se sont rétablies à peu près au bout de deux mois. Les populations soumises à la concentration élevée ne s'étaient pas encore rétablies lors de l'engel en novembre 1979.

Les populations de rotifères n'ont pas été diminuées par suite de l'exposition aux faibles concentrations de pesticides et leur nombre est passé de 1 000 à 2 500 x 10^3 m^{-3} une semaine après le traitement. La perméthrine en forte concentration avait une faible action sur les populations de rotifères, provoquant une légère baisse de leurs nombres au commencement. Cependant, deux mois après le traitement, la population atteignait un maximum de 7 000 x 10^3 m^{-3}. Les causes probables sont une diminution de la prédation ou de la compétition

exercée par les autres populations zooplanctoniques tuées par le pesticides.

Les résultats obtenus à partir des échantillons du printemps suivant seront aussi étudiés.

EVALUATION OF IMPACT OF A SYNTHETIC PYRETHROID, PERMETHRIN, ON A LAKE ECOSYSTEM USING LIMNOCORRALS
II. EFFECTS ON ZOOPLANKTON

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SUMMARY - The effect of a pesticide, permethrin, on an aquatic ecosystem was studied in large-volume (125 m^3), flexible enclosures (limno-corrals) located in one basin of a small Southern Ontario lake. Three limnocorrals were treated with 50 Hg/L of permethrin (technical), another three received 5 Hg/L and three remained as controls. The taxonomic composition and abundance of the zooplankton populations contained in all corrals were studied on a weekly basis during Aug. 1979 - April 1980. Replication in corrals treated in a similar manner was shown to be very good.

Immediately following application of both concentrations of permethrin, populations of crustaceans declined drastically from 40 - 400 animals x 10^3 m^{-3}. Recovery of populations in the low concentration corrals occurred within approximately 2 months following application. Populations in the high concentration corrals showed no recovery at the time of ice cover in Nov. 1979.

Populations of rotifers were not reduced by the low concentration of pesticide, and, numbers increased from 1 000 animals x 10^3 m^{-3} to 2 500 animals x 10^3 m^{-3}, one week following treatment. The high concentration of permethrin had a slight effect on population of rotifers causing an initial drop in numbers. However, 2 months following treatment, numbers of rotifers peaked to approximately 7 000 animals x 10^3 m^{-3}. The probable causes are lack of predation and/or competition by other zooplankton populations reduced by the pesticide.

Results from the following spring samples will be discussed.

LE PHOTOTROPISME CHEZ LES CRUSTACES UTILISE COMME INDICATEUR EFFICACE DE L'EXPOSITION CHRONIQUE A DES METAUX OU: Y A-T-IL DE L'ESPOIR?

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Universite de Guelph

RESUME - On a émis l'hypothèse que les changements sublétaux de comportement constituent l'un des indicateurs les plus efficaces de l'action des substances toxiques. Ainsi, nous nous demandons si une réponse comme la phototaxie manifestée par le zooplancton crustacéen, très marquée et importante sur le plan écologique, ne pourrait pas constituer un moyen très efficace d'évaluer la toxicité de diverses substances dans l'eau. Nous avons étudié par des expositions chroniques de 16 jours les effets du plomb et du cadmium sur le phototropisme de trois espèces étudiées dans la nature et d'une espèce étudiée en laboratoire. Nous avons choisi l'intensité lumineuse et la longueur d'ondes de façon à maximiser la phototaxie des groupes témoins, après avoir expérimenté pour chaque espèce 8 seuils d'intensité pour chacune de 8 longueurs d'ondes. Nous avons arrêté notre choix d'une température commune d'expérimentation après avoir évalué complètement la toxicité aigue du plomb et du cadmium à plusieurs températures choisies dans la plage normale des températures in situ de chaque espèce.

Dans presque tous les cas, il a suffi d'un écart de la concentration des métaux de 2 ordres de grandeur pour s'assurer après 16 jours, dans chacune des trois séries de tests, qu'au moins une des concentrations ne suscitait pas de réponse tellement différente de celle des témoins, alors qu'au moins une autre concentration induisait une diminution de plus de 50% de la phototaxie. Les organismes ont mis du temps à répondre au stress que constituent les métaux en faibles concentrations. Les tests de plages multiples ont montré qu'il fallait au moins huit jours (et plus souvent qu'autrement, seize jours) avant que

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les groupes expérimentaux ne manifestent une réponse distincte de celle des groupes témoins. Après 16 jours d'exposition, toutes les courbes du tropisme en fonction des métaux s'exprimaient sur échelle log-log par des droites; les coefficients de corrélation rendaient les résultats très significatifs et il y avait des changements significatifs des pentes et des coordonnées à l'origine entre les espèces et les métaux. La compilation des concentrations efficaces a montré que le degré de sensibilité aux espèces était à peu près le même pour les deux métaux, que le cadmium était en moyenne 5 fois plus toxique que le plomb, que les populations de l'hypolimnion froid étaient beaucoup plus sensibles que les populations de l'épilimnion chaud, ou que les sujets de laboratoire; enfin, les phototropismes sont modifiés par de très faibles concentrations de métaux, des concentrations égales à 0.025 de la CL50-96 h provoquant des différences significatives en 16 jours. Les concentrations du plomb provoquant des écarts significatifs étaient de l'ordre de 1.25 µg/L alors que celles du cadmium étaient de l'ordre de 0.16 µg/L. Une série d'expériences portant sur la réponse en fonction du temps a montré que le plomb agit davantage sur la réponse physiologique à la lumière alors que le cadmium agit davantage sur la vitesse de la réponse à un photo-stimulus.

THE CRUSTACEAN PHOTORESPONSE AS A SENSITIVE INDICATOR OF CHRONIC METAL EXPOSURE, or/ IS THERE ANY LIGHT AT THE END OF THE TUNNEL?

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University of Guelph

SUMMARY - It has been suggested that sublethal changes in behaviour patterns represent some of the most sensitive indicators of toxicant effects. It is, therefore, suggested that such a strongly-developed and environmentally-important response pattern as the phototaxis of crustacean zooplankton could provide a very sensitive basis for aquatic toxicity evaluations. The effects of lead and cadmium on the photoresponses of three field species and one lab. species were studied during 16 days chronic exposures. The selection of intensities and wavelengths for 8 intensity response surfaces for each species. The selection of a common test temperature was based on a complete acute toxicity evaluation of both lead and cadmium at several temperatures covering each species' normal in situ range.

In almost all cases, a 2 order-of-magnitude range in metal concentrations satisfied the requirements that at least one metal concentration show no significant difference from the controls, and that at least one metal concentration effect a greater-than 50% reduction in the light response in each of three replications after 16 days. The metal response in the low concentrations used in this study was slow to develop. Multiple range tests indicated that at least 8 (and more often 16) days were required for a clear response distinct from controls to develop. After 16 days exporure, all metal-response relationships were log-log linear with highly significant correlation coefficients and some significant variations in slope and intercept between species and metals. Tabulation of effective concentrations indicated that the species order of sensitivity was the same for both metals, that cadmium was, on average, approximately 5 times more toxic than lead, that cold hypolimnetic animals are much more sensitive than warm epilimnetic or lab. animals, and that photoresponses are extremely sensitive to very low metal concentrations,

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with 16 days significant effect concentrations as low as .0025 of the 96hr.LC50. Significant effect concentrations for lead were as low as 1.25 µg/l while those for cadmium were as low as 0.16 µg/l. A series of timeresponse experiments indicated that lead had a greater effect on the physiological light response, while cadmium had a greater effect on the rate of response to a light stimulus.

EFFET DU CONDITIONNEMENT ALIMENTAIRE ET DE LA TENEUR EN GLUCIDES ACCESSIBLES SUR LA TOLERANCE AU CUIVRE DANS L'EAU DE LA TRUITE ARC-EN-CIEL.

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RESUME - Quatre groupes de poissons dont le poids moyen au début des expériences était d'à peu près 2.9 g ont été nourris pendant 6 à 7 semaines suivant un régime isocalorique et isoazoté, mais ont reçu des rations alimentaires complétées par 0 ou 21% de cérélose (d-glucose). La concentration létale liminaire de cuivre pour le groupe recevant le régime pauvre en glucides était de 408 µg/L, soit beaucoup plus que les 246 µg/L pour les groupes du régime riche en glucides.

Les deux autres groupes étaient à un régime qui prévoyait à peu près 20% de glucides sous forme d'amidon, mais dont les portions alimentaires étaient obtenues soit par extrusion, soit par granulation à la vapeur. Le conditionnement des aliments par extrusion se fait dans des conditions de température, d'humidité et de pression plus élevées que celles de la granulation à la vapeur et accroît, en outre, la disponibilité des glucides.

La concentration létale liminaire de cuivre pour les poissons nourris aux granules préparés à la vapeur était de 350 µg/L, c'est-à-dire qu'elle était beaucoup plus élevée que les 276 µg/L des poissons nourris aux aliments préparés par extrusion. Le glycogène du foie et les rapports masse du foie/masse corporelle variaient proportionnellement à la concentration de glucides disponibles. Il ressortait nettement une corélation significative entre la tolérance au cuivre et la concentration

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de glycogène du foie (suivant des équations de régression, la CL50 égale 424-15.8 (% de glycogène du foie), r^2= .997).

Les résultats montrent que le régime alimentaire peut avoir une action très importante sur la tolérance des poissons aux substances toxiques; en outre, ils fournissent peut-être des indices concernant une certaine variabilité des résultats d'études de toxicité entre laboratoires, de même qu'ils indiquent certaines irrégularités des résultats d'études sur le terrain et en laboratoire. Ces résultats peuvent aussi être employés pour optimiser la production dans les établissements pisicoles alimentés par des eaux de qualité incertaine, de même qu'ils peuvent servir à des programmes de repeuplement.

INFLUENCE OF DIET PROCESSING AND AVAILABLE DIETARY CARBOHYDRATE CONTENT ON THE TOLERANCE OF WATERBORNE COPPER BY RAINBOW TROUT.

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SUMMARY - Four groups of fish, with initial mean weights of approximately 2.9 g were maintained for 6 to 7 weeks on diets which were isocaloric and isonitiogenous but varied in carbohydrate content. The first two groups were fed diets supplemented with either 0 or 21% cerelose (d-glucose). The incipient lethal level (ILL) of copper for the low carbohydrate group was 408 µg/L, significantly higher than ILL of 246 µg/L shown by fish on the high carbohydrate diet.

The remaining two groups were fed diets that contained approximately 20% carbohydrate as starch, but which were processed by either extrusion or steam pelleting. Extrusion processing requires a higher level of heat, moisture and pressure than steam pelleting, and increases the carbohydrate availability of the diet.

The ILL of copper for fish on the steam pelleted diet was 350 µg/L, significantly higher than the ILL of 276 µg/L shown by fish receiving the extruded diet. Liver glycogen and liver-body weight ratios were directly proportional to available carbohydrate concentration. A significant correlation between copper tolerance and liver glycogen level was evident (regression equation LC50= 424 - 15.8 (% liver glycogen), r^2 = .997.)

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The results indicate that diet can have a significant impact on telerance of toxicants by fish, a potential explanation for some of the variability noted in inter-laboratory toxicity studies, as well as discrepancies in field-lab studies. The results may be applicable to optimizing production in hatcheries receiving water of marginal quality, as well as fish stocking programmes.

THEME II

Mécanismes de toxicité

Conférencier invité.

Toxic mechanisms

Invited speaker.

Dr. GABRIEL PLAA
Université de Montréal

RÉSUMÉ - L'évaluation des dangers pour la santé, associés à la présence de polluants chimiques dans les voies d'eau, suscite un intérêt croissant. Il y a lieu de considérer les interactions entre les divers agents chimiques. Nous avons étudié un de ces cas, soit l'interaction entre un pesticide (Mirex ou Kepone) et un haloalcane (le chloroforme). Chez la souris, le Kepone, mais non le Mirex, agit comme renforçant des propriétés hepatoxiques du chloroforme. Cette potentiation est due en partie à la production accrue d'un métabolite toxique du chloroforme. Par ailleurs, le Kepone semble aussi aggraver la susceptibilité des constituants cellulaires aux effets toxiques du chloroforme.

SUMMARY - There is a growing interest in evaluating the potential health risk involved because of the presence of chemical pollutants in waterways. Interactions between chemicals must be considered. We have studied such an interaction between a pesticide (Mirex or Kepone) and a haloalkane (chloroform). In mice Kepone, but not Mirex, potentiates the hepatotoxic properties of chloroform. This potentiation is due, in part, to an enhanced formation of a toxic metabolite of chloroform. However, Kepone also seems to enhance the susceptibility of cellular constituents to the toxic actions of chloroform.

INTERACTIONS POSSIBLES D'ORDRE TOXICOLOGIQUE DE DIVERS POLLUANTS DE L'EAU

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Durant la dernière décennie, un intérêt grandissant s'est fait sentir en regard de l'évaluation des risques impliquant les polluants présents dans nos cours d'eau. En Amérique du Nord plusieurs agences gouvernementales travaillent constamment à l'établissement de standards sécuritaires au point de vue de l'approvisionnement de l'eau. Un des problèmes majeurs lors de ces études réside dans le fait que les polluants que l'on retrouve normalement dans l'eau sont en présence d'autres substances. Ces interactions et leurs effets biologiques causent de sérieux problèmes en ce qui a trait à l'ensemble des évaluations des standards sécuritaires. Notre laboratoire s'est penché sur un des nombreux aspects de ce problème, i.e. l'interaction de deux insecticides (Mirex et Képone) et leurs effets sur les propriétés toxicologiques d'un polluant, un hydrocarbure retrouvé couramment, le chloroforme. L'aspect toxicologique qui nous intéresse ici est l'hépatotoxicité.

Avant de pouvoir déterminer s'il peut y avoir un risque dans des conditions spécifiques d'utilisation, il faut au préalable évaluer cette toxicité. Donc, nos études ont été planifiées en foction des conditions de laboratoire, lesquelles sont facilement contrôlables, et non en fonction d'un modèle qui mime les expositions réelles dans l'environnement. En effet, les pesticides ont été administrés à des souris par voie orale. Le chloroforme a été administré plus tard, de la même manière. Nous avons varié les doses de chaque agent dans le but d'obtenir de bonnes courbes dose-effet. Des doses finales ont été choisies de manière à observer et mesurer facilement les effets toxicologiques. Dans ces conditions, le Képone et non le Mirex, démontrait une potentialisation de l'hépatotoxicité causée par le chloroforme. Cette potentialisation était directement proportionnelle à la dose de Képone. Selon le degré de potentialisation de Képone, la dose-seuil du chloroforme était abaissée.

Pour ce qui est du mécanisme d'action, d'autres études nous ont montré que la potentialisation n'était due ni à une hausse générale du cytochrome P-450 ni à une augmentation de l'activité des oxydases à fonction mixte, dans le foie. Le Mirex augmentait le contenu en cytochrome P-450 et l'activité des oxydases à fonction mixte sans qu'il y ait potentialisation. Le Képone n'avait aucun effet sur ces deux paramètres mais potentialisait la toxicité du chloroforme. Cependant, seul le Képone augmentait la liaison covalente du chloroforme aux macromolécules hépatiques. La potentialisation due au Képone semble être due à une augmentation de la formation d'un métabolite toxique du chloroforme. Toutefois, les observations morphologiques dues à la potentialisation du Képone indiquent que la lésion obtenue n'est pas identique à celle causée par des doses élevées de chloroforme. Il

existe donc la possibilité que le Képone modifie aussi la susceptibilité de certains constituants cellulaires à l'action toxique de ce métabolite.

Le détail et les résultats de ces recherches ont été publiés dans les articles suivants:

W.R. Hewitt, H. Miyajima, M.G. Côté and G.L. Plaa: Acute alteration of chloroform-induced hepato and nephrotoxicity by Mirex and Kepone. Toxicol. Appl. Pharmacol. 48: 509-527 (1979).

D.J. Cianflone, W.R. Hewitt, D.C. Villeneuve and G.L. Plaa: Role of biotransformation in the alterations of chloroform hepatotoxicity produced by Kepone and Mirex. Toxicol. Appl. Pharmacol. 53: 140-149 (1980).

Maintenant il nous apparaît possible d'ébaucher de nouveaux protocoles expérimentaux qui s'avéreraient plus compatibles avec les conditions de l'environnement dans le but de déterminer si ces mêmes interactions peuvent être observées.

COMPARAISON DES REPARTITIONS A LONG TERME ET A COURT TERME POUR LE MERCURE METHYLE ET LE MERCURE INORGANIQUE CHEZ LA TRUITE S. GAIRDNERI

C. Thellen[1], G. Joubert[2] et R. Van Coillie[1]

R E S U M E

Afin de préciser certains facteurs déterminants pour l'absorption du mercure chez la truite arc-en-ciel, deux formes chimiques de mercure ($HgCl_2$ et CH_3HgCl) ont été utilisées lors d'expériences à long terme et à court terme menées en parallèle dans des conditions expérimentales contrôlées.

1. Une contamination de 1,0 ppm de mercure organique dans la nourriture s'accumule rapidement au niveau des tissus musculaires et des viscères des spécimens. Comparativement, le mercure inorganique présent dans la nourriture est peu accumulé.

2. Les deux formes de mercure peuvent être bioconcentrées lors d'expériences à long terme avec un excès continu sublétal d'environ 0,25 ppb de mercure. Leur distribution devient distincte dans l'organisme. De fait, l'accumulation du mercure organique est plus élevée dans les viscères que dans les tissus musculaires des spécimens. Par ailleurs, le chlorure mercurique, qui se localise principalement dans les viscères, se stabilise dans le tissu musculaire à une concentration environ deux fois moindre que celle de la forme organique.

1) C.I.L. (Canadian Industries Limited), Eco-Recherches, 121 boulevard Hymus, Pointe Claire, Québec, H9R 1E6

2) Ministère de l'Environnement du Québec, Complexe scientifique du Québec, 2700 rue Einstein, Sainte-Foy, Québec, G1V 4C7.

3. A court terme, le mercure inorganique s'accumule d'abord au niveau des branchies, des structures ostéoïdes et du foie et ensuite graduellement dans les muscles tandis que le mercure organique se fixe directement dans ceux-ci.

Ces constatations explicitent l'importance de trois facteurs dans l'absorption mercurielle chez les poissons, à savoir : forme chimique du mercure, mode de contamination, répartition versus temps dans l'organisme.

SUMMARY

Following the precise identification of the determinant factors in the absorption of mercury by rainbow trout, two chemical forms of mercury ($HgCl_2$ and CH_3 HgCl) were used to conduct simultaneous long and short term investigations under controlled experimental conditions.

1) A 1,0 ppm organic mercury contamination in the diet accumulates rapidly in the muscular tissue and organs of the specimens. Relatively little of the inorganic mercury in the diet accumulates.

2) The two forms of mercury can be bioconcentrated during long term investigations under a continuous sublethal exposure to 0,25 ppb of mercury. The distributions of the two forms can be distinctly seen in the organism. The accumulation of organic mercury is more pronounced in the organs than in the muscular tissue of the specimens. However, the mercuric chloride, which is also localized principally in the organs, is found in the muscular tissue in concentrations half the amount of those for the organic form.

3) In the short term, inorganic mercury accumulates externally in the branchial and osteoidal structures as well as the liver and afterwards in the muscle. Organic mercury is fixed directly in the muscles.

This facts explain the importance of three factors in the absorption of mercury in fish which are:

- chemical form of mercury,
- the contamination pathway,
- and the distribution with time in the organism.

INTRODUCTION

Bien que le cheminement du mercure dans l'environnement ait fait l'objet de nombreuses recherches depuis une quinzaine d'années (Krenkel et al, 1973; C.N.R.C., 1979), certains points demeurent encore peu connus. Entre autres, constate t'on des différences de répartition interne du mercure chez les poissons lorsque cet élément est imposé à long terme ou à court terme, sous forme inorganique ou organique et via la nourriture ou l'eau ? Les données à ce sujet sont assez limitées (Hannerz, 1968; Norstrom et al, 1975; Mc Kim et al, 1976; C.N.R.C., 1979), notamment pour la truite Salmo gairdneri (Gibblin et Massaro, 1973; Olson et al, 1973) qui a été choisie comme organisme-test pour les bioessais légaux d'écotoxicité par Environnement Canada. La présente publication vise dès lors à préciser la répartition du mercure chez cette dernière espèce en fonction de ces différents facteurs.

MATERIEL ET METHODES

a) expérimentation à long terme

Les conditions et procédures techniques adoptées pour celle-ci furent les suivantes :

- 50 truites Salmo gairdneri juvéniles/bassin
- eau : filtrée et traitée avec rayons ultra-violets
  : oxygène dissous entretenu à 8,6 ppm $\pm$ 1,2*

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* Ecart type

: température ajustée à 13° C ± 1,2*

: débit de 500 ml ± 19*/minute

: photopériode de 12 heures

: dureté de 45 mg ($CaCO_3$)/ml

: pH à 7,1 ± 0,3*

- nutrition quotidienne (poudre alimentaire appropriée) limitée à 2% de la biomasse piscicole présente dans le bassin et siphonage régulier des restes et excréments
- dans 2 bassins, addition de 1 ppm de CH_3HgCl à un débit de 0,5ml/minute afin d'obtenir une concentration de 0,25ppb de mercure méthylé à flux continu, laquelle fluctua en fait quelque peu (0,26 ppb** ± 0,1*) et était voisine du seuil de concentration maximale acceptable à long terme, tel que défini par Mc Kim et al (1973) à 0,29ppb
- dans 2 bassins, ajout de 1 ppm de $HgCl_2$ avec même débit pour avoir une teneur de 0,25 ppb de mercure inorganique à flux continu (celle-ci équivalait en fait à 0, 23ppb** ± 0,05*)
- dans 2 bassins, addition de 1 ppm de CH_3HgCl à la nourriture (on y détectait alors 1,06 ppm Hg**)
- dans 2 bassins, ajout de 1 ppm de $HgCl_2$ à la nourriture (teneur décelée en Hg : 1,12 ppm Hg**)
- dans 2 bassins, conditions témoins

** Spectrophotométrie en absorption atomique réalisée aux laboratoires de Pêches et Océans Canada à Longueuil (Québec) selon les techniques reconnues.

- durée de l'expérimentation : 211 jours (30 semaines)
- retrait de 5 poissons/bassin aux jours 7, 21, 43, 71, 100, 128, 156 et 211 (soit un intervalle moyen de 26 jours), prèlèvement de leurs muscles et de leurs entrailles (tube digestif et foie), congélation de ces prises et analyse du mercure présent à ces niveaux par spectrophotométrie en absorption atomique**.

b) expérimentation à court terme

On applique pour celle-ci le protocole ci-après :

- 50 truites Salmo gairdneri juvénibles/aquarium
- eau ayant des caractéristiques assez similaires à celles de l'expérimentation à long terme
- 6 aquaria : 2 avec 25 ppb de CH_3HgCl
  : 2 avec 25 ppb de $HgCl_2$
  : 2 témoins
- durée de l'expérimentation : 24 heures
- retrait de 5 poissons/aquarium après 0, 12 et 24 heures, prèlèvement de leurs muscles, foie et écailles, congélation de ces prises et détection du mercure présent dans ces tissus par microanalyse bidimensionnelle (200 μ x 200 μ) aux rayons X (30 Kv, 10 n A, 200 secondes) en longueur d'onde dispersive (raie Lα) en microscopie électronique par balayage sur coupes enrobées de glycol méthacrylate (Van Coillie, 1973 et 1976).

RESULTATS ET DISCUSSION

a) répartition du mercure après incorporation à long terme

Signalons d'abord que les traitements sublétaux imposés n'ont pas été nocifs pour les poissons. En effet, leur croissance en longueur et en poids et leur taux de survie étaient significativement semblables après 211 jours en absence et en présence de mercure inorganique ou organique ajouté à l'eau ou à la nourriture.

Le mercure en excès sublétal dans l'eau s'incorpore davantage à long terme dans les entrailles des truites que dans leurs muscles (voir figure 1).

De fait, pour une durée voisine de 200 jours d'un traitement continu avec 0,25 ppb de mercure organique ou inorganique, cette incorporation peut se chiffrer comme suit :

	mercure organique			mercure inorganique		
	jour 211	jour 10	différence	jour 211	jour 10	différence
entrailles :	2470ppb	90ppb	2380ppb	2670ppb	90ppb	2580ppb
muscles :	1680ppb	30ppb	1650ppb	840ppb	10ppb	830ppb

Le mercure ajouté à la nourriture tend également à se fixer

un peu plus dans les entrailles que dans les muscles à long terme chez les truites (voir figure 2).

Les résultats alors acquis pour l'incorporation du mercure inorganique de la nourriture se trouvent à un bas palier de valeurs (en dessous de 0,25ppm de façon générale). Ceci montre que la pénétration du mercure inorganique par voie digestive est très réduite, voire marginale. Par ailleurs, à ce bas palier, la dispersion des résultats augmente car des faibles différences spectrophotométriques y occasionnent des écarts prononcés. Bien que cette dispersion et cette petite échelle des valeurs soient peu propices à une quantification, une approximation de l'incorporation du mercure inorganique chez la truite à partir de la nourriture a été effectuée pour faire ressortir qu'elle s'avère minime par rapport à celle du mercure organique de la nourriture, tel qu'indiqué ci-dessous :

	mercure organique			mercure inorganique		
	jour 211	jour 10	différence	jour 211	jour 10	différence
entrailles :	1520ppb	212ppb	1308ppb	135ppb	67ppb	68ppb
muscles :	1310ppb	115ppb	1195ppb	19ppb	2ppb	17ppb

Comparons à présent les fixations de mercure organique et inorganique survenues durant 200 jours chez les truites au niveau de leurs entrailles et de leurs muscles par voie branchiale (et cutanée) à partir de l'eau et par voie digestive à partir de la nourriture (voir tableau 1).

Compte tenu des différences de concentrations du mercure dans l'eau (0,25ppb) et dans la nourriture (1 ppm), la comparaison doit être limitée aux rapports Hg organique/Hg inorganique et aux rapports Hg entrailles/Hg muscles. Nonobstant cette limite, elle fournit trois séries d'indications :

1 (i) l'absorption du mercure organique est supérieure de façon générale à celle du mercure inorganique;

(ii) la pénétration préférentielle de Hg organique par rapport à Hg inorganique apparaît nettement plus prononcée à partir de la nourriture qu'à partir de l'eau : ceci est principalement du au fait que l'absorption intestinale de Hg inorganique s'avère minime;

2 (i) on retrouve davantage de mercure dans les entrailles que dans les muscles;

(ii) cette fixation préférentielle du mercure dans les entrailles par rapport aux muscles semble indépendante du mode d'absorption du mercure car elle est sensiblement égale lorsque celui-ci provient de l'eau ou de la nourriture;

3 (i) le rapport Hg d'origine organique/Hg d'origine inorganique se révèle plus élevé pour les muscles que pour les entrailles;

(ii) le rapport Hg entrailles/Hg muscles a des valeurs plus hautes pour le mercure d'origine inorganique que pour celui d'origine organique.

Ces indications permettent d'établir que l'accumulation et la répartition à long terme du mercure chez les truites dépendent surtout de la forme chimique de cet élément comme le font ressortir les trois intégrations suivantes de nos résultats :

- le mercure inorganique s'incorpore moins dans les truites que le mercure organique;
- la pénétration de Hg inorganique s'y effectue beaucoup plus au niveau des branchies (et peau) qu'au niveau de l'intestin alors que celle de Hg organique est intense dans les deux cas;
- le mercure d'origine inorganique s'y retrouve davantage dans les entrailles que dans les muscles tandis que celui d'origine organique y est seulement quelque peu supérieur dans les premiers par rapport aux seconds.

En général, le mercure accumulé à long terme par les poissons s'y trouve surtout sous forme méthylée (C.N.R.C., 1979). Ceci provient de trois facteurs :

(i) le mercure est méthylé par la méthylcobalamine et/ou des microorganismes présents dans le milieu (Krenkel et al, 1973);

(ii) les lois de la perméabilité cellulaire favorisent l'entrée du mercure organique (Webb, 1966);

(iii) les poissons peuvent réaliser une méthylation de cet élément (Hannerz, 1968). Chez ces derniers, la méthylation du mercure s'effectue notamment à deux niveaux : d'une part, le mucus branchial (et cutané) grâce aux microorganismes associés à celui-ci (Jernelov, 1968) et, d'autre part, le foie grâce à l'anabolisme de maturation de ses acides ribonucléiques (Van Coillie et Thellen, 1981). Ces deux possibilités de méthylation expliquent, au moins partiellement, que le mercure inorganique pénètre principalement par les branchies chez la truite et s'y retrouve plus dans le foie au sein des entrailles que dans les muscles. Mais, malgré ces possibilités, l'incorporation du mercure inorganique y reste inférieure à celle du mercure méthylé, ce qui rejoint d'autres observations analogues (Olson et al, 1973; C.N.R.C., 1979).

b) répartition du mercure après incorporation à court terme

Les sites d'accumulation rapide pour le mercure chez les poissons étant, en plus des branchies et du sang, le foie, les muscles et les écailles (Van Coillie et al, 1974 ; C.N.R.C., 1979), nous avons recherché cet élément dans ces trois derniers tissus chez les truites après un traitement où la durée était raccourcie à 12-24 heures et la concentration amplifiée à 25ppb de mercure méthylé ou inorganique. On obtint alors les résultats exprimés au tableau 2.

Ils révèlent que les phénomènes suivants survenaient durant 24 heures chez les truites :

- l'absorption du mercure méthylé y était globalement plus élevée que celle du mercure inorganique;
- Hg d'origine organique s'y fixait préférentiellement dans les muscles et ensuite le foie tandis que Hg d'origine inorganique s'y localisait surtout dans celui-ci et les écailles.

La fixation du mercure inorganique dans les écailles peut, à première vue, étonner. Mentionnons cependant ici que, comme toute structure ostéoïde, les écailles ont un renouvellement minéral; de ce fait, un remplacement limité et réversible de certains métaux par d'autres s'y effectue et des métaux lourds peuvent alors y être provisoirement incorporés (Flemming, 1974; Van Coillie et al, 1974).

Dans ces conditions, on comprend que le mercure inorganique se retrouve rapidement et partiellement dans les écailles de truite.

Si l'on met en parallèle les résultats acquis lors de l'expérimentation à long terme avec ceux obtenus lors du bioessai à court terme, les points suivants ressortent :

- l'incorporation du mercure méthylé l'emportait sur celle du mercure inorganique dans les deux cas chez la truite;
- le mercure inorganique s'y fixait plus dans le foie (et les entrailles) que dans les muscles durant les deux expérimentations, ce qui est à relier à la méthylation du mercure s'effectuant dans le foie;
- Hg organique se localisait à court terme davantage dans les muscles que dans le foie (et entrailles) chez la truite tandis qu'à long terme cette répartition avait tendance à s'inverser.

C O N C L U S I O N S

1) A long terme (200 jours) comme à court terme (24 heures), l'absorption du mercure organique méthylé (CH_3HgCl) s'avère plus élevée que celle du mercure inorganique ($HgCl_2$) chez les truites Salmo gairdneri.

2) En présence d'un très faible excès de 0,25ppb d'Hg organique ou inorganique dans l'eau, elles accumulent le métal jusqu'à 0,83 à 2,58ppm durant 200 jours. Cette accumulation est plus poussée dans leurs foie et entrailles (2,58ppm pour Hg inorganique et 2,38ppm pour Hg organique) que dans leurs muscles (1,65ppm pour Hg organique et 0,83ppm pour Hg inorganique).

3) Si parallèlement leur nourriture quotidienne contient un excès de 1ppm d'Hg organique ou inorganique, elles incorporent surtout le mercure méthylé jusqu'à 1,19 à 1,31ppm durant 200 jours tandis que l'absorption intestinale du mercure inorganique s'y révèle très réduite (0,02 à 0,07ppm). Le métal se fixe alors également davantage dans leurs foie et entrailles (1,31ppm pour Hg organique et 0,07ppm pour Hg inorganique) que dans leurs muscles (1,19ppm pour Hg organique et 0,02ppm pour Hg inorganique).

4) Lors de ces expérimentations à long terme, le mercure organique s'accumule plus que le mercure inorganique dans leurs muscles. Par contre, lorsqu'il a été absorbé par voie branchiale (et cutanée) à partir de l'eau, ce dernier se fixe plus que le mercure méthylé dans leurs foie et entrailles.

5) Pendant des courts traitements de 24 heures avec 25ppb de Hg organique ou inorganique dans l'eau, le mercure méthylé est davantage incorporé dans leurs muscles que dans leur foie (laquelle répartition s'inverse lors de l'expérimentation à long terme) tandis que le mercure inorganique se retrouve plus dans leur foie (et leurs écailles provisoirement) que dans leurs muscles.

6) L'absorption du mercure inorganique qui s'effectue beaucoup plus au niveau de leurs branchies (et peau) qu'au niveau de leur intestin ainsi que sa localisation préférentielle dans leur foie pourraient être reliées à une méthylation du mercure inorganique dans leur mucus branchial (et cutané) et dans leur métabolisme hépatique.

R E M E R C I E M E N T S

Cette recherche a été initiée aux laboratoires de recherche des Services de Protection d'Environnement Canada à Longueuil (Québec) et poursuivie à ceux de C.I.L. Eco-Recherches à Pointe Claire (Québec).

Nous tenons vivement remercier Messieurs N. Bermingham, C. Blaise, R. Legault, P. Sloterdijk et autres personnes d'Environnement Canada et Pêches-Océans Canada qui ont apporté leur support et collaboration à ce projet. Nous exprimons également notre reconnaissance sincère aux laboratoires de Pêches et Océans Canada à Longueuil et de l'Institut national de la recherche scientifique de l'Université du Québec pour leur contribution analytique à nos travaux. Notre gratitude s'adresse enfin à Madame Baron-Van Coillie qui a assumé la dactylographie du présent texte.

FIGURE 1 - Teneur en mercure (µg/g, pds frais) mesurée dans les muscles (———) et les entrailles (0- - - - 0) des truites lors du traitement de 0.25 ppb de mercure dans l'eau.

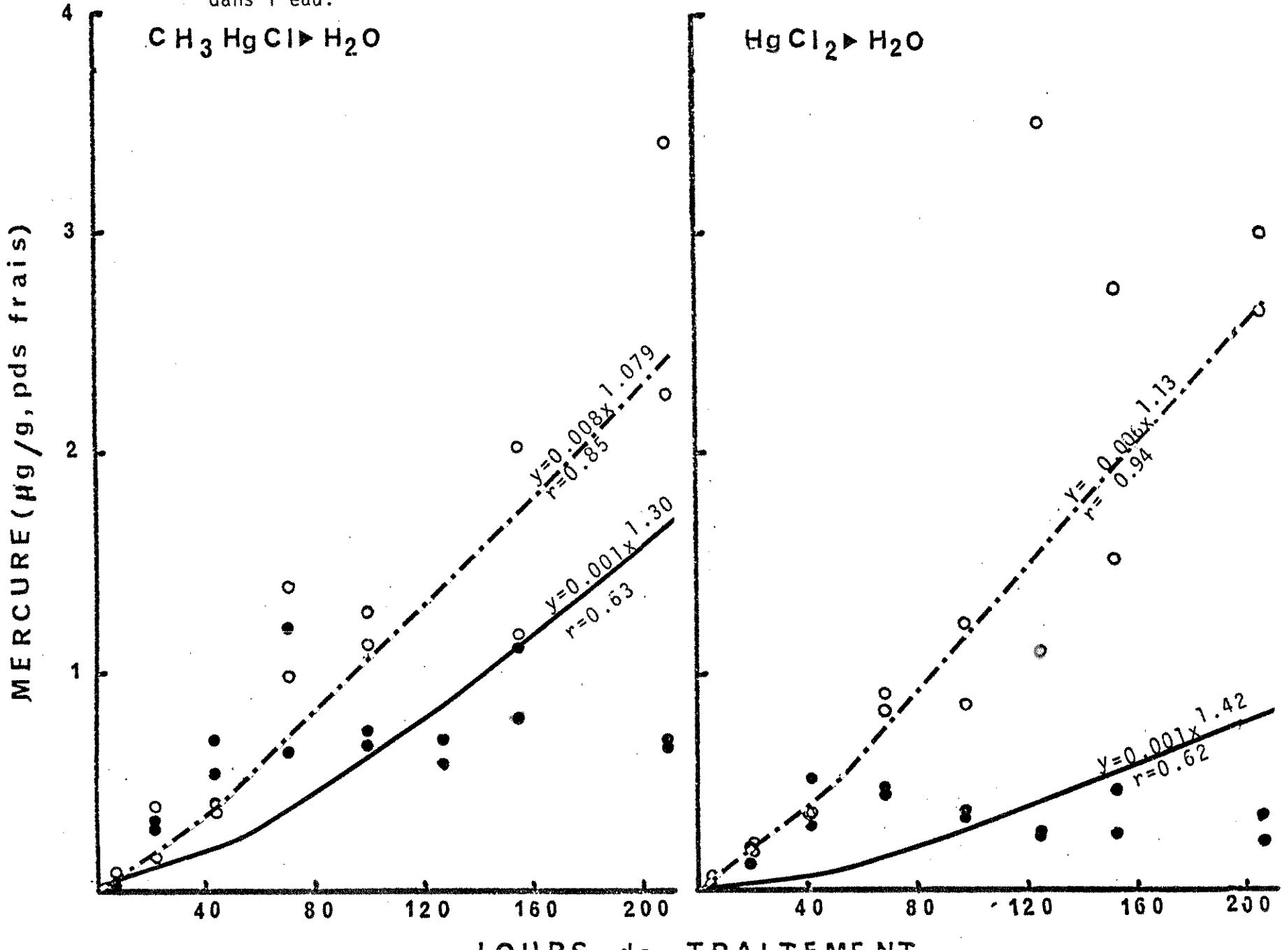

FIGURE 2- Teneur en mercure (µg/g, poids frais) mesurée dans les muscles (●——●) et les entrailles (○——○) des truites lors du traitement de 1 ppm de mercure dans la nourriture.

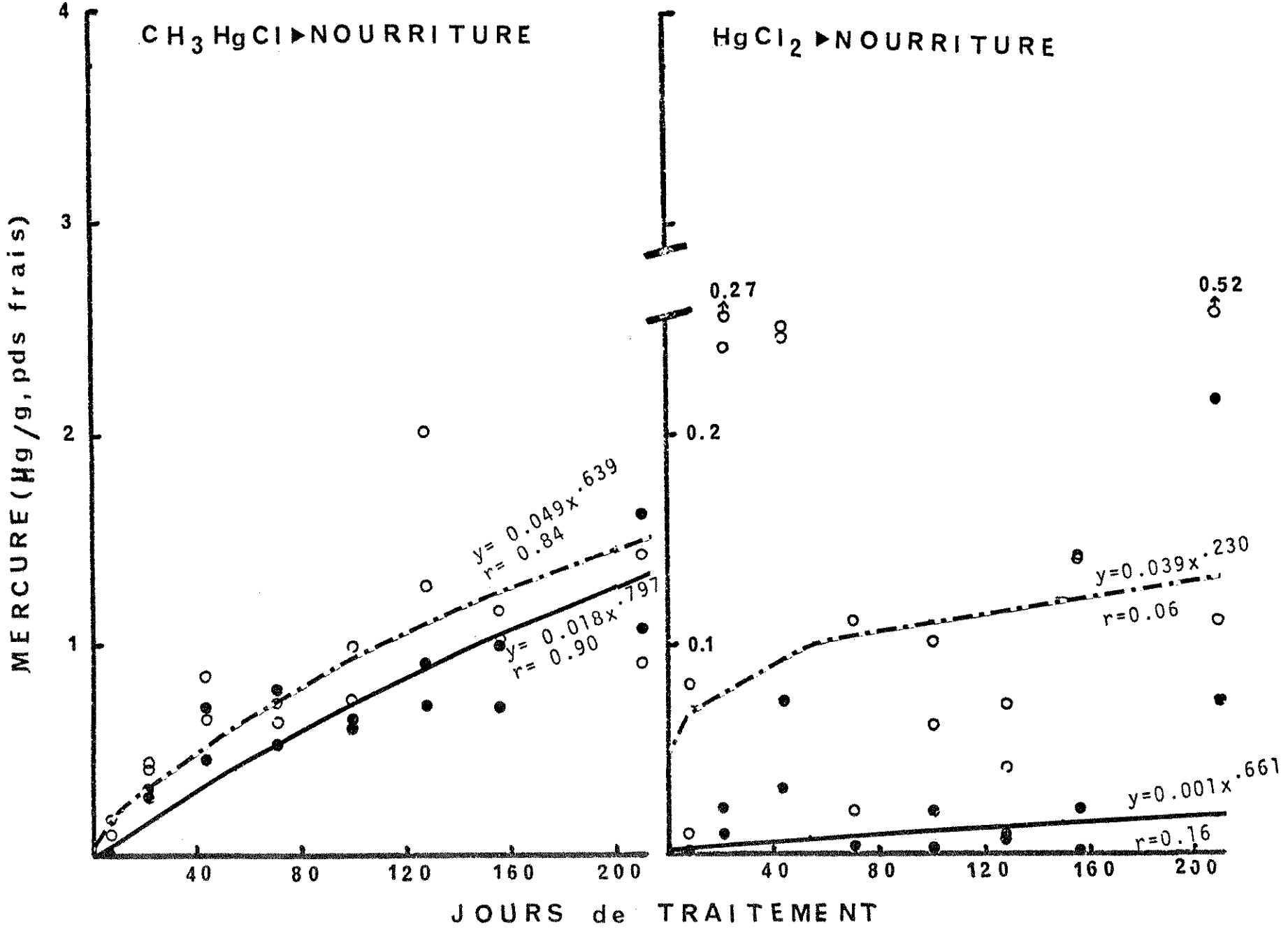

Tableau 1 Incorporation du mercure organique et inorganique durant 200 jours dans les entrailles et les muscles des truites à partir de l'eau et de la nourriture

EAU (0,25 ppb CH_3HgCl ou $HgCl_2$)

	mercure organique (1)	mercure inorganique (2)	rapport (1)/(2)
ENTRAILLES (a)	2380ppb	2580ppb	0,9
MUSCLES (b)	1650ppb	830ppb	2,0
RAPPORT (a)/(b)	1,4	3,1	

NOURRITURE (1ppm CH_3HgCl ou $HgCl_2$)

	mercure organique (1)	mercure inorganique (2)	rapport (1)/(2)
ENTRAILLES (a)	1308ppb	68ppb	19,2
MUSCLES (b)	1195ppb	17ppb	70,3
RAPPORT (a)/(b)	1,1	4,0	

Tableau 2 Incorporation du mercure organique et inorganique durant 24 heures dans le foie, les muscles et les écailles des truites à partir de l'eau

	mercure organique 25ppb CH_3HgCl (1)	mercure inorganique 25ppb $HgCl_2$ (2)	rapport (1)/(2)
FOIE			
0 h	1,08 ± 0,11 (N.B.)	1,08 ± 0,11 (N.B.)	1,00
12 h	1,13 ± 0,09	1,22 ± 0,15	0,93
24 h	1,21 ± 0,17	1,40 ± 0,12	0,86
	(Δ 12%)	(Δ 30%)	
MUSCLES			
0 h	1,02 ± 0,07	1,02 ± 0,07	1,00
12 h	1,18 ± 0,12	1,04 ± 0,09	1,13
24 h	1,49 ± 0,20	1,09 ± 0,13	1,37
	(Δ 46%)	(Δ 7%)	
ECAILLES			
0 h	1,03 ± 0,14	1,03 ± 0,14	1,00
12 h	1,05 ± 0,11	1,10 ± 0,08	0,95
24 h	1,05 ± 0,09	1,17 ± 0,11	0,89
	(Δ 2%)	(Δ 14%)	

N.B. Valeurs semi-quantitatives P/B obtenues par balayage microanalytique aux rayons X à longueur d'onde dispersive en microscopie électronique ± intervalles de confiance (P 0,95, n = 10).

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MESURE DIRECTE DE L'ABSORPTION D'AGENTS CHIMIQUES XENOBIOTIQUES AU NIVEAU DE L'EPITHELIUM DU SYSTEME RESPIRATOIRE DE L'OMBLE DES FONTAINES (SALVELINUS FRONTINALIS)

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RESUME - Un montage in vivo autour du tissu branchial de poissons a été constitué pour permettre de suivre l'absorption dans le sang d'agents chimiques xénobiotiques dispersés dans l'eau à travers l'épithélium du système respiratoire de poissons. Il fallait séparer l'eau aspirée et expirée par les poissons et déterminer la teneur en agents xénobiotiques de chacune des fractions. La différence de concentration correspondait à l'absorption de l'agent xénobiotique. Un circuit à écoulement continuel (400 ml/min.) a été mis en place; il était constitué de façon à permettre d'évaluer l'effet de différentes variables du milieu (par exemple, l'oxygène dissous, la température, le pH, la dureté de l'eau) sur le transport des agents chimiques de part et d'autre de l'interface eau-sang des branchies.

Les auteurs ont choisi des ombles de grande taille (450-550 g); le débit et le volume ventilatoire, le rythme cardiaque, l'absorption d'oxygène, la consommation d'oxygène et le volume urinaire de poissons retenus dans le montage ont été comparés à ceux de poissons nageant librement, certaines données provenant de la littérature dans le second cas. Le fait de retenir les poissons dans le montage n'a eu aucun effet observable sur la fonction respiratoire des poissons, ni sur leur fonction cardio-vasculaire, au cours de la période d'expérimentation. Bien mieux, la consommation moyenne d'oxygène des ombles de l'expérience (51.3 ± 9.5 (E.-T.) mg O_2/kg/h) se situait en plein dans les plages connues de métabolisme typiques des ombles des fontaines aux températures expérimentales.

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L'absorption d'endrine à deux concentrations sublétales dans l'eau a été mesurée sur neuf ombles à 11-12°C pendant une période d'exposition atteignant cinq jours. L'absorption d'endrine aux deux concentrations dans l'eau de 0.046 et de 0.072 g d'endrine/L a été de 82 ± 11% et de 79 ± 11%, respectivement, cela indique que la concentration d'endrine dans l'eau n'a aucun effet déterminant sur l'absorption. Quand l'eau était saturée en oxygène et à 11-12°C, il a été calculé que la concentration moyenne totale d'endrine absorbée au niveau des branchies se chiffrait à peu près à 6.9 µg/jour à la plus faible concentration d'endrine dans l'eau et à 12.6 µg/jour à la plus forte concentration.

Un stress dû aux variations de teneur en oxygène dissous a été provoqué à certains moments lors de l'exposition des sujets à l'endrine afin de juger de l'effet de l'hypoxie sur le volume respiratoire, l'absorption d'oxygène et l'obsorption d'endrine. Ce stress a été appliqué par étapes, soit à 80%, 50% et 30% de saturation. Le volume ventilatoire moyen s'est accru de 72 ± 21 ml/min. à saturation à 310 ± 67 ml/min. à 30% de saturation. L'absorption d'oxygène a diminué passant de 60 ± 11% à saturation à 44 ± 10% à 30% de saturation, alors que l'absorption d'endrine est passée de 80 ± 11% à saturation à 47 ± 11% à 30% de saturation. L'absorption d'oxygène et d'endrine n'a pas tellement changé à 80% de saturation, mais elle a été considérablement réduite à 50% de saturation et moins.

Ces premières données montrent que les montages in vivo autour de branchies peuvent constituer un instrument efficace de mesure directe de l'absorption d'agents xénobiotiques au niveau des branchies et que les variables du milieu comme l'oxygène peuvent modifier la faculté d'absorption des branchies. Les changements à ce niveau ont des effets marqués sur l'absorption totale d'un agent xénobiotique. Il est essentiel

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de poursuivre les travaux de recherche dans cette voie (1) afin de parvenir à constituer des modèles de bioconcentration chez les poissons, (2) de comprendre les effets des variables du milieu sur les mouvements de substances toxiques au niveau des branchies, et (3) de mettre au point des modèles pharmacodynamiques pour les poissons.

A DIRECT MEASURE OF THE UPTAKE EFFICIENCY OF XENOBIOTIC CHEMICALS ACROSS THE RESPIRATORY EPITHELIUM OF BROOK TROUT (SALVELINUS FRONTINALIS

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SUMMARY - An in vivo fish gill preparation was developed for monitoring the movement of xenobiotic chemicals from the aqueous environment across the respiratory epithelium into the blood. The procedure involved separating a transected fish's inspired and expired water and determining the xenobiotic content of each fraction. The difference between these two fractions became the uptake efficiency (%) for the xenobiotic. A continuous-flow system (400 ml/min.) was used and designed to allow a determination of the effects of fluctuating environmental variables (i.e., dissolved oxygen, temperature, pH, hardness, etc.) on the movement of chemicals across the blood-water interface of the gills.

Large brook trout (450-550 g) were used for these experiments, and values for ventilation rate, ventilation volume, heart rate, oxygen uptake efficiency, oxygen consumption, and urine volume were compared between transected and untransected fish, which included literature values for untransected fish. Spinal transection had no observable effects of respiratory or cardio-vascular function in these fish over the experimental period. In fact, the mean oxygen consumption of these transected brook trout (51.3 ± SD 9.5 mg O_2/Kg/hour) fell well within the published ranges for the standard metabolic rate of brook trout at similar temperature.

The uptake efficiency of endrin at two sublethal water concentrations was measured in nine individual trout at 11-12^{o}C over an exposure period of up to five days. The endrin uptake efficiency at the

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two water concentrations of 0.046 and 0.072 μg endrin/l was 82 ± 11% and 79 ± 11%, respectively, indicating no significant effect of water endrin concentration on putake efficiency. Total mean endrin taken up across the gills of these brook trout with oxygen at saturation and the temperature at 11-12°C was calculated to be approximately 6.9 μg/day at the low endrin concentration and 12.6 μg/day at the high concentration.

A dissolved oxygen stress was introduced at selected intervals during the endrin exposures to determine the influence of hypoxia on respiratory volume, oxygen uptake efficiency and endrin uptake efficiency. The oxygen stress was applied in a stepwise fashion at 80%, 50%, and 30% of saturation. Mean ventilation volume increased from a low of 72 ± 21 ml/min at saturation to 310 ± 67 ml/min at 30% of saturation. Oxygen uptake efficiency decreased from a mean of 60 ± 11% at saturation to 44 ± 10% at 30% of saturation, while endrin uptake efficiency dropped from a mean of 80 ± 11% at saturation to a low of 47 ± 11% at 30% of saturation. Oxygen and endrin uptake efficiencies were not significantly altered at 80% of saturation, but were significantly reduced at 50% of saturation and below.

These initial data indicate that an in vivo fish gill preparation can be used effectively to measure the direct uptake of xenobiotics across the gills, and that changes in environmental variables such as oxygen can alter the uptake efficiency of the gills. In turn, these changes in uptake efficiency have significant effects on the total uptake of a xenobiotic. Further studies of this kind are essential (1) for the building of fish bioconcentration models, (2) for understanding the effects of environmental variables on the movement of toxics across the gills, and (3) for developing future pharmacodynamic models with fish.

ABSORPTION DU ZINC PAR LE SAUMON COHO

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RÉSUMÉ - L'absorption du zinc par des saumons Coho juvéniles a été mesurée en eau douce et en eau dure à 12°C, avec le ^{65}Zn. Les poissons ont été exposés au zinc sous forme de $ZnSO_4$ (32 à 600 ppm) pendant des périodes variant de 15 à 480 minutes. L'absorption du zinc par les tissus branchiaux dépendait à la fois de la durée d'exposition et de la concentration. L'absorption au niveau des branchies précédait la détection du zinc radioactif dans le plasma sanguin et les autres tissus. Les reins contenaient plus de zinc par unité de poids que le foie, l'intestin ou l'estomac; très peu du radioisotope s'est concentré dans les muscles. L'absorption était freinée par la présence de calcium dans l'eau, mais non par le cuivre aux mêmes concentrations. Comme il ressort d'autres études, nous avons montré que l'absorption varie considérablement selon le nombre de battements des opercules dépendant eux-mêmes d'un certain nombre de paramètres constituant des stress. Bien qu'il soit impossible d'obtenir des mesures cinétiques absolues de l'absorption du zinc en raison de cela, la méthode que nous décrivons permet d'obtenir des mesures relatives exactes de l'absorption du zinc et peut constituer un outil commode pour quantifier les facteurs agissant sur l'absorption et la toxicité du zinc.

ZINC UPTAKE BY COHO SALMON

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SUMMARY - The kinetics of zinc uptake by juvenile coho salmon have been measured in hard and soft water at 12°C using ^{65}Zn. Fish were exposed to zinc as $ZnSO_4$ (32 to 600 ppb) for periods from 15 min to 480 min. Zinc uptake into gill tissue was both time and concentration dependent. Uptake into gill preceded the appearance of radioactive zinc in plasma and other tissues. Kidney had higher zinc levels per unit weight than liver, intestine or stomach and muscle contained minimal radioisotope. Zinc uptake was inhibited by aqueous calcium but not affected by similar concentrations of aqueous copper. In agreement with the results of others it was found that uptake varied widely with opercular rate which in turn was affected by a number of stress parameters. Although for this reason absolute kinetics of zinc intake could not be measured, the procedure described here provides an accurate relative measure of zinc uptake and may be a usefull method when used to quantitate factors affecting the uptake and toxicity of zinc.

CONSTRICTION DES TESTS DANS LE LAC ONTARIO

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RESUME - De nombreux cas de constriction des testes de truites grises capturées dans le lac Ontario et dans le lac Michigan ont été rapportés dernièrement. Nous avons étudié les conséquences de ces constrictions sur le succès de la reproduction de l'espèce avec huit truites grises provenant du bassin Est du lac Ontario.

Nous avons confirmé par des coupes histologiques le retardement de la spermatogénèse chez toutes les truites atteintes de ces constrictions, par rapport à des sujets normaux. Les constrictions sont produites quand un nouveau cycle de la spermatogénèse n'est pas amorcé. Les spermatogonies couvrant les parois des tubules séminifères sont incapables de se diviser par mitose et finissent par dégénérer. Ensuite, beaucoup de tubules des régions touchées s'effondrent, ce qui conduit éventuellement à une constriction des testes. Le potentiel reproductif est davantage réduit par la libération prématurée des cellules germinales en voie de maturation dans les vésicules, notamment les permatocytes et les permatides. Nous avons trouvé que ces cellules constituent la moitié du volume contenu dans le canal déférent; celui-ci ne contient habituellement que le sperme. Nous avons enregistré plusieurs cas de développement anormal des cellules germinales dnas les régions entourant les constrictions. Un index de la reproduction établi en tenant compte du tissu reproductif non développé, demeuré intact à la fin de la spermatogénèse, montre que 40% des spermatozoïdes des truites atteintes de constrictions ne pouvaient plus fertiliser les oeufs. Il s'agit d'une estimation prudente dans laquelle il n'est pas tenu compte des régions d'où proviennent les cellules à maturation anormale, ni des régions de libération prématurée des cellules germinales. Il y aurait lieu d'entreprendre immédiatement des études pour trouver la cause de ces constrictions.

GONODAL CONSTRICTIONS IN LAKE ONTARIO

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Summary - A high incidence of constricted testes has been recently reported in Lake trout captured from Lake Ontario and Lake Michigan. In this study the implications of these constrictions on the reproductive success of the species have been investigated in eight lake trout taken from the Eastern basin of Lake Ontario.

Delayed cycles of spermatogenesis were confirmed histologically in all constricted testes when compared with non-constricted forms. Constrictions are produced when lake trout fail to initiate a new cycle of spermatogenesis. Spermatogonia lining the seminiferous tubules are unable to undergo mitosis and subsequently degenerate. This is followed by the collapse of many tubules in the affected area and eventually leads to the formation of a constriction in the testis. The reproductive potential is further reduced by premature release of developing germ cells including spermatocytes and spermatids, from cysts in which they normally develop. These cells were found to constitute 50% of the volume of the main collecting sperm duct. This duct is normally occupied only by sperm. Abnormal development of germ cells in regions surrounding constrictions was also frequently recorded.

A reproductive index based upon the remaining undeveloped reproductive tissue at the end of the cycle of spermatogenesis indicates that available sperm for fertilization of eggs was reduced by approximately 40% in constricted forms. This estimate is conservative and does not include areas of abnormal cell development or prematurely released germ cells. Immediate studies should be initiated to determine the cause of these constrictions.

BIOACCUMULATION DU ZINC CHEZ CHLAMYDOMONAS VARIABILIS EN FONCTION DES ESPECES DU ZINC

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RESUME - Nous avons entrepris des études pour établir la vitesse d'adsorption et d'assimilation du zinc en fonction des espèces sur Chlamydomonas variabilis en culture continue, en culture semi-continue et par lots à pH déterminé. La concentration du zinc pouvait atteindre 1000 parties par milliard sans aucun effet sur la progression exponentielle du nombre de divisions cellulaires dans les cultures par lots, bien que le nombre de cellules obtenues au cours de la phase stationnaire ait diminué aux concentrations les plus élevées de zinc.

Le phénomène de bioaccumulation du zinc a été divisé en deux: 1) la fraction absorbée par les membranes cellulaires (par extraction à l'EDTA), et 2) la fraction transportée à travers les membranes cellulaires (concentration restante de zinc après extraction à l'EDTA). Une durée d'extraction de 10 minutes est optimale.

Il s'est avéré que l'adsorption du zinc sur les membranes cellulaires et son assimilation sont en relation directe avec la concentration de zinc jusqu'à concurrence de 1000 parties par milliard. L'adsorption et l'assimilation se produisaient en quatre phases au cours des six heures d'incubation: 1) l'adsorption et l'assimilation rapides du zinc durant la première minute, 2) un rythme constant et élevé de 1 à 60 minutes, 3) rupture du rythme d'adsorption et d'assimilation entre 60 et 120 minutes, période au cours de laquelle une partie du zinc adsorbé retournait en solution, et 4) reprise de l'adsorption et de l'assimilation en progression linéaire, mais plus tentement qu'à la phase 2).

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Au commencement, il y avait plus de zinc adsorbé qu'assimilé, mais l'écart s'amenuisait à mesure que durait l'incubation. Il y a un rapport direct et significatif entre la quantité de zinc adsorbée et la quantité de zinc assimilée. Tout le zinc libéré par la membrane cellulaire au cours de la phase 3 s'est retrouvé en solution. Nous étudions le mécanisme de dégagement du zinc et son rôle possible de protection de la cellule contre la toxicité du zinc.

Nous discuterons des résultats de nos expériences à l'aide d'un modèle préparé pour distinguer entre les étapes biologiques et physicochimiques limitant la vitesse de bioaccumulation des métaux à l'état de traces dans le phytoplancton.

ZINC BIOACCUMULATION BY CHLAMYDOMONAS VARIABILIS AS A FUNCTION OF ZINC SPECIATION.

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SUMMARY - Studies were undertaken to determine rates of zinc adsorption and assimilation as a function of zinc speciation using Chlamydomonas variabilis grown in pH-controlled batch, semi-continuous and continuous cultures. Concentrations of zinc up to 1000 ppb did not alter the exponential cell division rate in batch culture, although the cell yield obtained during the stationary phase was diminished at the higher zinc concentrations.

The bioaccumulation of zinc was separated into two fractions: (1) that which was bound to the cell membrane (determined by extraction with EDTA), and (2) that which was transported across the cell membrane (zinc remaining after extraction with EDTA). An extraction time of 10 min proved optimal.

The adsorption of zinc onto cell membranes and the assimilation of zinc proved to be linear with increasing zinc concentration up to 1000 ppb. Both zinc adsorption and assimilation could be described by four phases during a 6 h incubation period: (1) an initial rapid adsorption and assimilation during the first minute, (2) a constant elevated rate from 1 min to 60 min, (3) a discontinuity in adsorption and assimilation from 60 min to 120 min during which time zinc was released from the cell membrane, and (4) a resumption of linear adsorption and assimilation, but at a slower rate than during phase (2). The quantity of adsorbed zinc was initially greater than that assimilated but this difference diminished with incubation time. Significant positive correlations were found between

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quantities of adsorbed and assimilated zinc. All of the zinc lost from the cell membrane during phase (3) could be accounted for as an increase in zinc concentration in solution. The mechanism of zinc release and its possible rose in protecting the cell from zinc toxicity are being investigated.

Results of these experiments will be discussed in relation to a model which attempts to distinguish biological from physiochemical rate-limiting steps for the bioaccumulation of trace metals by phytoplankton.

EFFETS DE LA TEMPÉRATURE SUR LA TOXICITÉ DU PENTACHLOROPHÉNOL POUR LES OEUFS, LES ALEVINS VÉSICULÉS ET LES ALEVINS DE LA TRUITE ARC-EN-CIEL (SALMO GAIRDNERI)

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RÉSUMÉ - Deux groupes de truites arc-en-ciel (Salmo Gairdneri) ont été exposés aux concentrations nominales de pentachlorophénol (PCP) de 0, 10, 32 et 100 µg/L, l'un des groupes depuis la fertilisation des oeufs jusqu'à 4 semaines après que les alevins se soient mis à nager, et l'autre depuis l'éclosion jusqu'à 4 semaines après que les alevins se soient mis à nager. Le premier groupe d'oeufs a été incubé à 12°C et, après l'éclosion, la température a été portée à 20°C. Le deuxième groupe a été incubé à 6°C et la température a été portée à 10°C après l'éclosion.

Il n'y a pas eu d'augmentation significative (niveau de probabilité: 0.01) de la mortalité et du temps mis pour éclore pour les oeufs exposés au PCP, alors que le poids humide à l'éclosion était réduit de manière significative. Par rapport au groupe témoin, les oeufs incubés dans l'eau froide perdaient plus de poids que les oeufs incubés dans l'eau chaude. Durant la phase de résorption du sac vitellin, il y a eu davantage de mortalités et la croissance a été davantage réduite par le PCP chez les sujets en eau froide, mais la teneur en humidité n'a pas changé. L'efficacité de la résorption du sac vitellin, une mesure de l'efficacité de la croissance entre l'éclosion et le moment où les poissons se mettent à nager, a aussi été diminuée par le PCP. Après que les poissons se soient mis à nager, durant la période où ils sont alimentés, il y avait beaucoup de mortalité chez les groupes exposés à 100 ug de PCP/L, mais il n'y avait pas d'effets dus à la température. Les vitesses de croissance ont été significativement ralentis par le PCP, les effets les plus importants étant observés dans les eaux les plus chaudes.

L'impact global du PCP et des interactions entre le PCP et la température a été évalué par le calcul de la biomasse produite par 1000 oeufs à l'éclosion, quand les poissons se mettent à nager et après

quatre semaines pendant lesquelles ils ont été nourris, alors que les sujets avaient été exposés aux effets de la température et du PCP combinés.

Compte tenu des taux de mortalité et du poids humide observés, nous savons que la biomasse finale des poissons élevés en eau chaude était réduite par rapport à celle des poissons élevés aux températures de référence, quelles que soient les concentrations choisies de PCP, alors que la biomasse des poissons élevés en eau froide avait augmenté chez le groupe exposé à 10 ug de PCP/L et avait diminué chez le groupe exposé à 100 µg de PCP/L.

L'exposition au PCP des poissons dès le jour où les oeufs ont été fertilisés a eu des effets beaucoup plus considérables que l'exposition à partir de l'éclosion. Le groupe le plus durement touché était celui incubé en eau froide et exposé à 100 µg de PCP/L dès la journée de la fertilisation: les poissons de ce groupe n'ont jamais pu nager.

TEMPERATURE EFFECTS ON PENTACHLOROPHENOL TOXICITY TO EGGS, SAC-FRY AND FRY OF RAINBOW TROUT (Salmo gairdneri)

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SUMMARY - Rainbow trout (Salmo gairdneri) were exposed in two groups from egg fertilization until four weeks after swim-up and from hatch until four weeks after swim-up to nominal pentachlorophenol (PCP) concentrations of 0, 10, 32 and 100 µg/L. The first group of eggs was incubated at a temperature of 12°C and, following hatch, the temperature was raised to 20°C. The second group was incubated at 6 and 10°C in the egg and post-hatch stages respectively.

Eggs of rainbow trout had non-significant (.01 probability level) increases in mortality and time to hatch when exposed to PCP, as well as a significant reduction in wet weight at hatch. Cold eggs lost more weight relative to controls than did warm eggs. During yolk sac resorption, mortality and reduced growth due to PCP were again greatest in the cold regime, but percent moisture was unaffected. Sac resorption efficiency, a measure of growth efficiency between hatch and swim-up, was also reduced by PCP. After swim-up, during the feeding stage, high mortality continued at 100 µg PCP/L but there were no temperature interactions. Growth rates were significantly depressed by PCP with the greatest effects in the warm regime.

The overall impact of PCP and PCP-temperature interactions was assessed by calculating the biomass produced by 1000 eggs at hatch, at swim-up and after 4 weeks of feeding after exposure to each PCP-temperature combination.

Based on observed mortality rates and wet weights, the final biomass of warm fish was reduced from control levels at all PCP concentrations, whereas that of cold fish was enhanced by 10 µg PCP/L and reduced at 100 µg PCP/L.

Exposure of fish to PCP from the day of egg fertilization had a much greater effect than did exposure from the day of hatch. The most severely affected group of fish were those in the cold regime, exposed from the day of fertilization to 100 µg PCP/L - this group failed to attain swim-up.

ADDENDUM TO ABSTRACT - Threshold PCP concentrations causing adverse effects were estimated to be 20 and 10 µg/L for the cold and warm fish respectively.

Key words: Pentachlorophenols, temperature, chronic, toxicity, fish, time.

THEME III

Toxicologie marine. | Marine toxicology.

Conférencier invité. | Invited speaker.

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RÉSUMÉ - Un document de fond portant sur les questions d'actualité concernant la toxicologie en milieu marin est présenté en vue de remettre ces questions dans leur perspective lors de la Session de toxicologie en milieu marin. Différentes questions précises sont considérées, notamment les polluants marins les plus dangereux, la toxicité en eau douce par rapport à la toxicité en eau salée, certains besoins de recherche, et plus particulièrement de tests de toxicité en milieu marin effectués en laboratoire. D'autres questions sont brièvement étudiées, dont les efforts déployés pour contrôler les déversements de produits nuisibles et la préparation des futurs toxicologues des milieux marins.

SUMMARY - A discussion paper on current issues in marine toxicology is presented, to provide a perspective on the field for the session on Marine Toxicology at the Workshop. Marine pollutants of concern, the issue of freshwater versus saltwater toxicities, and some research needs, especially in marine toxicity testing in the laboratory, are discussed. Other issues of concern, such as the continued objective of the control of harmful discharges and the education of future marine toxicologists, are briefly mentioned.

INTRODUCTION AND OVERVIEW OF PRESENTATION - The presence or threat of marine pollution is not a new concern for Canada and Canadians. We have a very long coastline on three oceans; only two provinces in fact do not have saltwater coastlines. There are a substantial number of people and communities on the coasts, and with them pockets or concentrations of industrial activity. At the present time, there is considerable activity

in the offshore, outer continental shelf areas for oil and gas, and the continual major discharges from large rivers (Fraser, Mackenzie, St. Lawrence, Saint John, St. Croix, etc.) carry loadings of persistent pollutants and organic nutrients into the estuaries and coastal zones.

Along with the long-term, small-volume and often subtle inputs of man-made materials, there have in the past been numerous massive and well-publicized occurrences of marine pollution. These have occurred on east and west coasts. Examples are: pulp-mill discharges on both coasts, phosphorous discharges from ERCO in Placentia Bay, Newfoundland, The Arrow and Kurdistan oil spills off Nova Scotia, many closures of shellfisheries due to paralytic shellfish poisoning, the presence of heavy metals in lobsters, to name just a few.

As a result of the presence or threat of known inputs, a large number of studies have been conducted by Canadian scientists, especially recently, to define the various hazards associated with specific materials in marine systems. People such as Waldichuk, Davis, Alderdice, Sprague, Zitko, McLeese, etc., are amongst the major contributors in recent years. Marine pollution research in Canada accelerated in the 1970's; many of the laboratories and people involved are identified in the two past Aquatic Toxicity Proceedings (Hodson et al. 1979; Klaverkamp et al. 1980, in press). Canada has contributed to the work of international bodies dealing with ocean pollution (GESAMP, I.J.C., I.C.E.S., F.A.O., UNEP, etc.), and nationally, federal legislation has come into being or has been revised (Fisheries Act, Ocean Dumping Control Act, Contaminants Act, being amongst them).

All of this activity, the concern about pollutants, the people and their studies, and the legislation, has resulted in a subsection of aquatic toxicology - namely marine toxicology. Legitimately a subsection or not, marine toxicology can be defined very broadly as being the branch of the marine sciences dealing with the chemical and biological assessment of known or suspected pollutants entering estuarine and marine coastal waters. The assessment includes the physico-chemical fate of compounds, biodegradation, bioaccumulation, biological effects, both lethal and sublethal, and derivation

of "safe" concentrations. It's prime responsibility is to describe levels of materials causing significant impacts on marine organisms - individuals, populations, communities - and to contribute to the establishment of marine water quality criteria by defining acceptable threshold concentrations, if they exist, for recognized toxic substances. Marine toxicologists are attempting to protect sensitive and vulnerable estuarine, coastal and offshore areas from permanent significant damage to productivity and fisheries (Fig. 1).

Marine toxicology is a field of aquatic science that has matured enormously throughout the 1970's. This is due to the application of many new and established methods in analytical chemistry, the development and practical use of many acute and chronic sublethal bioassay techniques, the recognition of the value of field monitoring and biomonitoring techniques, and the wide adoption of the interdisciplinary approach.

The topic of marine toxicology has been brought up in individual papers and discussions at previous Aquatic Toxicity Workshops (Craig 1976, Davis et al. 1978). Dr. M. Waldichuk, a marine scientist with a long and devoted concern about marine pollution, stated " ..... we need more bioassay data obtained in parallel experiments with freshwater and marine systems, preferably using the same species (Coho salmon, for example) acclimated to the particular water. As we have found with boron and some of the "neutral" fractions from KME (kraft mill effluent), we may have higher toxicity for some substances in seawater than in freshwater because of some peculiar mode of toxic action. We need to know more about the mechanism of this 'increased seawater toxicity'. Clearly, we should not be using freshwater toxicity data to develop criteria for seawater" (Waldichuk, in Craig 1976). At the same meeting in 1975, it was stated that most of the standard bioassay procedures incorporate freshwater organisms, but because of marked physiological differences between freshwater and marine organisms, there is a continual need for bioassays utilizing sensitive life stages of marine invertebrates and fish (Wells, in Craig 1976). One of the viewpoints at the Fourth Annual Aquatic Toxicity Workshop in Vancouver in 1977, during the discussion on receiving water standards and utilization of laboratory data,

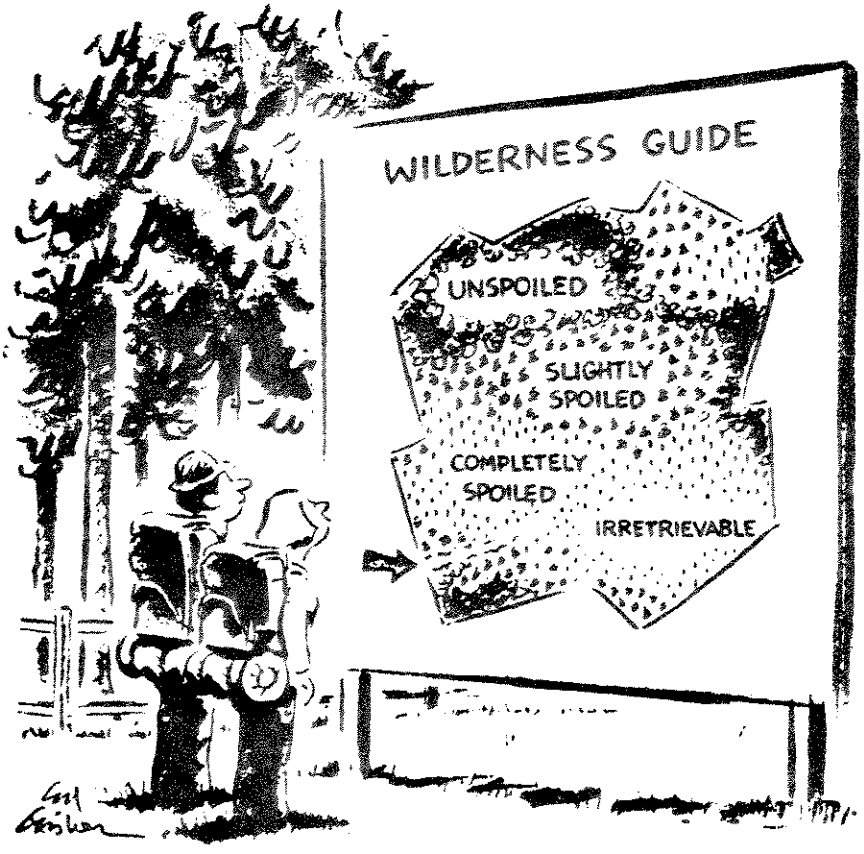

Figure 1. An example of what marine toxicologists are trying to prevent - substitute OCEAN for WILDERNESS.

was that "application of freshwater organism test data to the marine environment is a questionable practice and is not scientifically justified" (Davis et al. 1978).

For the first time at these workshops, we have a session devoted to the topic of marine toxicology, and have a full and interesting program planned today. To introduce the topic to this forum and to provide points for discussion or thought, my paper will briefly describe three current issues in the field:

(1) Marine pollutants of concern.

(2) Toxicities in freshwater versus seawater.

(3) Identification of some major research trends and needs.

MISE EN CIRCULATION, BIOABSORPTION ET EFFETS SUBLETAUX DE CONTAMINANTS PROVENANT DE SEDIMENTS MARINS.

E.R. McGreer, *B.J. Reid * et H. Nelson**
*EVS Consultants Ltd.

RESUME - L'expansion prévue d'une cale sèche flottante dans le port de Vancouver, Colombie-Britannique, rend obligatoire l'enlèvement des sédiments par dragage. Le Service de protection de l'environnement d'Environnement Canada s'est inquiété des risques liés à la mise en circulation et à l'absorption éventuelle de BPC et de métaux provenant des sédiments contaminés du secteur. Cette étude subventionnée porte sur les effets possibles du dragage sur les biotes avoisinants et sur l'inocuité du rejet au large des matières draguées.

Les sédiments ont été prélevés aux alentours des installations actuelles et ont été étudiés en laboratoire. Des études antérieures ont montré que les sédiments contenaient des BPC et des métaux lourds en fortes concentrations. Des bio-essais d'absorption en 30 jours par des coques (Macoma balthica) et des moules (Mytilus edulis) se poursuivent actuellement. Les tissus seront examinés au commencement et à la fin des bio-essais pour déterminer leur teneur en BPC, Hg, Cd, As, Cu, Pb et Zn. La salinité pour les tests varie entre 10 et 25 parties par millier et la température est fixée à 10°C. En outre, des échantillons d'eau de mer seront prélevés dans chaque contenant au bout de 24 heures, de 10 et de 30 jours et les contaminants qu'ils contiendront seront dosés pour déterminer le lessivage. Des sédiments estuariens non contaminés serviront de témoins.

Nous étudierons aussi les effets sublétaux des sédiments contaminés sur le comportement de la coque Macoma balthica quand elle s'enfouit et quand elle fuit une menace. Des études ont montré que le

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temps mis par M. balthica pour s'enfouir dépend entre autres de la concentration des métaux dans les sédiments (McGreer, 1979). La vitesse d'enfouissement dans des sédiments contaminés sera comparée à celle observée dans des sédiments normaux.

Nous étudierons aussi comment les coques enfouies sont attirées ou repoussées par les sédiments contaminés et des sédiments témoins, au moyen de la méthode de McGreer (1979). Les résultats permettront d'évaluer les risques de bioaccumulation dans des organismes marins de contaminants industriels provenant des sédiments.

Référence: McGreer, E.R. 1979. Sublethal Effects of Heavy Metal Contaminated Sediments on the Bivalve, Macoma balthica (L.); Mar. Pollut. Bull., 10:259-262.

MOBILIZATION, BIOUPTAKE AND SUBLETHAL EFFECTS OF CONTAMINANTS FROM MARINE SEDIMENTS

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SUMMARY - Plans for expansion of a floating drydock in Vancouver Harbour, B.C. required extensive dredging of nearshore sediments. The Environmental Protection Service of Environment Canada expressed concern over the potential release and subsequent uptake of PCB's and metals from contaminated sediments in the area. This study was funded to predict impacts of the dredging operation on resident biota and suitability of the dredge spoil for ocean dumping.

Sediments were colledted from sites adjacent to the present ship repair facility and returned to the laboratory. Previous studies showed high levels of PCB's and heavy metals. Thirty-day uptake bioassays are currently being conducted, using clams (Macoma balthica) and mussels (Mytilus edulis). Tissues will be analyzed at the beginning and end of the bioassays for PCB's, Hg, Cd, As, Cu, Pb and Zn. Tests are being carried out at salinities of 10 and 25 ppt, at a temperature of 10^{o}C. In addition, seawater samples for contaminants analyses will be drawn off each test container at 24h, 10 and 30 days to assess the rate of leaching from the test sediments. Uncontaminated, estuarine sediments will be run as a control.

Sublethal effects of contaminated sediment on the burrowing and avoidance behaviour of the clam, Macoma balthica, will also be determined. The time required for M. balthica to burrow has previously has shown to be affected by the concentration of metals in the sediment (McGreer, 1979). The rate of burrowing in contaminated sediment

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will be compared to that of a central (clean) sediment. Preference/ avoidance studies on burrowed clams will also be assessed for the contaminated and control sediments using the method of McGreer (1979). Test results will be used to assess potential for bioacculumation of contaminants in marine organisms from industrially polluted sediments.

Ref. McGreer, E.R. 1979. Sublethal Effects of Heavy Metal Contaminated Sediments on the Bivalve, Macoma balthica (L.) Mar. Pollut. Bull. 10:259-262.

MOBILIZATION, BIOACCUMULATION AND SUBLETHAL EFFECTS OF CONTAMINANTS FROM MARINE SEDIMENTS

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McGREER, E. R., B. J. REID and H. NELSON. 1980. Mobilization, Bioaccumulation and Sublethal Effects of Contaminants From Marine Sediments.

Laboratory studies were conducted to determine the mobilization and bioaccumulation of contaminants (Cd, Cu, Pb, Zn, As, Hg and PCB's) from marine sediments sampled near a ship repair facility. The deposit-feeding clam Macoma balthica and the filter-feeding mussel Mytilus edulis were used to assess bioaccumulation potential. Lead and the polychlorinated biphenyl (PCB) Aroclor 1254 were bioaccumulated to the greatest extent in laboratory tests. No direct relationship between salinity and contaminant uptake was apparent. Analysis of resident biota indicated significant accumulations of lead, Aroclor 1254 and copper in invertebrate tissues. The time required for 50% of the clam population to burrow (ET 50) ranged from 1.2 h in the control to 5.2 h in the contaminated substrate.

Key words: contaminated sediments, bioaccumulation, heavy metals, PCB's, burrowing behaviour.

INTRODUCTION

Plans for an expanded floating drydock and ship repair facility at Burrard Yarrows Drydock in Vancouver Harbour will require extensive blasting and dredging of nearshore bottom sediments. Concern was expressed about the potential for release and subsequent bioaccumulation of contaminants from the dredging operations. Sediments sampled in the vicinity of other ship repair facilities in Vancouver Harbour have contained extremely high levels of PCB's and heavy metals (Munday and McGreer, 1979), and sediments adjacent to the Burrard Yarrows facility were known to be similarly contaminated (H. Nelson, pers. comm.). The present study was initiated at the request of the Environmental Protection Service and was designed to assess the leaching and bioaccumulation of select contaminants from marine sediments collected in the area of Burrard Drydock. Contaminant levels in laboratory invertebrates were also compared with concentrations in resident biota collected near the drydock facility.

METHODOLOGY

FIELD COLLECTIONS

Collections of resident biota were made at locations shown in Figure 1. Samples of the mussel, Mytilus edulis, were collected from wooden pilings. Teredo worms, Bankia setacea, were removed from a broken piece of log obtained in a dredge sample. The crab, Cancer magister, was obtained from baited crab traps. After collection, all animals were immediately frozen whole in heat-treated (105°C for 12 h) aluminum foil for PCB samples, and "Whirlpak" plastic bags for metal samples.

Clams (Macoma balthica) for laboratory tests were collected from an uncontaminated area of the Fraser River estuary. Sediments containing M. balthica were sieved through a 5 mm mesh sieve, and clams remaining on the screen were retained for testing. Mussels (Mytilus edulis) over 3 cm in length were collected by SCUBA diver from the west side of Boyer Island, Howe Sound, B.C. Clams and mussels were returned to the lab and maintained in running seawater (25 ppt) at 10± 1° C. Polypropylene containers used in transporting and holding the bivalves were pre-treated with dilute nitric acid and rinsed in pesticide grade hexane/acetone mixture to prevent contamination.

Contaminated sediments were collected by Ponar grab from two sites adjacent to the Burrard Yarrows ship repair facility (Fig. 1). Sediment for metal analysis (100 g) was placed in a 'Whirlpak' bag and frozen. Sediment analyzed for PCB's was stored in pre-treated, foil-lined sealed glass jars. Sediments used in control tests were collected from the same uncontaminated area in the Fraser River estuary as the clams, M. balthica.

LABORATORY METHODS

Thirty-day, static bioassays were conducted in 77 L polyethylene containers. Tests were carried out at 10± 0.5°C at salinities of 10 and 25 ppt. All containers, and other equipment were pre-cleaned as previously described. Five litres of either contaminated or uncontaminated (control) sediment were

placed on the bottom of each container. Seawater from Burrard Inlet was adjusted to the desired salinity with dechlorinated tapwater and siphoned into the containers. One hundred M. balthica and fifty M. edulis were placed in each test container. Mussels were suspended in plastic baskets 20 cm from the sediment interface. Aeration was provided through airstone, and dissolved oxygen and pH were checked every 72 h. Water samples for metal and PCB analysis were drawn off using a pre-cleaned glass pipette, at intervals of 1, 10, and 30 days. Samples for metals were stored in acid-washed, distilled-rinsed plastic bottles; and samples for PCB analysis in hexane/acetone-treated, foil-capped glass bottles. After 30 days, all test organisms were removed and allowed to depurate for 48 h in clean seawater. Test species were segregated into individual 'Whirlpak' bags (for metals) and heat-treated foil (for PCB's) and immediately frozen. Frozen whole clam and mussel tissue was taken for analysis.

The rate of burrowing for M. balthica was determined using the methods described by McGreer (1979). Test containers were polypropylene tubs (approx. 43 x 14 x 10 cm) filled to 5 cm with either contaminated and uncontaminated sediment and covered by 2.5 L of seawater. Ten clams were placed on the sediment and the time required for animals to burrow was recorded.

ANALYTICAL TECHNIQUES

Copper, lead, zinc and cadmium in seawater were analyzed after chelation and extraction using APDC/MIBK by direct atomic absorption spectrophotometry. Mercury was analyzed by AAS using a cold-vapour technique, and arsenic by hydride generation atomic absorption. Sediment samples were digested with nitric/perchloric acids and analyzed as described above.

Tissue samples were homogenized, then digested using nitric/perchloric acids. Copper and zinc were analyzed by direct atomic absorption spectrophotometry; lead and cadmium by graphite furnace atomic absorption; and mercury and arsenic as described above.

Water samples were extracted with hexane and sediment samples with hexane/acetone. The resulting extracts were "cleaned up" by column chromatography using florisil. Sulfur was removed by extraction. Tissue samples were homogenized, then extracted with petroleum ether and "cleaned up" by liquid-liquid partition and column chromatography using florisil. All extracts were analyzed by gas-liquid chromatography with electron capture detection.

RESULTS AND DISCUSSION

Background concentration of metals and PCB's in sediments are shown in Table 1. The levels of all contaminants were considerably higher in the Burrard sediments compared to the control.

CONTAMINANT MOBILIZATION

The mobilization of contaminants from sediments into seawater during the 30 day bioaccumulation experiments is indicated in Table 2. Cadmium, mercury

and PCB concentrations were less than analytical detectable limits in all samples tested.

The concentration of copper, lead, zinc and arsenic in seawater overlying Burrard sediment increased over 30 days at 25 ppt salinity (Table 2). A similar increase in the seawater concentration of lead and arsenic at 25 ppt was observed in the control tests at the end of 30 days. Arsenic was the only metal which showed a significant increase in concentration (14-16 ug/L) over initial seawater levels after one day of exposure to Burrard sediments (Table 2). In several tests (i.e. Cu, Zn at 10 ppt; Pb, As at 25 ppt), the metal level in seawater of the control exceeded that of the Burrard (contaminated) sediment at the end of the test.

A number of possible mechanisms governing the mobilization of trace metals from marine sediments have been identified and include diffusion, desorption, dissolution, redox reaction, complex formation, biological effects and physical disturbance (Lu and Chen, 1977). The interaction of these different mechanisms is influenced by other factors such as the concentrations and form of metal in the sediment, the amount and type of organic material present, and changing environmental conditions (e.g. salinity and dissolved oxygen). Relatively few studies have been conducted on the release of metals from marine sediments, and these have often reported widely varying results for the same metals (e.g. Bindra and Hall, 1977; Burrows and Hulbert, 1975), especially when "polluted" sediments are compared.

The lack of apparent leaching observed in the present study with respect to cadmium was consistent with the findings of Burrows and Hulbert (1975), who found that sediments removed this metal from overlying waters under oxygenated conditions. Lu and Chen (1977) found no significant change in the concentration of mercury in interfacial water after five months' exposure of sediment to seawater. Results of copper, zinc and lead in the present study agree with those reported by Lu and Chen (1977) for oxygenated conditions. The greater release of metals from control than polluted sediments has also been reported by other reseachers, and the organic content of the sediment or seawater has been cited as the determining factor (Bindra and Hall, 1979; Burrows and Hulbert, 1975).

BIOACCUMULATION

Data from the laboratory bioaccumulation experiments are illustrated in Figures 2 to 8. Cadmium, lead, arsenic and mercury were bioaccumulated from the contaminated sediments to varying degrees during the 30 day test period. Lead was taken up most readily in both M. balthica and M. edulis (Fig. 4). Two PCB's, Aroclor 1254 and 1260, were also measured in tissues at concentrations above background levels (Fig. 8). The filter feeder M. edulis accumulated cadmium, lead, zinc and mercury to a greater level than did the deposit- feeding M. balthica, which accumulated arsenic and the Aroclor compounds to a greater degree. Cadmium, lead, and PCB's reached their highest tissue levels at 10 ppt salinity, while zinc, arsenic and mercury showed more accumulation at 25 ppt. A number of metals (Cd, Pb, Zn, As, Hg) were shown to bioaccumulate from control sediments to levels exceeding those from the contaminated substrate.

Concentrations of contaminats in tissues of resident biota collected near the Burrard drydock (Fig. 1) are also shown in Figures 2-8. Compared to background concentrations recorded for M. edulis used in laboratory tests, the resident mussels showed no significant accumulation of cadmium, arsenic or mercury; however, elevated levels of zinc, copper, lead and Aroclor 1254 were found. Accumulation in other invertebrates sampled was significant only for copper (Fig. 3).

The availability of trace metals to organisms may be affected by four general factors: 1) the physiological and ecological characteristics of the organism; 2) the forms of the dissolved metals; 3) the forms of trace metals in ingested solids; and, 4) the chemical and physical characteristics of the water (Jenne and Luoma, 1977). Luoma and Jenne (1976, 1977) studied the uptake of cadmium, zinc, cobalt, and silver in Macoma balthica and showed that the availability of metals was dependent upon the metal sediment association, with zinc being absorbed more readily than cadmium from iron-oxide particules. Similarly, the bioavailability of dissolved metals is affected by specification of the metal, and the presence of various organic and inorganic complexes (Jenne and Luoma, 1977). The presence of organic chelating agents may explain the lack of significant bioaccumulation of certain metals compared to their concentration in Burrard sediments, and to their uptake from control sediments in the present study. The lack of a direct correlation between metal uptake and sediment concentration has been observed in other studies (e.g. Hall and Bindra, 1979), and points to the need for bioaccumulation tests in addition to chemical data in assessing contaminant effects.

Two PCB's, Aroclors 1254 and 1260, were bioaccumulated from contaminated sediments in the present study (Fig. 8). This result was surprising in that only Aroclor 1260 was detected in the contaminated sediment sampled (Table 1). Failure to detect all organic compounds in a sample with routine analytical techniques (i.e. gas-liquid chromatography with electron capture detection) can occur when one compound is present in a concentration much higher than the others (J. Park, pers. comm.). In such cases, more sophisticated analytical procedures (e.g. gas chromatography coupled with mass spectrometry) are required. Such problems underline the need for bioaccumulation studies in assessing environmental effects of organic compounds. Langston (1978) demonstrated selective accumulation of lower chlorinated Aroclor compounds (e.g. Aroclor 1254) compared to more highly chlorinated Aroclors (e.g. A.1260) in M. balthica. Results of the present study also showed a preferential uptake for Aroclor 1254 over Aroclor 1260 in both laboratory and resident biota.

There was relatively good agreement in the degree of bioaccumulation between laboratory test animals and resident biota for cadmium, lead, zinc, arsenic, and PCB compounds (Fig. 2-8). The greatest discrepancy was for copper which accumulated to high levels in resident mussels (173 ppm) and crabs (133 ppm), but which did not bioaccumulate appreciably in laboratory tests. One explanation is that a second source of copper (e.g. contamination by industrial effluents, marine paints, etc.) was present at the site.

SUBLETHAL EFFECTS

Burrowing of Macoma balthica (Table 3) was shown to be inhibited in contaminated sediments (2.7-5.2 h) compared to that of the control (1.2 h). In a previous study, McGreer (1979) showed the ET50 value (4.8 h) for burrowing of M. balthica in metal-contaminated sediments to be significant (p 0.05) from controls (0.17h), and cadmium was implicated as the metal which most affected burrowing behaviour.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. Waldichuk, Department of Fisheries and Oceans for his advice during this study. We also acknowledge Can Test Ltd. of Vancouver, B.C. for performing the chemical analyses. Finally, the assistance of the following members of E.V.S. Consultants Ltd. staff is greatly appreciated: J.B. Coustalin, D.R. Munday, P.M. Chapman, G.A. Vigers, D. Prissick and C. Davidson.

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TABLE 1

BACKGROUND METAL AND PCB LEVELS IN
BURRARD AND CONTROL SEDIMENTS

Sample	Metals (ug/g dry weight)						Polychlorinated Biphenyls (ug/g dry weight)	
	Cd	Cu	Pb	Zn	As	Hg	Aroclor	Concentration
Burrard	2.04	975	215	2110	318	4.48	1260	17
Control	<0.2	12.5	<3	41.9	3.02	0.05	1260	<0.005

TABLE 2

CONCENTRATIONS OF HEAVY METALS AND POLYCHLORINATED BIPHENYLS IN SEAWATER DURING 30 DAY BIOACCUMULATION EXPERIMENTS

Salinity (ppt)	Time (days)	Test	Metals (ug/L) Cd	Cu	Pb	Zn	As	Hg	Polychlorinated Biphenyls Aroclor	Concentration (ug/L)
10	Initial	Seawater	<1	1	1	131	1	<0.2	-	<0.05
25	Initial	Seawater	<1	3	1	250	1	<0.2	-	<0.05
10	1	Burrard	<1	<1	1	41	16	<0.2	-	<0.05
10	10	Burrard	<1	3	4	42	10	<0.2	-	<0.05
10	30	Burrard	<1	<1	<1	19	2	<0.2	-	<0.05
10	30	Control	<1	6	<1	56	2	<0.2	-	<0.05
25	1	Burrard	<1	1	2	52	14	<0.2	-	<0.05
25	10	Burrard	<1	7	3	108	9	<0.2	-	<0.05
25	30	Burrard	<1	14	7	96	12	<0.2	-	<0.05
25	30	Control	<1	7	15	59	13	<0.2	-	<0.05

TABLE 3

THE MEDIAN EFFECTIVE TIME (ET50) AND 95% CONFIDENCE LIMITS FOR THE BURROWING OF *Macoma balthica* IN BURRARD AND CONTROL SEDIMENTS

Sample	ET50(h)	95% Confidence Limits	$\bar{x}$ for replicates A and B
Burrard Replicate A	2.7	(0.63 - 10.0)	3.9
Burrard Replicate B	5.2	(1.92 - 14.04)	
Control	1.2	(0.28 - 5.0)	

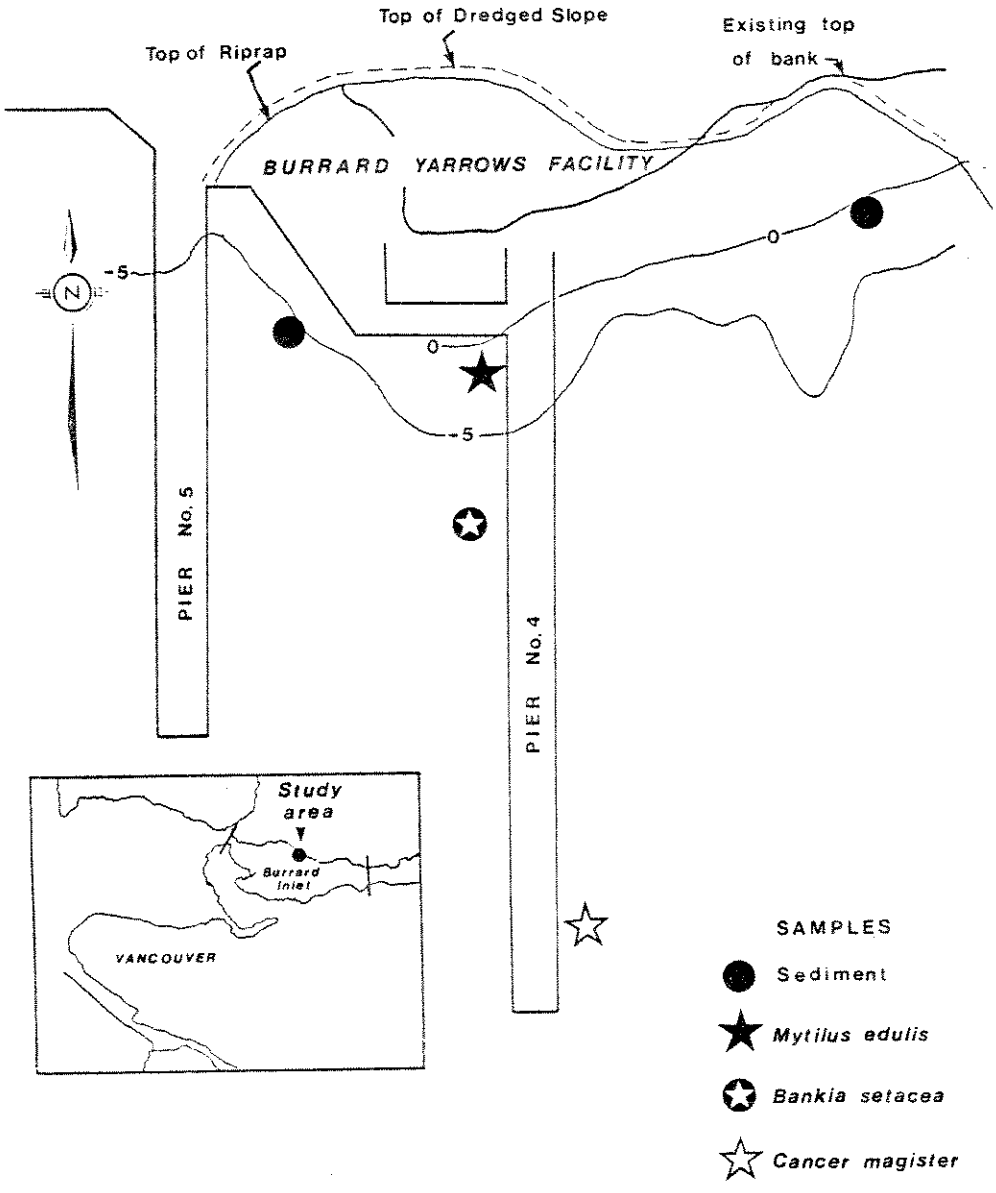


Figure 1

Location of study area and sampling sites
(Schematic diagram only)

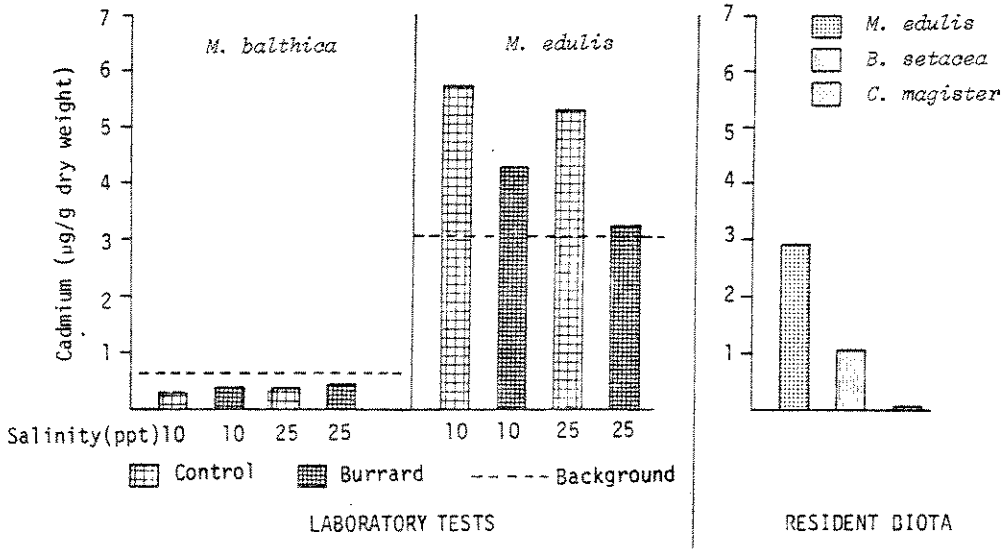


Figure 2

Bioaccumulation of cadmium

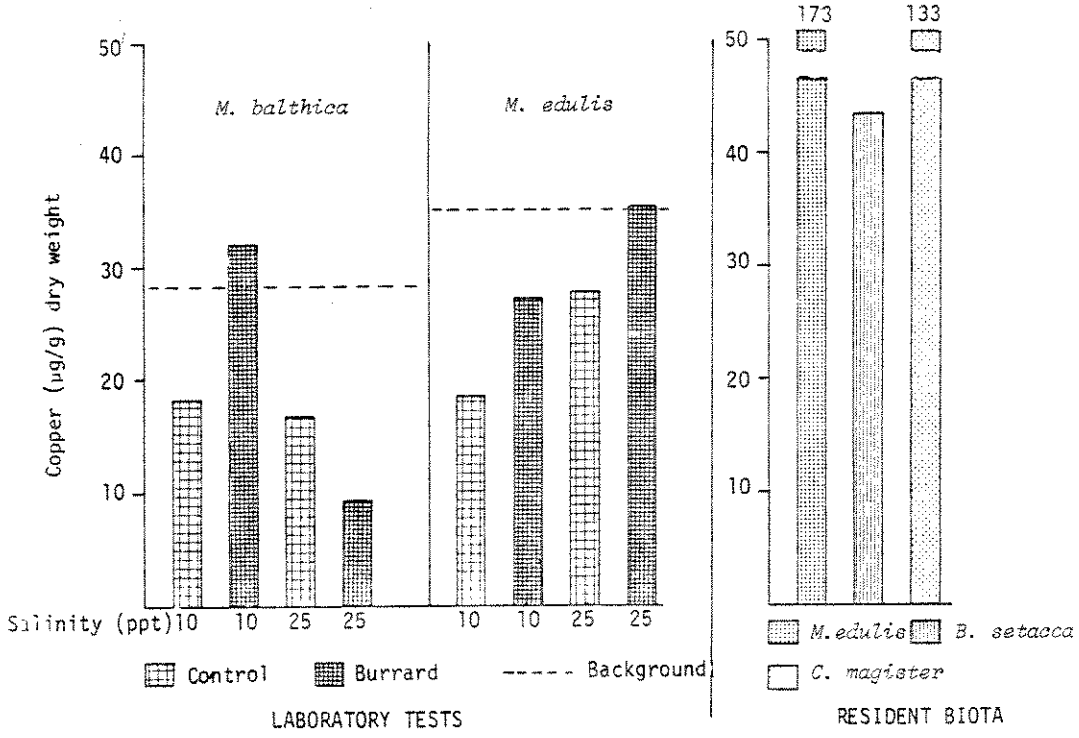


Figure 3

Bioaccumulation of copper

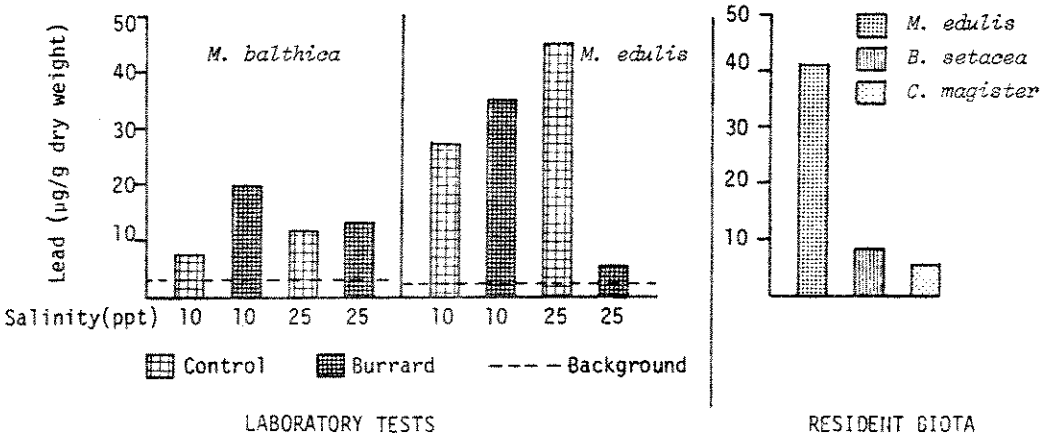


Figure 4

Bioaccumulation of lead

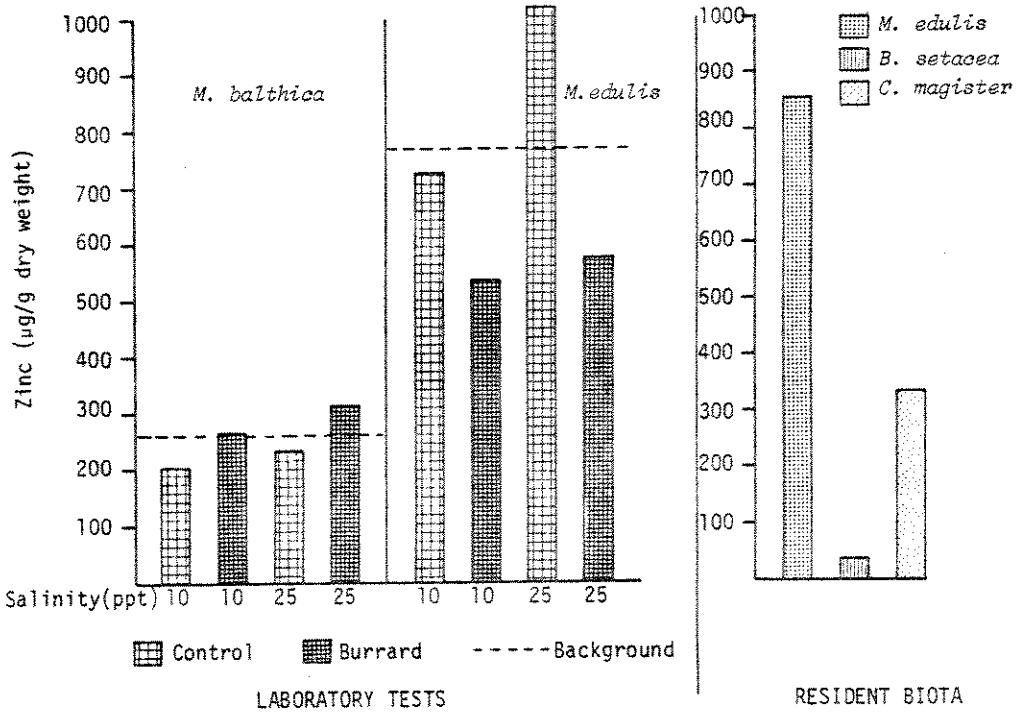


Figure 5

Bioaccumulation of zinc

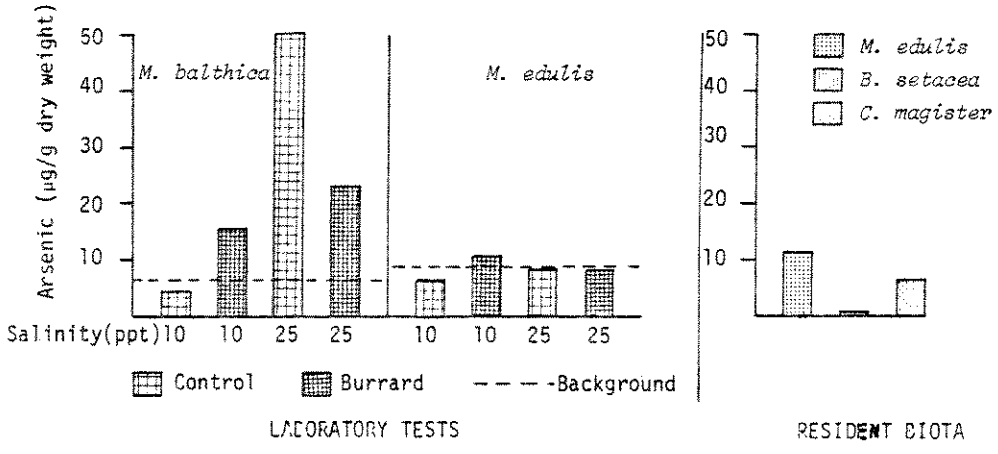


Figure 6

Bioaccumulation of arsenic

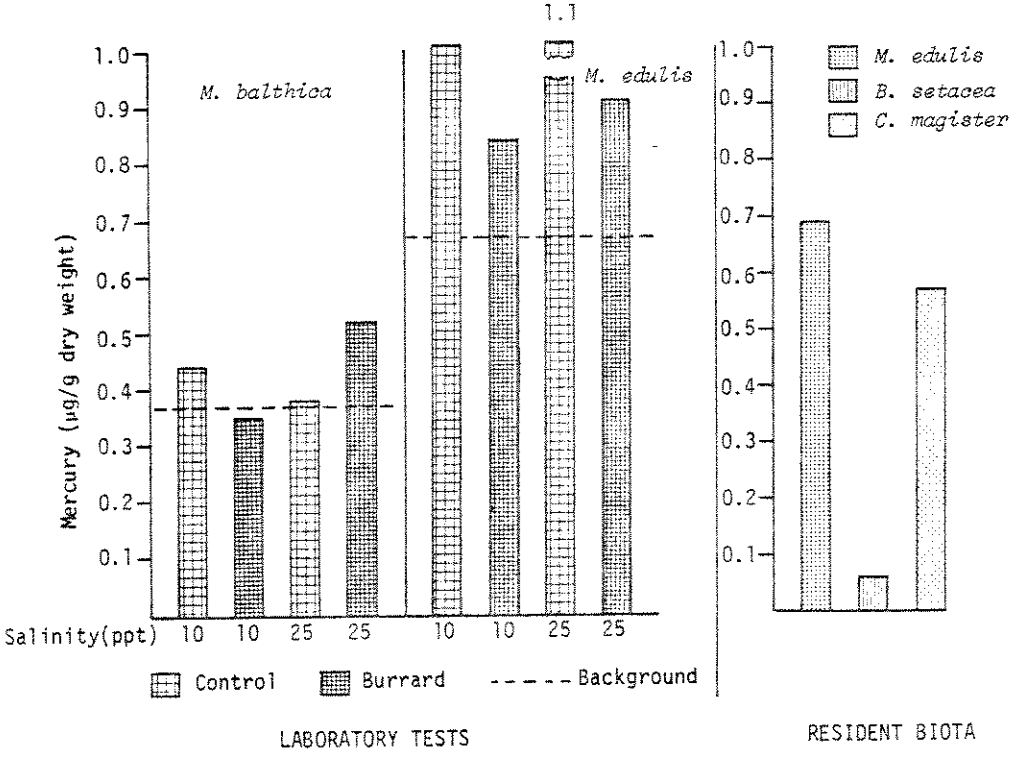
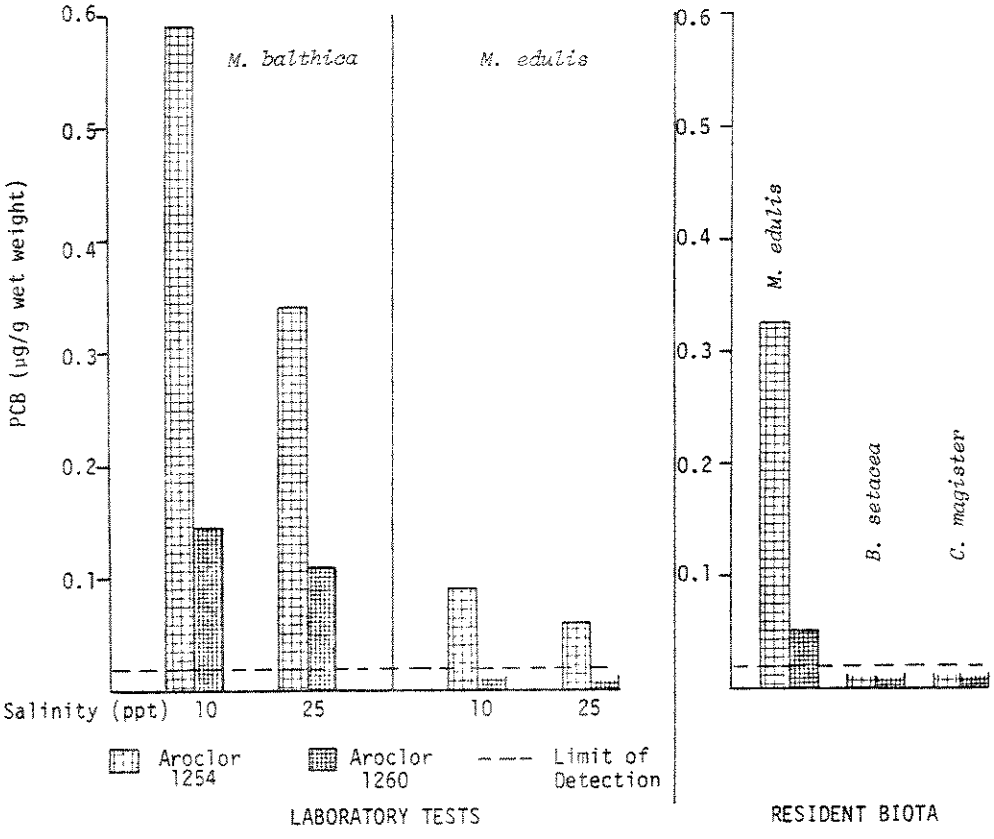


Figure 7

Bioaccumulation of mercury



Concentration of PCB's in Control test organisms at or below detection limit of 0.02 ug/g.

Figure 8

Bioaccumulation of PCB's from Burrard sediments

MESURES IN SITU DE L'EXCRÉTION D'AXOTE ET DE LA CONSOMMATION D'OXYGÈNE POUR MESURER LE STRESS CHEZ ASTERIAS VULGARIS.

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RÉSUMÉ - La mesure de la consommation d'oxygène et d'excrétion des produits métaboliques a été effectuée in situ afin de connaître l'état de stress de Asterias vulgaris. Des sujets ont été soumis à des faibles concentrations d'oxygène dissous dans des enceintes isolées à la station Ocean Pulse de Jeffries Ledge, dans le golfe du Maine (42°46.5'N, 70°14.5'0), par 36 mètres de fond. Le rapport atomique de l'oxygène consommé à l'azote excrété avait diminué d'un ordre de grandeur quand la concentration d'oxygène dissous était abaissée à 0.15 ml/L, comparativement aux incubations aux concentrations supérieures à 0.5 ml/L.

L'altération expérimentale de la qualité de l'eau peut être réalisée in situ par l'introduction de substances toxiques dans une cage d'incubation contenant les sujets qui, sauf pour le confinement dans l'enceinte, sont toujours dans leur milieu naturel.

IN SITU MEASUREMENTS OF NITROGEN EXCRETION AND OXYGEN CONSUMPTION AS DETERMINATION OF STRESS IN ASTERIAS VULGARIS.

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SOMMARY - Monitoring of oxygen consumption and excreted metabolic products has been used in situ to determine the state of stress in Asterias vulgaris. Animals were subjected to low dissolved oxygen concentrations in closed chambers at the Ocean Pulse station on Jeffries Ledge in the Gulf of Maine (42°46.5'N, 70°14.5'W) in water approximately 36 m deep. The atomic ratio of oxygen consumed to nitrogen excreted was found to decrease by an order of magnitude when dissolved oxygen concentrations decreased to 0.15 ml/l as compares with incubations in which the concentration was maintained above 0.5 ml/l.

The experimental alteration of natural water quality can be accomplished in situ by the introduction of toxic substances into an incubation chamber containing the test organism, which, except for isolation in the chamber, has not left its natural environment.

In situ Measurements of Nitrogen Excretion and Oxygen Consumption as Determinations of Stress in *Asterias vulgaris*

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National Marine Fisheries Service
Northeast Fisheries Center
Biological Oceanography Investigation
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ABSTRACT

Monitoring of oxygen consumption and excreted metabolic products was used *in situ* to determine the state of stress in *Asterias vulgaris*. Animals were subjected to low dissolved oxygen concentrations in closed chambers on Jeffreys Ledge in the Gulf of Maine (42°46.5'N, 70°14.5'W) in water approximately 36 m deep. The atomic ratio of oxygen consumed to ammonium-nitrogen excreted appeared to decrease by an order of magnitude when dissolved oxygen concentrations decreased to 0.15 ml/l as compared with incubations in which the concentration was maintained above 0.5 ml/l.

The experimental alteration of natural water quality can be accomplished *in situ* by the introduction of toxic substances into an incubation chamber containing test organisms, which, except for being isolated in the chamber, remain in their natural environment.

Introduction

To adequately monitor the relative "health" of an ecosystem one must determine the baseline or "normal" levels of abiotic variables in the system and the responses of the system's inhabitants to changes in these variables. The reactions of the biota to the alteration of a single variable may be reflected by changes in community composition, rates of growth, reproduction or metabolism. The synergistic effects of changes in more than one variable may result in different reactions. While the measurement of abiotic variables is basically straightforward in spite of the sophisticated instrumentation and techniques that may be required, adequate description and definition of the responses by the biota to changes in their environment is not. Since it is difficult to determine the response of each species in the community to each variable and combinations of variables, a selected group of species and controllable variables must be chosen. Another approach would be to consider the whole community as a single entity and measure the community's response to environmental changes. Such responses might be manifested in species composition changes or alterations in community metabolic rates.

Our species selection criteria for this study were that the animals be typical and permanent members of the community, have similar species present in both pristine and polluted environments, be capable of being manipulated without injury, produce significant changes in selected variables in a reasonable time interval and not be highly motile. The echinoderm Asterias vulgaris met these criteria. To avoid synergistic effects we altered only

one variable, the dissolved oxygen concentration. We chose dissolved oxygen because its concentration varies in nature and oxygen depletion has historically been responsible for extensive faunal mortalities (May, 1973; Young, 1973; Azarovitz et al., 1980). Boesch (1978) documented the extirpation of echinoderms including A. forbesi during the hypoxic event off the coast of New Jersey during the summer of 1976. As death is the end product of unalleviated lethal stress, an understanding of the species reactions to this stress, before death, is required so that a simple monitoring of the species may indicate stressful changes in the environment.

In light of the use of fauna and flora to indicate environmental quality, Bayne (1975) modified the definition of stress by Brett (1958) to read: "Stress is a measurable alteration of a physiological, or behavioral, or biochemical, or cytological steady-state which is induced by environmental change, and which renders the individual (or the population, or the community) more vulnerable to further environmental change" (Mann, 1977).

Various indices of stress have been reported in the literature including activity (Savage, 1976; Olla et al., 1978), morphometrics (Cooper et al., 1964; Mann, 1977), and metabolism (Snow and Williams, 1971; Jefferies, 1972; Bayne, 1977; Mann, 1977; Spaargaren, 1977; Widdows, 1978). The metabolic indices of stress appeared to be the most applicable to echinoderms. Adult echinoderms are oxygen conformers and as their oxygen consumption is proportional to the ambient oxygen concentrations, a low rate of oxygen consumption alone would not indicate stress. These animals are, however, ammonotelic and the O:N ratio should be a good metabolic indicator of stress.

The atomic ratio of oxygen consumed to nitrogen excreted (O:N) is documented in the literature as a useful indicator of stress in bivalves (Ansell and Sivadas, 1973; Bayne, 1975; Widdows, 1978). A relatively low O:N ratio is indicative of high protein catabolism usually associated with stress, whereas a higher O:N ratio is the result of normal or unstressed, predominantly carbohydrate and lipid, catabolism.

We chose to conduct the incubations in situ to avoid removing the test animals from their natural environment (except for the confinement of the incubator) and to minimize handling and transport. Bayne et al. (1976) have approached the problem of extrapolating physiological results obtained in the laboratory by interpreting the physiological responses of natural populations in field experimentation. In our opinion in situ experimentation may be used effectively to complement and verify laboratory physiological experimentation and this study is an initial effort toward that end.

Materials and Methods

Incubators made of black plexiglas were placed by divers on the rock substrate of Pigeon Hill in approximately 36 m of water. Pigeon Hill rises off Jeffreys Ledge in the Gulf of Maine at 42°46.5'N latitiude and 70°14.5'W longitude approximately 31 km ENE of Cape Ann, MA (Figure 1). The incubators were cylindrical (28 cm diameter, 15 cm height) with a flat bottom and removable flat top having a volume of 9.2 l. Each top contained two nipples which were connected by non-toxic plastic tubing to a submersible recirculating pump (Figure 2) which maintained water flow within the incubator to prevent stratification. After the incubators were set by the divers, the top was removed and the surface water, with which the incubator had been filled to facilitate transport to the bottom, was replaced by ambient bottom water. The divers then located a starfish which was active and apparently in good health, gently placed the animal inside the incubator and secured the top. Three to five replicate 35 ml bottom water samples were taken for dissolved oxygen analyses using the azide modification of the iodometric method (Am. Public Health Assoc., 1975) except that amylose was used in place of starch and 0.025 N phenylarsine oxide was used in place of sodium thiosulfate (Kroner et al., 1964; U. S. EPA, 1974). Samples from the incubators were taken for both dissolved oxygen and nutrient analysis immediately after closing the starfish inside and at the end of the incubation periods. The samples were drawn using 50 ml syringes fitted with 3-way valves which could be coupled to the nipples on the incubator top. In taking a sample, the excurrent tube was crimped and

detached from the incubator nipple. The syringe was then attached to the nipple and flushed with incubator water using the 3-way valve. After the sample was taken and the valve closed to seal the syringe, the excurrent tube was reconnected to the nipple. In the case where samples were drawn during an incubation, approximately 90 ml of incubated water was removed which required that an equal amount of ambient water enter the incubator. This amounted to only 0.97% of the total incubated volume. To determine the dissolved oxygen concentration of the incubated water 0.2 ml of Winkler reagents were added to 35 ml of the sample by injecting the reagents via a small syringe directly into the 50 ml syringe, replacing the 3-way valve and shaking. The samples were then titrated.

The nutrient samples, with the exception of those for ammonium-nitrogen, were frozen in 30 ml polypropylene tubes that had been cleaned with hydrochloric acid and deionized water. These samples were analyzed ashore on a Technicon Autoanalyzer II.[1] Nitrite and nitrate were estimated using the naphthylethylenediamine-sulfanilamide system with cadmium reduction of nitrate after Wood et al. (1964). The inorganic phosphorus analysis utilized the molybdate-ascorbic acid procedure after Murphy and Riley (1962). The reactive dissolved silicone procedure is based on the use of oxalate to reduce a silicomolybdate complex and at the same time decompose any phospho- or arseno-molybdates (Mullin and Riley, 1955). The urea analysis is an adaptation to seawater of Marsh et al.'s (1965) blood urea method in which diacetylmonoxime reacts with urea in the presence of thiosemicarbizide and ferric ion intensifiers. Autoanalyzer standardizations were made in artificial seawater (31 g NaCl + 10g $MgSO_4 \cdot 7H_2O$ + 0.04 g $NaHCO_3$).

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[1]Does not constitute an endorsement statement by the National Marine Fisheries Service.

Results and Discussions

All animals survived the experiment and recovered when placed in oxygen saturated surface water. It is unlikely that starvation played any part in producing stress as Jangoux and Van Impe (1977) have shown that in Asterias rubens (L.), a short duration starvation (one week) is very unlikely to induce caecal autolysis and since starvation of medium duration (2 to 4 weeks) is required for the pyloric caeca to liberate their reserve material and ensure the animal's survival. The longest incubation was 240 hours (1.43 weeks) with the average being 126 hours (0.75 weeks). The animals were not removed from the benthic environment in which the temperature ranged from 9°C to 11°C and therefore were never thermally stressed. The physical stress caused by handling the animals was minimal as the animals were carefully picked up off the substrate and gently placed in the incubators. Hypoxia then was the only obvious stress responsible for any changes that occurred in the oxygen consumption rates (VO_2), nitrogen excretion rates and the O:N ratios.

Obtaining more definitive data on the echinoderm's basic, unstressed oxygen consumption and nitrogen excretion at ambient dissolved oxygen (D.O.) concentrations proved impossible due to periods of inclement weather. A literature search to provide these data resulted in only six references concerning Asterias spp. and of these only three were usable, as the others did not contain temperatures or weights. The usable data (Table 1) required Q_{10} recalculations so that they could be compared with ours (VO_2 in ml O_2/gm wet-weight/hr at 9°or 11°C). According to Farmanfarmaian (1966), Meyer (1935) reported the Q_{10} value of A. rubens within the range of 10° to 25°C to be between 2 and 3.

We used the average Q_{10} value of 2.5 to calculate the VO_2 for A. rubens and A. forbesi at 9° and 11°C. The range of VO_2 for the three literature values at 9°C is .016 to .019 with an average of 0.018 ml O_2/gm wet-weight/hr and the range at 11°C is .018 to .023 with an average of 0.021 ml O_2/gm wet-weight/hr. From Table 2 (in which the test organisms are designated stars I, II, III and IV) the VO_2 for stars I (corrected to 9°C), II (first 96 hours only) and III are each below the calculated VO_2 for 9°C and, after rounding, average 0.009 which is 0.009 below that expected at 9°C. (The similarity of these numbers is a coincidence and has no significance.) Upon correction of the oxygen uptake values for star I (weight = 36.7 grams) from 11°C to 9°C using the aforementioned Q_{10} of 2.5, the calculated rate of 0.336 ml O_2/hr/animal compares exactly with that of star III (weight = 35.8 grams) which was incubated at 9°C. This demonstrates a consistency in the VO_2 of the animals over an average change in dissolved oxygen concentrations of 4.47 ml/l. Star II (weight = 47.7 grams) has a whole animal rate slightly higher than stars I and III. This is probably due to star II's larger size since the rate becomes slightly lower when placed on a per gram wet-weight basis. According to Farmanfarmaian (1966), Koller and Meyer (1933) have shown that the rate of oxygen consumption by A. rubens decreases as the weight increases. Their interpretation is that as the weight of an A. rubens increases, the respiratory mechanism quantitatively becomes limiting. This appears to be corroborated by our data for star II and for our one specimen of Porania insignus (star IV) in which the consumption rate of 0.333 ml O_2/hr/animal is only 0.003 below the rate of stars I and III and 0.026 below the average rate for stars I, II and III (0.359 ml O_2/animal/hr), despite the fact that it weighs 3 times the average of stars I, II and III (120.3 grams vs. 40.1 grams).

That adult asteroids are oxygen conformers and their oxygen consumption rates are therefore dictated by the ambient dissolved oxygen concentration has been well documented. Binyon (1972) states that some investigators claim A. rubens can utilize the last traces of dissolved oxygen from the environment approaching 100% utilization. Farmanfarmaian (1966) reports that Koller and Meyer (1933) have demonstrated that A. rubens is a strict conformer in the oxygen concentration range of 12 to 2 cc/l. Still others, according to Binyon (1972), found a 95% extraction capability, which, for the initial ambient oxygen of our experiment, would reduce the oxygen in the incubator to about 0.26 ml/l (Table 3). Only in the second incubation of star II was the oxygen concentration of the incubated water permitted to fall below the 95% extraction level (to 0.15 ml/l).

Since adult asteroids are oxygen conformers, the rate of oxygen consumption alone does not necessarily indicate stress in the organism. However, a comparison of the ratios in Table 3 for A. vulgaris, stars II and III, does indeed suggest stress in the 23-29 Oct. incubation of star II, in which the dissolved oxygen was permitted to fall below the 95% extraction level. While no statistical significance can be attached to these data due to the low number of replications, a trend between stressed and unstressed conditions is apparent especially with regard to oxygen consumption, increased excretion of urea, phosphate excretion, and the loss of nitrate from the incubated water. As ammonium is the primary nitrogenous excretion product of Asteroidea, it is sufficient to use the ratio of $O:NH_4$ as the O:N indicator of stress. The low $O:NH_4$ value of 4.5 for the low D.O. incubation of star II, when compared to the much higher ratio values of 42 and 191 for the higher D.O. incubations of star II and star III respectively, indicates the stressed condition of star II during the second part of the incubation.

Conclusions

The O:N ratio successfully indicated a sublethal stressful condition in the echinoderm *Asterias vulgaris* whereas a determination of the animal's rate of oxygen consumption alone did not. A natural extension of this investigation would be to determine the changes in VO_2 and O:N ratio of *A. vulgaris* when subjected to stressful conditions other than hypoxia. These could include man-induced conditions such as exposure to varying concentrations of heavy metals and/or petrochemicals. Baseline studies into the *in situ* VO_2 and O:N ratios of other "indicator" animals should also be accomplished and may include *Mytilus* spp., *Modiolus modiolus*, *A. forbesi*, *Spisula solidissima* and other species.

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detached from the incubator nipple. The syringe was then attached to the nipple and flushed with incubator water using the 3-way valve. After the sample was taken and the valve closed to seal the syringe, the excurrent tube was reconnected to the nipple. In the case where samples were drawn during an incubation, approximately 90 ml of incubated water was removed which required that an equal amount of ambient water enter the incubator. This amounted to only 0.97% of the total incubated volume. To determine the dissolved oxygen concentration of the incubated water 0.2 ml of Winkler reagents were added to 35 ml of the sample by injecting the reagents via a small syringe directly into the 50 ml syringe, replacing the 3-way valve and shaking. The samples were then titrated.

The nutrient samples, with the exception of those for ammonium-nitrogen, were frozen in 30 ml polypropylene tubes that had been cleaned with hydrochloric acid and deionized water. These samples were analyzed ashore on a Technicon Autoanalyzer II.[1] Nitrite and nitrate were estimated using the naphthylethylenediamine-sulfanilamide system with cadmium reduction of nitrate after Wood et al. (1964). The inorganic phosphorus analysis utilized the molybdate-ascorbic acid procedure after Murphy and Riley (1962). The reactive dissolved silicone procedure is based on the use of oxalate to reduce a silicomolybdate complex and at the same time decompose any phospho- or arseno-molybdates (Mullin and Riley, 1955). The urea analysis is an adaptation to seawater of Marsh et al.'s (1965) blood urea method in which diacetylmonoxime reacts with urea in the presence of thiosemicarbizide and ferric ion intensifiers. Autoanalyzer standardizations were made in artificial seawater (31 g NaCl + 10g $MgSO_4 \cdot 7H_2O$ + 0.04 g $NaHCO_3$).

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Ammonium-nitrogen samples were stabilized by freezing in glass serum bottles with the phenol-alcohol reagent (Degobbis, 1973). Immedidately upon return to the laboratory the thawed samples were carried through the remainder of the phenylhypochlorite method of Solorzano (1969) as modified by Liddicoat et al. (1975). Calibration was done by standard addition to replicates of a surface sea water sample.

The bottom temperature was recorded at the beginning and end of all incubations. The incubations were terminated when the dissolved oxygen concentrations reached between 0.9 and 0.15 ml/l. Upon completion of an experiment the animal was brought to the surface, placed in oxygen saturated surface water and observed for signs of revival to show that death had not occurred. Once assured the animal was still living, it was frozen for a later wet-weight measurement. The rate of oxygen consumption by the bottom water alone was simultaneously measured in another incubator and then subtracted from the rate of oxygen consumption obtained by incubating the animal and entrapped water. Thus, the reported VO_2 values of ml O_2/hr/animal and ml O_2/gm wet-weight/hr are for the animals only.

The oxygen consumption rates were then converted to micromoles/gram wet-weight/hour (μM/gm/hr) and microequivalents/gram wet-weight/hour (μE/gm/hr) to make them compatible with the nitrogen excretion data so that the atomic O:N ratio could be calculated.

Table 1. VO_2 data from the literature corrected to 9° and 11°C.

	VO_2 ml/gm/hr	C°	Corrected VO_2 using Q_{10} = 2.5	Reference
Asterias rubens	0.024	15	0.016 @ 9°C	Bock & Schlieper (1953)[1]
Asterias rubens	0.024	15	0.018 @ 11°C	" " "
Asterias rubens	0.03	15	0.019 @ 9°C	Meyer (1935)[2]
Asterias rubens	0.03	15	0.023 @ 11°C	" "
Asterias forbesi	0.074	25	0.019 @ 9°C	Maloeuf (1937)[1]
Asterias forbesi	0.074	25	0.023 @ 11°C	" "

[1]From Farmanfarmaian (1966) Table 10-7 (values reported in CC O_2/gm wet-wt/hr).

[2]From Handbook of Respiration (1958) Table 148.

Table 2. Environmental conditions and rates of oxygen consumption and nitrogen excretion.

	H_2O Only 12-16 Oct.	A. vulgaris 12-16 Oct. Star I	A. vulgaris 19-23 Oct. Star II	A. vulgaris 23-29 Oct. Star II	A. vulgaris 29 Oct.-3 Nov. Star III	Porania insignis 29 Oct.- 3 Nov. Star IV
Weight (grams)	-	36.70	47.70	47.70	35.75	120.25
Temperature (°C)	11.0	11.0	9.0	9.0	9.0	9.0
Range D.O. (mg/l) (ml/l)	7.57-7.22 5.30-5.06	7.57-1.27 5.30-0.89	7.31-0.96 5.12-0.67	0.96-0.21 0.67-0.15	7.11-0.66 4.98-0.46	7.11-0.71 4.98-0.50
ΔDO (ml/l)	0.24	4.41	4.45	0.52	4.52	4.48
ΔT (hours)	96	92.75	96	144	116.25	116.25
95% extraction (ml/l)	-	-	0.26	0.26	0.25	0.25
VO_2 ml O_2/animal/hr[1]	-	0.416 (0.336 @ 9°C)	0.406	0.010	0.336	0.333
VO_2 ml O_2/gm wet/hr	0.0025 (ml/l/hr)	0.0113 (0.0092 @ 9°C)	0.0085	0.0002	0.0094	0.0028
O consumption uE/gm wet/hr	0.222 (uE/l/hr)	1.001 (0.821 @ 9°C)	0.759	0.0191	0.839	0.247
NH_4^+ excretion uM/gm wet/hr			0.0182	0.0042	0.0044	0.0270
NO_2^- excretion uM/gm wet/hr			0.0046	0.0032	0.00002	0.00086
NO_3^- excretion uM/gm wet/hr			-0.0022[2]	-0.0110	-0.0018	-0.0054
$\Delta NH_4^+ + NO_2^- + NO_3^-$ uM/gm wet/hr			0.0206	-0.0036	0.0026	0.0225
ΔUrea excretion uM/gm wet/hr			0.0020	0.0060	0.0014	0.0026
ΔN_{sum} uM/gm wet/hr			0.0234	0.0024	0.0040	0.0251
ΔP excretion uM/gm wet/hr			-0.0002	0.0005	*0.00002[3]	-0.005

[1] VO_2 (ml O_2/animal/hr) = $\frac{\Delta DO(ml/l)}{\Delta T(hours)}$ - .0025 (ml/l/hr) x 9.236(l)

[2] Negative signs indicate "uptake" rates.

[3] This number is so small it is not statistically significant and in fact could be negative.

Table 3. Ratios of oxygen consumed to nitrogen excreted.

	A. vulgaris 19-23 Oct. Star II	*A. vulgaris* 23-29 Oct. Star II	*A. vulgaris* 29 Oct.-3 Nov. Star III	*Porania insignus* 29 Oct.-3 Nov. Star IV
Temperature (°C)	9.0	9.0	9.0	9.0
Range D.O. (mg/l) (ml/l)	7.31-0.96 5.12-0.67	0.96-0.21 0.67-0.15	7.11-0.66 4.98-0.46	7.11-0.71 4.98-0.50
95% extraction (ml/l)	0.26	0.26	0.25	0.25
$O:NH_4^+$ uE/uM	42	4.5	191	9.1
$O:NO_2^-$ uE/uM	165	6.0	42000	274
$O:NO_3^-$ uE/uM	-345	-1.7	-466	-46
$O:NO_2 + NO_3 + NO_4$ uE/uM	37	-4.9	311	10.0
O:Urea uE/uM	271	3.2	599	95
O:Nsum uE/uM	32	8	210	9.8
O:P uE/uM	-3800	38	*42000	-490

* No significance can be attached to this number as the O:P ratio is derived from the statistically insignificant P (Table 2) and as such could be large and negative.

Figure 1. Study area.

	A. vulgaris 19-23 Oct. Star II	*A. vulgaris* 23-29 Oct. Star II	*A. vulgaris* 29 Oct.-3 Nov. Star III	*Eurantia brevipes* 29 Oct.-3 Nov. Star IV
Temperature (°C)	9.0	9.0	9.0	9.0
Range D.O. (mg/l) (ml/l)	7.31-0.96 5.12-0.67	0.96-0.21 0.67-0.15	7.11-0.66 4.98-0.46	7.11-0.71 4.98-0.50
95% extraction (ml/l)	0.26	0.26	0.25	0.25
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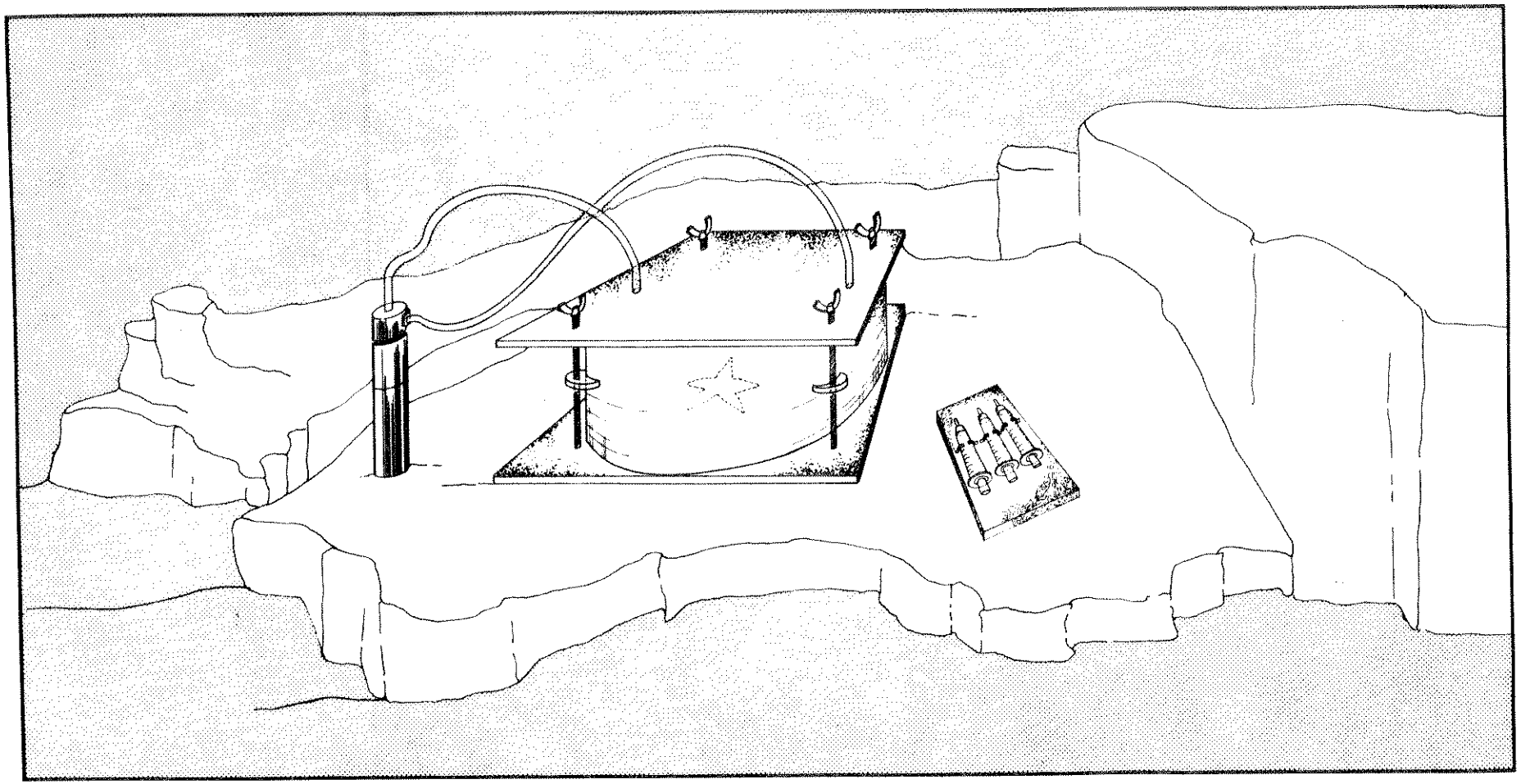
Figure 2. *In situ* incubator.



LE RECOURS AUX BIO-ESSAIS POUR EVALUER L'EFFET D'EFFLUENTS MUNICIPAUX ET D'UN EFFLUENT INDUSTRIEL SUR LA REPRODUCTION DU BAR RAYÉ

W.R. Parker et K.G. Doe

EPS

RESUME - Le bar rayé de la rivière Annapolis en Nouvelle-Ecosse ne parvient plus à se reproduire depuis plusieurs années. Des oeufs ont été recueillis dans la rivière au moyen de filets dérivants et ont continué leur maturation dans l'eau provenant de rivière Shubenacadie, dans laquelle le bar se reproduit très bien. Comme des eaux d'égoût chlorées de Bridgetown et l'effluent d'une usine de fabrication de matières élastiques sont soupçonnés de contribuer au déclin de ces populations, des stations ont été établies en aval de ces sites.

Six expériences redondantes sur l'éclosion des oeufs ont été effectuées: le pourcentage d'éclosion variait entre 43 et 62% selon les différents traitements. Les larves encore vivantes au jour 3 étaient dénombrées; les données étaient soumises à une analyse de la variance à double entrée. Il n'y avait pas de différences significatives entre les groupes quant au pourcentage de survie des larves ($F = 2.29$; et $= 0.05$).

Les larves étaient regroupées et élevées pendant plusieurs mois dans des conditions d'origine. Le taux de survie variait entre 0 et 2.8%.

Au niveau de l'éclosion et de la survie, les écarts étaient négligeables entre les groupes gardés dans l'eau des stations de la rivière Annapolis et les groupes gardés dans l'eau des stations de la Shubenacadie. Ceci indique que les substances ou le phénomène responsable de l'échec reproductif ont disparu rapidement, se sont transformées ou bien que la méthode de travail ne permettait pas de détecter la cause de l'échec reproductif du bar rayé.

USE OF BIOASSAYS TO DETERMINE THE EFFECTS OF A MUNICIPAL EFFLUENT AND AN INDUSTRIAL EFFLUENT ON THE REPRODUCTION OF STRIPED BASS.

W.R. Parker and K.G. Doe
EPS

SOMMARY - The striped bass in the Annapolis River, Nova Scotia have not reproduced successfully for several years. Striped bass eggs were collected from the river using drift nets and raised in water from various stations along it and water from the Shubenacadie River, where the bass are reproducing successfully. Since a chlorinated sewage effluent from Bridgetown and an effluent from an elastics plant were suspected of contributing to this decline, stations below these sites were included.

Six replicate hatching experiments were conducted and the mean percentage hatch ranged from 43% to 62% for the different treatments. Larvae alive at day three were counted and the data was analyzed by 2-way analysis of variance. There was no significant difference in percentage survival of larvae between the treatment groups ($F = 2.29$ $\alpha = 0.05$).

The larvae were pooled and raised in their respective treatments for several mounths. Survival ranged from 0% to 2.8% for the various treatment groups.

The differences in hatching and survival were negligible between the various Annapolis River stations and the Shubenacadie River. This indicates either that the substances or characteristics responsible for the reproductive failure are rapidly degraded or changed or that the test procedure was not suitable to detect a cause of the reproductive failure.

USE OF BIOASSAYS TO DETERMINE THE EFFECTS OF A MUNICIPAL EFFLUENT AND AN INDUSTRIAL EFFLUENT ON THE REPRODUCTION OF STRIPED BASS

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October 1980

INTRODUCTION

The Annapolis River flows southwesterly through the Annapolis Valley emptying into the Bay of Fundy via the Annapolis Basin. The river is about 140 kilometres in length and drains a water shed of 2,100 square kilometres (Jessop and Vithayasai, 1979). A barrage at the mouth of the river was constructed in 1962 to control flooding in the agricultural lands bordering the river. The barrage restricts the incoming tide and also prevents full flushing of the river at low tide. Saline waters do reach above Bridgetown, 29 kilometres upstream from the barrage. A distinct and permanent halocline exists at 1 to 2 metres below the surface in the stretch of river 20 kilometres to 30 kilometres from the mouth. The barrage has a fish passage-way to allow for the free movement of fish upstream and to the ocean (Martec, 1980).

The Annapolis River has historically been noted for a sport fishery for striped bass, *Morone saxitilis* (Walbaum). Since 1972, creel surveys and biological studies of the river have shown a lack of recruitment of striped bass. The angled fish were becoming older, larger and scarcer. No year class since 1972 was identified. Other anadromous fish such as shad, *Alosa sapidissima*; alewifes, *Alosa pseudoharengus*; and smelt, *Osmerus mordax*; appear to be maintaining healthy populations (Jessop and Doubleday, 1976; Jessop and Vithayasai, 1979).

The Department of Fisheries and Oceans requested assistance from Environment Canada with their continuing investigations into this apparent reproductive failure of the striped bass. They were concerned that water pollution from chlorinated municipal sewage from Bridgetown or the effluent from a textile plant downriver from Bridgetown may have been causing egg and/or larval mortality. Both effluents enter the river just below the area of the river where striped bass spawning occurs.

In May, 1979, EPS set up a mobile laboratory near Bridgetown and conducted a series of experiments in an attempt

to address the concerns expressed by the Department of Fisheries and Oceans. This report covers one aspect of our investigations, the bioassays conducted with striped bass eggs.

2 MATERIALS AND METHODS

Female striped bass release their eggs in flowing fresh water above the head of the tide. The eggs are immediately fertilized by male fish hovering in the current near the female. The pelagic eggs drift downstream with the current and usually hatch within 48 to 72 hours, depending on water temperature (Daborn, 1978).

One metre plankton nets were suspended in the river as shown in Figure 1. These drift nets were used to collect striped bass eggs moving with the current after spawning. The nets were placed in the river just below the mouth of Daniels Brook (Figure 2). This site was chosen based on reports of spawning in that area in previous years (Williams, 1978). The nets were checked each morning as spawning normally occurs at dusk. The collection bottles were emptied into polyethylene pails and the contents covered with river water. The pails were taken to the laboratory for sorting.

Striped bass eggs are about 3 mm in diameter, quite transparent with a distinctive oil globule (Mansueti, 1978). Healthy eggs were set aside for the bioassays. Dead eggs (ones which had turned opaque) were discarded.

The treatment groups for the bioassays consisted of two stations upriver from the spawning area, a station just below the outfall from the Bridgetown sewage lagoon and a station just below the outfall from the United Elactics textile plant. Three external controls were selected. The first was river water from the Shubenacadie River where striped bass continue to successfully reproduce. The other two control waters came from the Department of Fisheries and Oceans Fish Culture Station in Coldbrook, a fish hatchery with a good record of raising healthy trout and Atlantic salmon. The hatchery uses

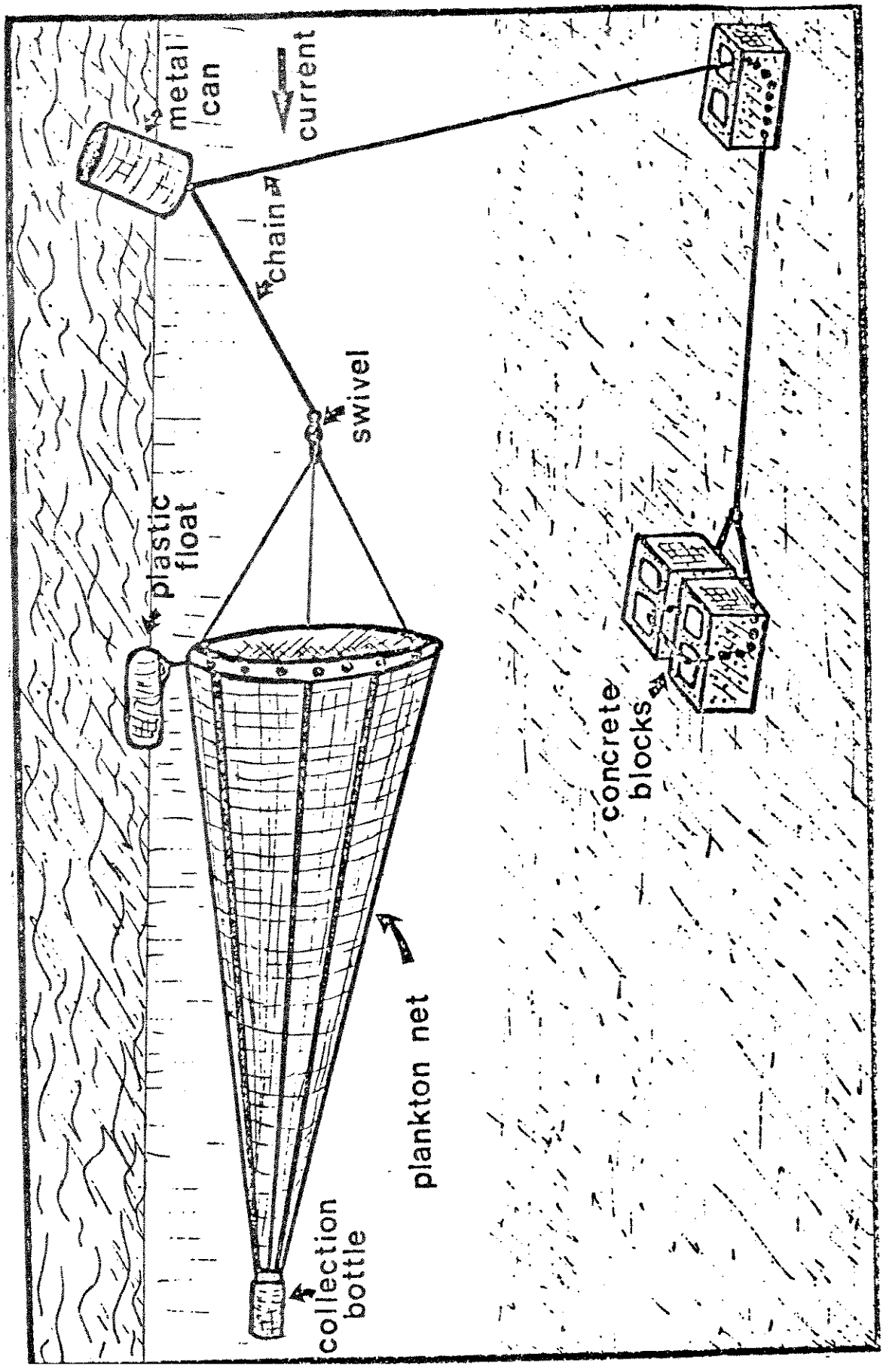


FIGURE 1 - DRIFT NET EQUIPMENT USED TO COLLECT STRIPED BASS EGGS

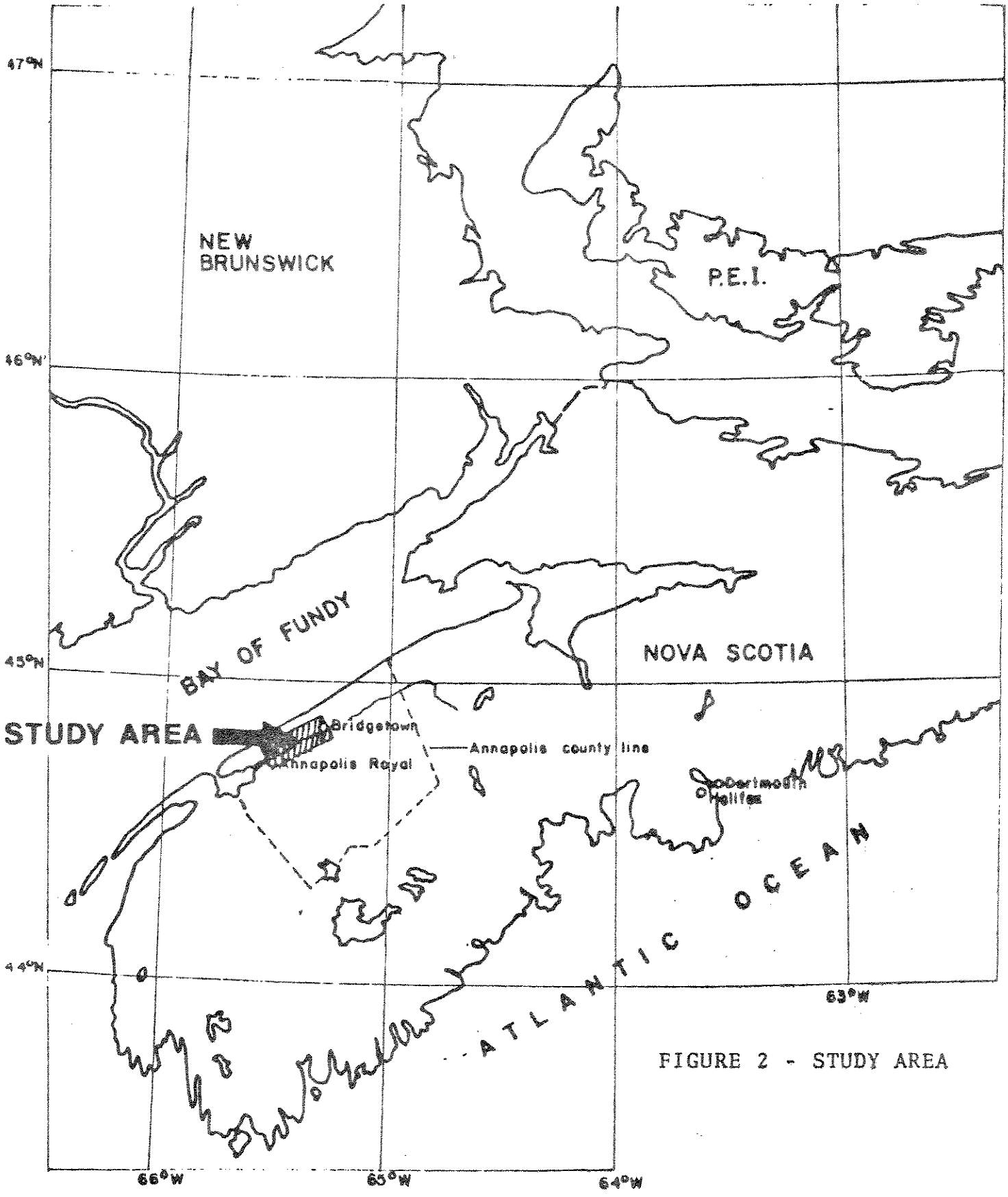
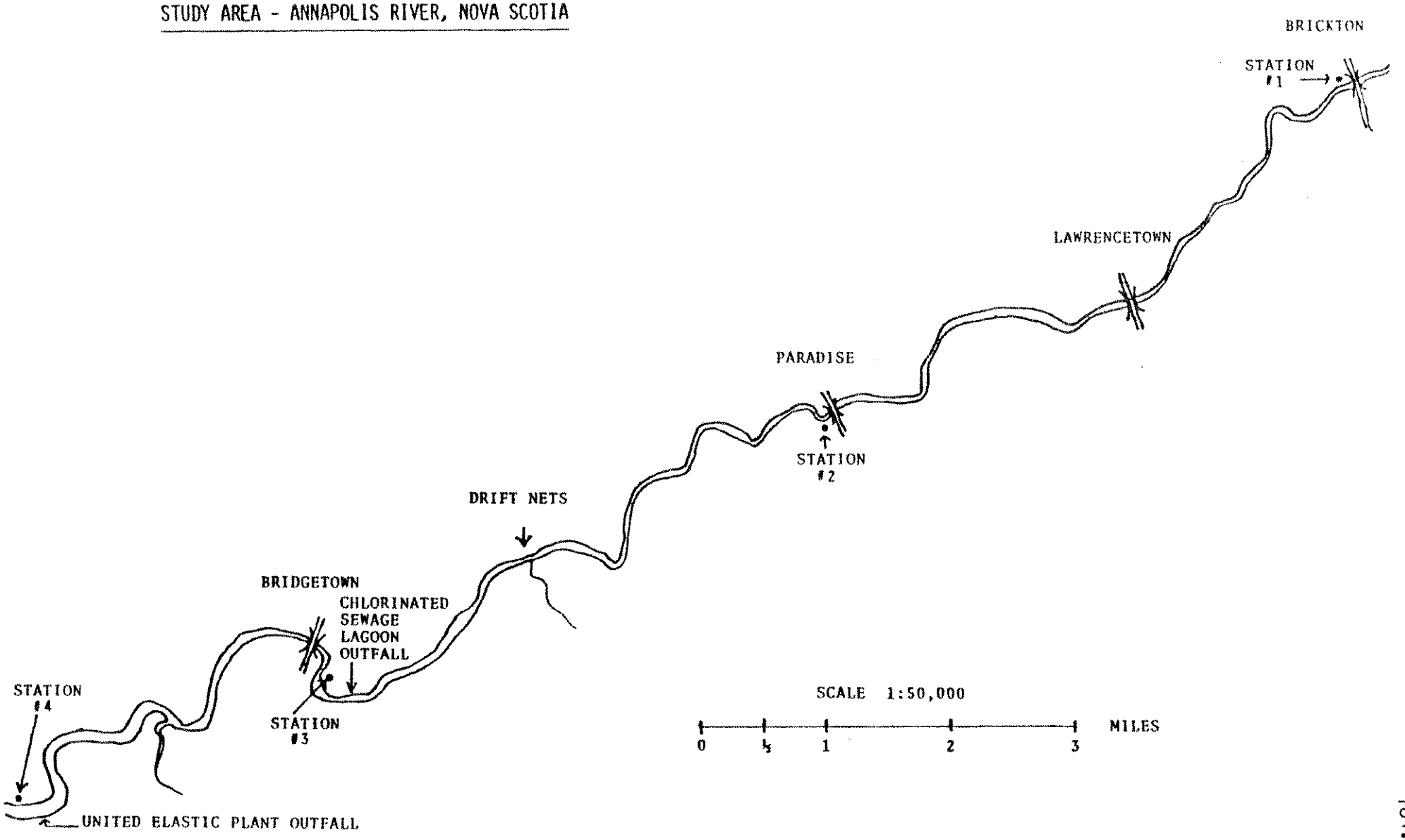


FIGURE 2 - STUDY AREA

STUDY AREA - ANNAPOLIS RIVER, NOVA SCOTIA



both surface water and well water for its operation and both waters were used in the striped bass experiments.

Egg hatching experiments were started on the same day as striped bass eggs were collected. Viable eggs were placed in glass jars of 2 or 3 liters in size at the rate of about 25 eggs per liter. The jars contained either water from one of the 4 river sites or the 3 control sites. The test solutions were lightly aerated to provide a current to keep the eggs suspended in the water column and to maintain dissolved oxygen at near saturation levels. The jars were maintained at about 18 degrees Celsius. The hatching experiments lasted for 3 days and were checked daily for hatching, egg mortality, larval mortality, temperature, dissolved oxygen and pH. These bioassays were static with no change in test solutions during the 3 days.

After 3 days, live larvae and eggs were transferred to larval holding tanks containing the same water treatments as the egg experiments. Larvae were handled carefully using glass pipettes and tanks were aerated gently. Tank volume was 20 liters. These tanks were checked daily for temperature, dissolved oxygen, pH, and egg and larval mortality. After 4 days the larvae were offered brine shrimp (*Artemia salina*) nauplii and fed daily (usually twice daily) thereafter. Tanks were cleaned as necessary or twice weekly, and one-half of the volume of solution was replaced twice weekly with fresh test solution. Dead larvae were removed daily. As each replicate of the egg experiment was completed, more eggs and larvae were added to the larval tanks; so that each batch of eggs was not followed individually after the egg experiments terminated.

3 RESULTS

Approximately 67,000 striped bass eggs were collected in the two drift nets between May 24 and June 19, 1979. Overall, at the time of sorting, 42% of the eggs were viable and 58% of the eggs had turned cloudy (i.e. the proteins had coagulated) or had disrupted membranes. A total of 5,328 viable eggs were used in the egg hatching experiments.

Egg catches were related to rising or peak temperatures in the river (Figure 3). The drift nets were placed in the river on May 23 for the first time and the first eggs were captured the next morning, May 24. It is therefore very likely that some spawning occurred before May 24. No eggs were captured after June 19 and the nets were removed from the river on June 25. Minimum temperature for spawning was 14 degrees Celsius.

Six comparable replicates for egg hatching experiments were obtained (Table 1). A different batch of eggs was used for each replicate. Egg hatching data is given in Table 2. Mean percent hatch ranged from 43% to 62% for the 7 treatment groups. Only 1 batch of eggs (Batch 4) hatched during the first 24 hours of the experiments suggesting that these eggs were older than eggs of the other 5 batches. These eggs (Batch 4) had the highest percent mortality of any egg batch at the time of sorting (Table 1).

At the end of the 3 day egg hatching experiments, the live larvae were counted and transferred to the 20 liter aquaria. This provided a reliable point in the experiment for comparisons (Table 3).

The overall results of the experiments ranged from a low of 0% survival of larvae to day 3 to a high of 96% survival. Overall mean survival for the 7 treatment groups ranged from 40% - 57%, again a fairly narrow range. The big differences are observed between egg batches where the mean survivals range from 20% - 82%.

Table 4 presents a summary of larval survival data up to August 14, the end of the experiments. Striped bass larvae raised in water from Station 1 (Brickton) did not survive past June 29, about 2 weeks post-hatch. Fish reared in Coldbrook Fish Culture Station well water had all died before August 14. All other treatments had survivals in the range of 4% to 10% by June 29 and 2% to 3% at the end of the tests on August 14. Table 5 shows the average sizes of the striped bass in the 5 remaining treatments on August 14.

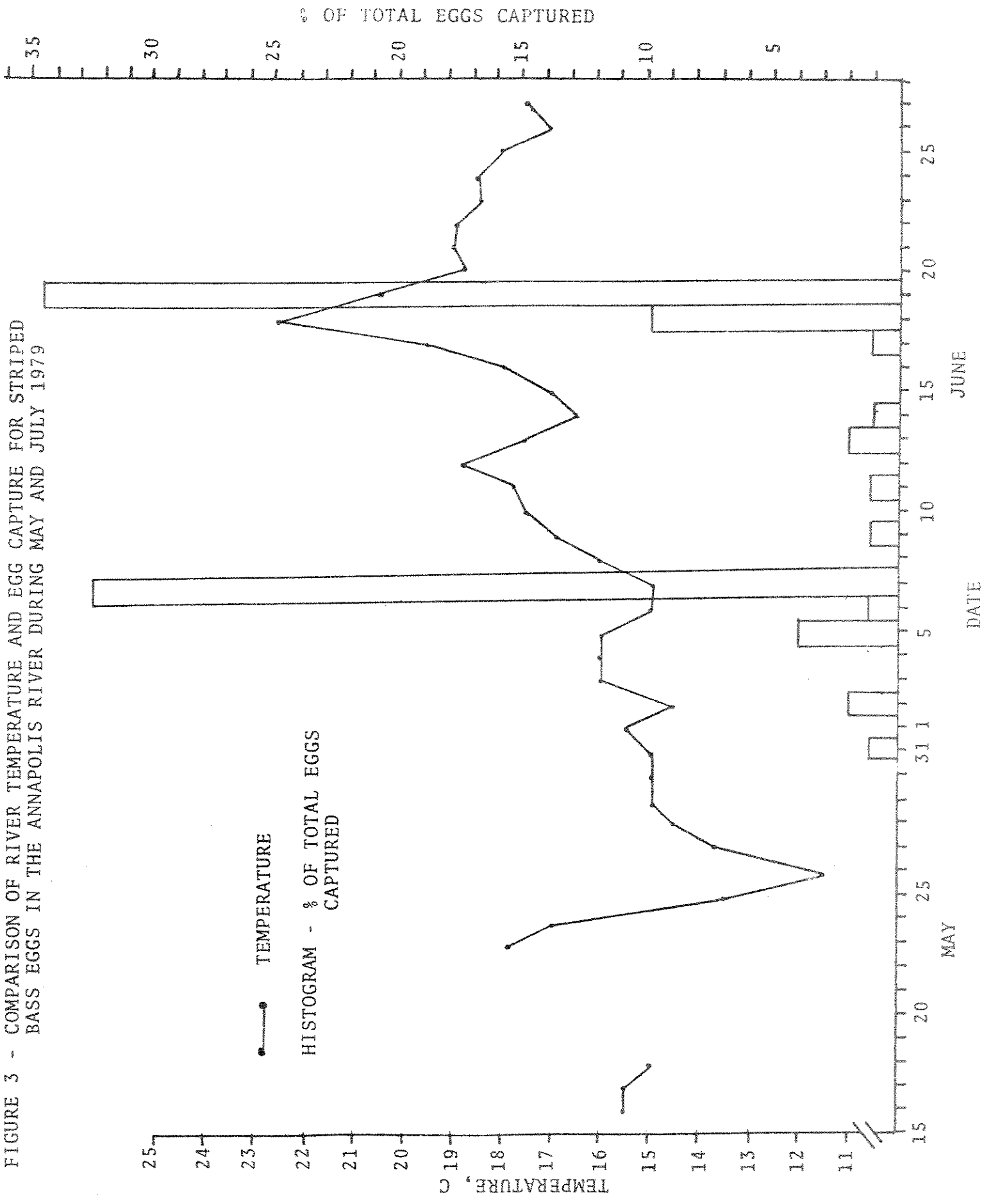


FIGURE 3 - COMPARISON OF RIVER TEMPERATURE AND EGG CAPTURE FOR STRIPED BASS EGGS IN THE ANNAPOLIS RIVER DURING MAY AND JULY 1979

TABLE 1 - BATCHES OF EGGS USED IN THE RANDOMIZED BLOCK DESIGN FOR TESTING DIFFERENCES IN THE MEAN SURVIVAL OF LARVAE AFTER THE THREE-DAY EGG HATCHING EXPERIMENTS. EACH EGG BATCH WAS USED IN ONE FULL REPLICATE OF THE EGG HATCHING EXPERIMENT

EGG BATCH	DATE COLLECTED	NUMBER COLLECTED	PERCENT ALIVE AT SORTING	NUMBER USED IN THE EXPERIMENTS
1	June 2	1305	50	400
2	June 4	1201	84	566
3	June 5	2840	87	404
4	June 8	22450	5	400
5	June 9	510	89	403
6	June 13	1539	64	560

TABLE 2 - SUMMARY OF EGG HATCH DATA EXPRESSED AS PERCENT OF EGGS ADDED. HATCH INCLUDED AN ESTIMATE OF LIVING LARVAE PLUS THOSE WHICH HAD DIED AND WERE REMOVED AND COUNTED

(i) Mean Percentage Hatch for Each Treatment (Average of the Six Egg Batches)

TREATMENT	MEAN* PERCENTAGE HATCH		
	DAY 1	DAY 2	DAY 3
Brickton	6.3 ± 15.5	33.3 ± 20.2	53.3 ± 33.9
Paradise	1.7 ± 4.1	32.5 ± 27.3	58.7 ± 27.3
Bridgetown	1.7 ± 4.1	42.8 ± 30.4	50.2 ± 38.4
Upper Granville	8.3 ± 20.4	59.0 ± 19.8	57.8 ± 24.8
Shubenacadie River	0 ± 0	48.5 ± 36.6	59.5 ± 20.0
Coldbrook F.C.S. - Pond	8.3 ± 20.4	62.0 ± 36.2	46.3 ± 36.9
Coldbrook F.C.S. - Artesian Well	17.7 ± 40.8	69.2 ± 26.4	62.2 ± 17.0

(ii) Mean Percentage Hatch for Each of the Egg Batches (Average of the Seven Treatments)

EGG BATCH	MEAN* PERCENTAGE HATCH		
	DAY 1	DAY 2	DAY 3
1	0 ± 0	23.8 ± 15.9	35.8 ± 20.5
2	0 ± 0	49.6 ± 29.6	60.4 ± 24.3
3	0 ± 0	77.3 ± 4.7	84.3 ± 14.5
4	33.5 ± 33.3	45 ± 36.9	36.5 ± 22.9
5	0 ± 0	45.8 ± 15.7	64.1 ± 18.4
6	0 ± 0	47.4 ± 40.6	42.3 ± 26.9

* Mean ± Standard Deviation

TABLE 3 - SURVIVAL DATA FOR LARVAE AT THE END OF THE THREE-DAY EGG HATCHING EXPERIMENTS. VALUES ARE EXPRESSED AS PERCENTAGES OF THE TOTAL NUMBER OF EGGS ADDED TO EACH JAR. ONLY THOSE REPLICATES IN WHICH ALL METHODS USED WERE THE SAME AND IN WHICH ALL TREATMENTS WERE USED ARE PRESENTED

TREATMENT	EGG BATCH						
	1	2	3	4	5	6	$\bar{x}$ ± S.D.*
Brickton	28	44	96	20	79	10	46.2 ± 34
Paradise	18	79	68	10	84	26	47.5 ± 33
Bridgetown	60	70	92	0	56	6	47.3 ± 37
Upper Granville	38	71	94	12	20	26	43.5 ± 32
Shubenacadie River	26	91	52	36	56	59	53.3 ± 22
Coldbrook F.C.S. Fish Pond	0	14	92	22	60	51	39.8 ± 34
Coldbrook F.C.S. Artesian Well	56	43	78	38	46	83	57.3 ± 19
$\bar{x}$ ± S.D.	32 ± 20	59 ± 24	82 ± 15	20 ± 13	57 ± 20	27 ± 26	---

* Mean ± Standard Deviation

TABLE 4 - SUMMARY OF LARVAL SURVIVAL UNTIL AUGUST 14, 1979

TREATMENT	NUMBER OF LARVAE ADDED	NUMBER OF EGGS ADDED	PERCENT SURVIVING TO JUNE 29	PERCENT SURVIVING TO AUGUST 14
Brickton	190	56	0	0
Paradise	183	47	4.3	1.7
Bridgetown	197	59	7.4	2.0
Upper Granville	143	36	7.8	2.8
Coldbrook Fish Pond	483	35	5.0	1.7
Coldbrook Artesian Well	531	4	4.7	0
Shubenacadie River	207	43	10.4	2.4

TABLE 5 - LENGTHS AND WEIGHTS OF STRIPED BASS RAISED DURING THE EXPERIMENTS. MEASUREMENTS RECORDED ON AUGUST 14, 1979, ABOUT 2 MONTHS POST HATCH

TREATMENT GROUP	LENGTH (cm) ± SD (N)	WEIGHT (g) ± SD (N)
Paradise	3.1 ± .08 (4)	0.3 ± .03 (4)
Bridgetown below STP	3.1 ± .32 (5)	0.29 ± .08(5)
Upper Granville	2.7 ± .20 (5)	0.21 ± .03(5)
Coldbrook FCS Pond Water	2.4 ± .12 (9)	0.14 ± .02(9)
Shubenacadie River	2.3 ± .15 (6)	0.11 ± .02(6)

4 DISCUSSION

The results of the drift net sampling confirm that the striped bass did spawn in the Annapolis River during the summer of 1979. So, although there has been no recruitment since 1972, sufficient adult fish are migrating upriver and spawning. Previous investigations had confirmed that spawning took place in 1976 and 1977 (Daborn, 1978). No studies were carried out in 1978.

Considering the physical stress the eggs undergo in being trapped in the net with other debris and the handling they undergo during sorting, 42% viable eggs after sorting is probably a good record overall and shows that the eggs spawned are fertile and developing.

The results of the egg hatching experiments give us 2 sets of data. The first is an estimate of the number of eggs that actually hatched. This value is achieved by accounting for dead eggs, live larvae and dead larvae in the hatching jars. Often when the eggs or larvae died, they quickly disintegrated and disappeared. In most egg hatching counts, we could not account for all of the eggs we had placed in the jar, so those missing eggs were counted as dead eggs (i.e. they did not hatch).

On the other hand, the second set of data is more accurate. It is the number of larvae which hatched from egg and survived until the end of Day 3 of the hatching experiments. These live larvae were counted and transferrred to the 20 liter aquaria for the second stage of the experiments. The results show very little difference between treatment groups but large differences between egg batches. Egg Batch 3 had the best overall percent survival while egg Batch 4 had the overall poorest survival. Egg Batch 4, as mentioned previously in the results were the poorest quality eggs collected and were the only batch to hatch on Day 1 of the tests.

This would seem to indicate that being in the river for a longer period of time was detrimental to the eggs. Once the

eggs were removed from the river and placed in aerating jars of river water, the hatching rate appears to be quite good.

The overall survival of the larvae was very poor. Percentage survival in the 5 remaining treatments ranged from 1.7% to 2.8%.

While these levels of survival are extremely low, the object of the experiment was to determine toxic effects of the water from the various stations on the striped bass eggs and larvae. More optimal fish culture techniques could have been used to raise the fish, but that would not have suited the aims of the experiments.

Eggs from these same batches were supplied to Dr. M. Wiles, a researcher at Saint Mary's University in Halifax, for studies on striped bass development and feeding habits. Verbal communication with him indicated that those eggs hatched with good success and that the larvae developed normally. At the end of August he had about 20% survival. The techniques used were similar to those in our experiments except that the eggs are hatched and the larvae are raised in water with a salinity of 6 to 8 ppt.

As shown in Table 5, there were some size differences in the fish at the end of the experiment. Fish raised in the 3 Annapolis River waters were larger than those raised in either Coldbrook Pond water or Shubenacadie River water.

5 CONCLUSIONS

1. Striped bass did spawn in the Annapolis River in 1979.
2. The striped bass eggs were fertile and viable and overall about 50% of the eggs in the experiments hatched.
3. Striped bass eggs did hatch and the larvae did survive and grow in water from the Annapolis River below the Bridgetown sewage lagoon outfall

and from below the outfall of the effluent from a textile plant. Hatching, survival and growth were similar for eggs and larvae raised in control waters. Therefore, within the limits of our experimental design, there does not appear to be a detrimental effect on the hatching of striped bass eggs or in the development of the larvae attributable to these 2 point sources.

4. There is some evidence to show that the river water does have a detrimental effect on the eggs. No explanations are forthcoming at present but future experimentation will be carried out during the summer of 1980 to investigate this problem further.

6 ACKNOWLEDGEMENTS

The authors would like to thank B. Jessop, Dr. M. Wiles and G. Trider for advice on the experimental design and methods. The able assistance of summer students G. Archambeault and J. Peck in collecting the data is much appreciated. Also we thank Mr. C. Inglis for allowing us to set up our mobile laboratory on his property in Tupperville and Mr. R. Corkum for the use of his boat dock.

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LES EFFETS DU Di-(2-ÉTHYLHEXYL)-PHTHALATE (DEHP) SUR LE MÉTABOLISME DES STÉROÏDES DE LA MORUE DE L'ATLANTIQUE GADUS MORHUA

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RÉSUMÉ - Les morues de l'Atlantique ont été mises à un régime de hareng dans lequel on avait introduit du DEHP encapsulé dans la gélatine à 0, 10, 100, 1000 µg/g de nourriture pendant 121 jours. Les gonades et les pronéphroses de poissons ayant atteint les premiers stages de développement des gonades ont été incubées in vitro dans des quantités équimolaires de (^{3}H)-prégnénolone et de (^{14}C) progesterone. Des autoradiographiques de chromatogrammes en couche mince, d'extraits stéroïdiens et des dosages des proportions isotopiques (^{3}H/^{14}C) des métabolites stéréoïdiens des testes et des pronéphroses de mâles mis à un régime de 0 (le groupe témoin) et de 1000 µg de DEHP/g n'ont montré aucune différence significative des profils du métabolisme des stéroïdes. Cependant, chez les femelles, il y a eu une altération significative des circuits de la biosynthèse des stéroïdes dans les pronéphroses et dans les ovaires des sujets ayant absorbé du DEHP. Bien que nous n'ayons pas observé de différence significative quant aux rapports isotopiques (^{3}H/^{14}C) du cortisol, les mêmes rapports pour le 11-désoxycortisol obtenus des sujets ayant ingéré 100 et 1000 µg de DEHP/g (17.39/L et 15.35/L, respectivement) étaient au moins deux fois plus importants que les rapports obtenus chez les groupes témoins et les groupes ayant absorbé 10 µg de DEHP/g (7.41/L et 6.14/L, respectivement).

THE EFFECTS OF Di-(2-ETHYLHEXYL)-PHTHALATE (DEHP) ON STEROID METABOLISM IN THE ATLANTIC COD GADUS MORHUA

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SUMMARY - Atlantic cod were maintained on a herring diet containing gelatin encapsulated DEHP at 0, 10, 100 and 1000 µg/g food for 121 days. Gonads and head kidneys of fish in the early stages of gonadal development were incubated in vitro with equimolar amounts of (^{3}H)-pregnenolone & (^{14}C)-progesterone. X-ray autoradiograms of the thin layer chromatograms of steroid extracts and analyses of isotope ratios (^{3}H/^{14}C) of steroid metabolites in the testes and head kidneys of male fish maintained on the 0 (control) and 1000 µg DEHP/g diets indicated no significant differences in steroid metabolic profiles. However, in female fish, there was a significant alteration of steroid biosynthetic pathways in the head kidneys and ovaries of the DEHP-fed fish. Although, there were no significant differences in the isotope ratios of cortisol in all groups, the ratios of 11-deoxycortisol from the 100 and 1000 µg DEHP/g groups (17.39/1 and 15.35/1, respectively) were greater than twice the ratios obtained from the control and 10 µg DEHP/g (7.41/1 and 6.14/1, respectively).

The Effects of Di-(2-ethylhexyl)-Phthalate (DEHP) on Steroid Metabolism in the Atlantic Cod Gadus morhua.

by

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INTRODUCTION

Due to their ubiquity as environmental contaminants and their wide industrial use, phthalic acid esters (PAEs) have been the object of extensive toxicological studies (Fishbein and Albro 1972; Peakall 1975). In the past, PAEs including DEHP which comprise approximately half of the world's production of PAEs have not been considered very toxic. Toxicological studies, mainly at acute levels in some mammalian species, have indicated that these compounds can be tolerated at very high levels. Indeed, one investigator remarked that the toxicological investigation of phthalates is like an "etiology looking for a disease". Recently, however, the subtle effects of PAEs on biochemical and physiological changes in various species (Thomas et al. 1978) indicate that these compounds are more toxic at chronic levels than was indicated by acute LD50 tests. The appearance of significant levels of DEHP in the aquatic environment including fish (Pfuderer et al. 1975; Zitko 1972; Williams 1973; Mayer et al. 1972; Stalling et al. 1973) and the high accumulation factors in some aquatic organisms (e.g., an accumulation factor of 3600 in the scud, Gammarus pseudolimnaeus, after a 14-day exposure to 0.1 µg DEHP/g) (Mayer and Sanders 1973) prompted our laboratory to investigate the effects of DEHP on fish.

At the workshop in Halifax in 1975, we described procedures by which alterations in steroid hormone metabolism in vivo and in vitro by the gonads and/or head kidneys of fish may be determined. We illustrated the sensitivity of the method in detecting the effects of sublethal contaminants (Freeman and Sangalang, 1976). In a preliminary study on the effects of DEHP on steroid metabolism in the Atlantic cod, we incubated testes and head kidneys with equimolar amounts of [^{3}H]-pregnenolone and [^{14}C]-progesterone with various amounts of DEHP in vitro (Freeman and Sangalang 1979).

The tissues were also incubated with [^{14}C]-DEHP under the same conditions. It was evident from the x-ray autoradiogram of the thin layer chromatogram (TLC) of extracts of the steroid metabolites in the head kidneys (Fig. 1) that there was no significant difference at the various treatment levels of 0, 1, 10, 100 and 1000 µg DEHP/g tissue. Likewise, the radioactive profiles of steroid metabolites in the testicular incubates indicated no effect of DEHP (Fig. 2). [^{14}C]-DEHP was not metabolized by either tissue, in vitro, as indicated by TLC chromatography of the tissue extracts in a solvent system known to separate some of the possible metabolites of DEHP (for example, mono-2-ethylhexyl phthalate and phthalic acid) from each other (Fig. 3). Isotope ratios of the steroid metabolites, cortisol (F), cortisone (E) from the head kidneys, and testosterone (T), 11β-hydroxytestosterone (11β-OH-T) and 11-ketotestosterone (11KT) from the testes, (Table 1) showed

little or no difference between the DEHP-treated and control tissues. Thus, the results suggested no effect of DEHP on the enzymes of steroid synthesis under the *in vitro* experimental conditions employed. Subsequently, we investigated the possibility that DEHP might exert adverse effects on steroid metabolism in cod *in vivo* through its metabolites. There is evidence that fish metabolize DEHP into several products (Stalling et al. 1973; Melancon and Lech 1976). At this time we would like to report the results of a study where we maintained cod with a herring diet containing various levels of DEHP for several months.

MATERIALS AND METHODS

Cod were caught by hand line off Terrence Bay, Halifax County, Nova Scotia and transported to our laboratory. Twenty-one to 24 fish, 800 to 2500 g and 45 to 65 cm long, were assigned to each of four experimental groups. The fish of each group were held two to three fish per tank: Each tank contained approximately 240 L of continuously flowing filtered sea water. The fish were held in these tanks for at least two weeks before the start of the feeding experiments.

For food, the fish were fed 10 g pieces of herring muscle containing gelatin capsules that held 0, 100, 1000 and 10,000 µg DEHP in 50 µL soybean oil and -cellulose as filler to make the appropriate diet levels. The experiment was started in early January of this year. The fish were fed approximately 3-4% of their body weight twice weekly. The general feeding performance per group was observed by recording the number of food pieces given at each feeding time and the number that were not consumed and therefore removed before the next feeding. Actual food intake of individual fish was to be approximated from DEHP levels in the tissues at the end of the experiment.

The feeding experiment was terminated in May after 121 days. The fish were killed with a sharp blow on the head. The fish were weighed and a gross examination of external appearance and internal organs were done. Gonads, head kidneys and livers were removed. The gonads were weighed. Samples of the various tissues were fixed in buffered 10% formalin for histological examination. The tissues were quickly frozen in dry ice and then stored at -30°C. Tissue steroidogenesis studies were done on the following day. For these studies, we were able to form comparable groups of at least four fish each in surviving

male fish from the 0 and 1000 µg DEHP/g diets and in female fish from all the diet groups. The fish were in the early stages of gonadal development and grouped according to similarity in gonadosomatic indices. Testes, ovaries and corresponding head kidneys were freed of extraneous tissues, thawed, pooled according to diet group, minced, and then thoroughly mixed to ensure homogeneity. One g tissue mixture per 10 mL incubation medium containing a NADPH-generating system as described by Sangalang et al. (1972) was incubated with equimolar amounts (0.024 µmole) of [^{3}H]-pregnenolone (5.22 µCi) + [^{14}C]-progesterone (1.24 Ci) at 4-5°C for 19 hours under an atmosphere of 95% O_2 + 5% CO_2. Incubations were stopped by freezing at -30°C.

After addition of approx. 30 µg of the appropriate authentic non-radioactive steroid carriers the incubates were extracted with dichloromethane (Sangalang et al. 1972). X-ray autoradiography of thin layer chromatograms of one-tenth of the extracts were prepared by Freeman and Sangalang (1976). Steroids from the remaining fractions were isolated and identified by sequential thin layer and paper chromatography to constant isotope (^{3}H/^{14}C) ratios and formation of acetate derivatives followed by recrystallizations to constant isotope ratios according to Axelrodet al. (1965). Radioactivity was measured by liquid scintillation spectrometry using a Packard TRI-CARB Model 3255.

For histological examinations, the formalin-fixed tissues were dehydrated, embedded in paraffin and then sectioned at 6 µm. The tissue sections were stained with Harris haematoxylin, counter stained with eosin and examined under a light microscope.

DEHP levels in the tissues were determined by electron-capture gas chromatography (GC) following clean-up of the liquid extracts by gel-permeation chromatography on Biobeads SX-3 and column chromatography on small alumina:sulfuric acid-impregnated alumina (layered) (Burns et al., (in press).

RESULTS AND DISCUSSION

The DEHP levels were determined in the livers, head kidneys and gonadal tissue from control and DEHP treated (1000 µg/g) male cod. DEHP levels of 2.43 µg/g (wet wt) in head kidneys and 0.908 µg/g in gonadal tissues of the DEHP treated fish did not differ significantly from the control. However, DEHP concentrations of 21.3 µg/g and 1.26 µg/g in the livers of DEHP treated and control fish, respectively, indicate a significant uptake of DEHP in the livers of the treated fish.

The autoradiogram of the TLC of one-tenth of the testicular and head kidney incubates (Fig. 4) did not reveal any significant differences in steroid metabolic profiles

between the control and the fish fed with 1000 µg DEHP/g. T, 11-KT, DHA and a number of other steroids were not detectable. In the more polar fractions, only 11β-OHT was present. There were no differences between the isotope ratios of 11β-OH-T in the control and 1000 µg DEHP/g group (Table 2).

The absence of T and 11-KT from the testicular extracts may be a reflection of the stage of sexual maturation of the fish. On histological examination, the testes were sexually immature and in the early stages of spermatogenetic development when only spermatogonia and spermatocytes and occasionally, spermatids, could be seen. Previous work in our laboratory have shown a direct correlation between the levels of T and 11-KT in the plasma of salmonids and cod with approaching sexual maturation (Idler et al. 1972; Sangalang and Freeman 1974; Freeman and Sangalang 1977). Analysis of T and 11-KT in the plasma of cod by radioimmunoassay has shown very low levels of these steroids in the plasma of sexually immature fish (Sangalang and Freeman 1979).

It is evident from the autoradiogram that steroid metabolites other than T, 11-KT and 11β-OH-T are the principal transformation products <u>in vitro</u> in the testes of sexually immature cod under our experimental conditions. It would be worthwhile to investigate the identities of these

metabolites as their presence may be correlated to the sexual development process in cod.

Oishi and Hiraga (1975a,b) reported significant effects of short-term DEHP administered via i.p. or in the diet on steroid metabolism (i.e. testosterone and/or urinary 17-keto steroids) in rats. These investigators used higher treatment levels of DEHP when compared to what we used in cod. DEHP (1 or 2%) administered in the diet of rats for 10 days caused changes in liver, kidney and testes weights and increased testicular concentrations of T. Daily i.p. injections at 1.25 g/kg into male rats for 5 days did not affect organ weights but decreased the plasma levels of T. The DEHP contents of the organs were not reported.

Seth et al. (1976) examined the effects of DEHP on rat gonads. They found increased levels of succinic dehydrogenase activity and focal degeneration of seminiferous tubules and edema of interstitium in the testes of rats treated with 5 ml/kg of DEHP intraperitoneally. We have not observed any testicular abnormalities in our control and DEHP-treated cod.

In the head kidney incubates of the male cod, F, B, S and DOC were detected in significant quantities with F, B and S as principal transformation products. There were no significant differences in the isotope ratios of corresponding steroids extracted from the head kidneys of the control and DEHP-fed groups, indicating no alterations in the biosynthetic routes (Table 2). The above observations were similar

to those obtained from incubations of testes and head kidneys of sexually mature cod with [^{3}H]-pregnenolone + [^{14}C]-progesterone and 0, 1, 10, 100 and 1000 µg DEHP added per g tissue. There were no abnormalities in the head kidneys of the fish under the light microscope. Thus, it would seem that, as far as steroid metabolism is concerned, there is little or no effect of DEHP in vivo or in vitro in the male cod at the various treatment levels under our experimental conditions.

Although our work on the effects of DEHP on the female cod is not yet complete, we would like to report the results obtained at this time, as they appear relevant to the conclusions we can draw regarding DEHP. The autoradiogram of one-tenth of the head kidney incubates from the fish that consumed foods at the various DEHP levels revealed some differences in steroid metabolic profiles in contrast to those observed in the male fish (Fig. 5). For example, the relative proportions of radioactivity associated with the areas isopolar with B, S and 11-KT and also those almost isopolar with DOC and 17α-OH-P in the tissue incubates from the DEHP group differed from those of the control. The differences from the control did not appear to follow a trend, although one can observe, by comparison with the control, the stimulation and/or inhibition of the production of certain metabolites in the DEHP group. There were no significant differences in the isotope ratios of F, but those of S, one of the principal transformation products, indicated

significant changes (Table 3). In the 100 and 1000 µg DEHP/g group, the isotope ratios of S were much higher than the initial $^{3}H/^{14}C$ precursor ratio and indicated that the synthesis of this F intermediate occurred predominantly from [^{3}H]-pregnenolone or via Δ^{5}-steroid intermediates. The lower ratios in the control and 10 µg DEHP/g group favoured a biosynthetic route from [^{14}C]-progesterone or via Δ^{4} intermediates. The significance of the change in the biosynthetic route is not known.

In the neutral steroid fractions of the ovarian incubates the radioactive profiles of metabolic products in the DEHP treated groups differed from that of the control (Fig. 6). Analysis of isotope ratios of some of the steroid metabolites are still in progress. However, the x-ray autoradiogram shows changes in the relative intensities of radioactive metabolites (areas 1, 2, 3, and 4) in the DEHP incubates compared with the control and suggests alterations in biosynthetic routes, at least in as far as the [^{14}C]-labelled metabolites are concerned. It is possible that these alterations in steroid metabolism could exert adverse effects on the reproductive (i.e. gonadal development) process in the female cod.

The ovaries used in the steroidogenesis studies were all in the early stages of maturation and at the early stages of oocyte vitellogenesis. The results, so far, indicate a greater sensitivity of female cod than male cod to DEHP in

the diet. However, until completion of the analysis of DEHP in the tissues of the female fish to relate the level of contaminant in the tissues to that in the food, conclusive proof of the effect of DEHP in the diet of female cod can only be deferred.

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FIGURE CAPTIONS

Fig. 1. Autoradiogram of the thin layer chromatogram of one-tenth of steroid extracts from head kidneys of sexually mature male cod incubated with [^{3}H]-pregnenolone + [^{14}C]-progesterone and 0, 1, 10, 100 and 1000 µg DEHP/g tissue *in vitro*.

Fig. 2. Autoradiogram of the thin layer chromatogram of one-tenth of steroid extracts from testes of sexually mature cod incubated with [^{3}H]-pregnenolone + [^{14}C]-progesterone and 0, 1, 10, 100 and 1000 µg DEHP/g tissue *in vitro*.

Fig. 3. Autoradiogram of the thin layer chromatogram of extracts of active and heat-inactivated head kidneys and testes of sexually mature cod incubated with [^{14}]-DEHP for 19 hr *in vitro*.

Fig. 4. Autoradiogram of the thin layer chromatogram of steroid extracts of the testes and head kidneys of sexually immature male cod incubated with [^{3}H]-pregnenolone and [^{14}]-progesterone *in vitro*. The fish were maintained on 0 (control) and 1000 µg DEHP/g herring diet for 121 days. Arrowheads indicate the positions of non-radioactive steroid carriers and the dark spots indicate those of radioactive steroid metabolites.

Steroid symbols and common names: $\Delta 4$-A=androstenedione DOC=11-deoxycorticosterone; 17-OH-Pe=17α-hydroxy pregnenolone; T=testosterone; DHA=dehydroepiandrosterone; 11β-OH-T=11β-hydroxy testosterone; 11KT =11-ketotestosterone; 11β-OH-A= 11β-hydroxyandrostenedione; 17-OH-P=17α-hydroxy-progesterone; B=corticosterone; S=11-deoxycortisol E=cortisone; F=cortisol.

Fig. 5. Autoradiogram of the thin-layer chromatogram of steroid extracts of head kidneys of sexually immature female cod incubated with [^{3}H]-pregnenolone and [^{14}C]-progesterone <u>in vitro</u>. The fish were incubated on 0 (control), 10, 100 and 1000 µg DEHP/g herring diet for 121 days. Arrow-heads indicate the position of non-radioactive steroid carriers and the dark spots indicate those of radioactive steroid metabolites.

Fig. 6. Autoradiogram of the thin layer chromatogram of the neutral fraction of steroid extracts of sexually immature and ovaries incubated with [^{3}H]-pregnenolone and [^{14}C]-progesterone <u>in vitro</u>. The fish were maintained on 0 (control), 10, 100 and 1000 µg DEHP/g herring diet for 121 days. Arrow-heads indicate the positions of non-radioactive steroid carriers and the desk spots indicate those of radioactive steroid metabolites.

Fig. 1

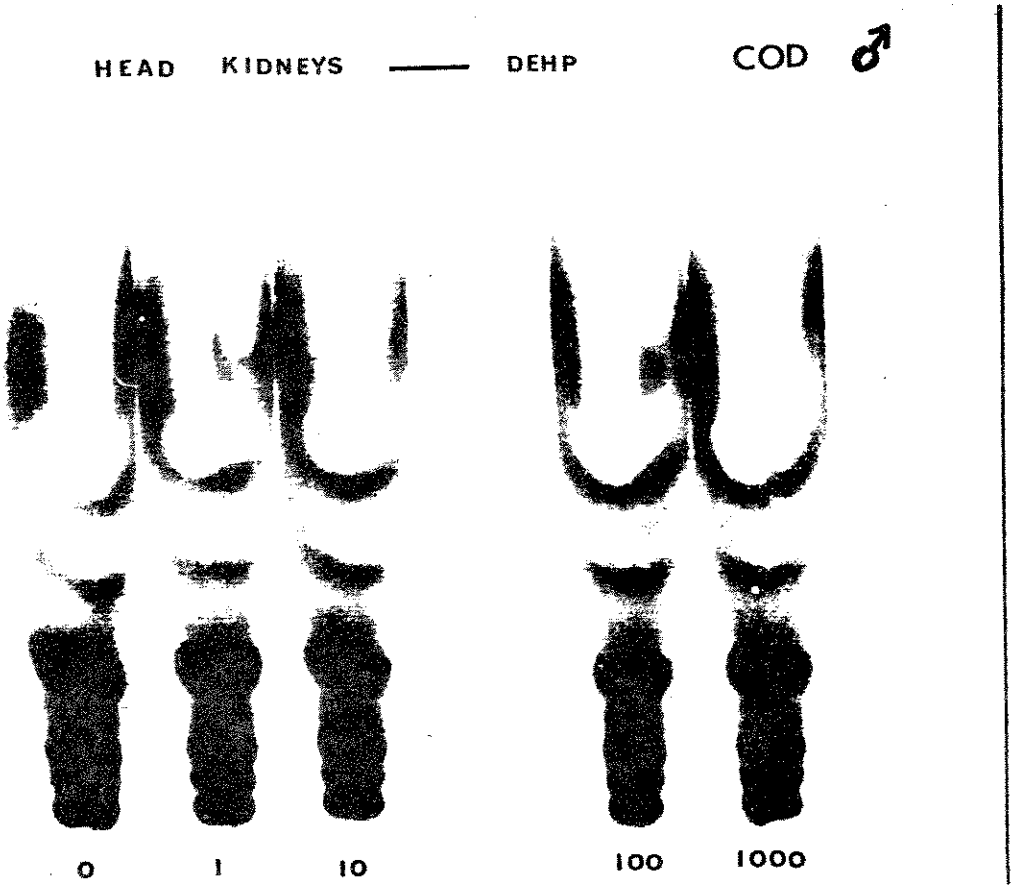


Fig. 2

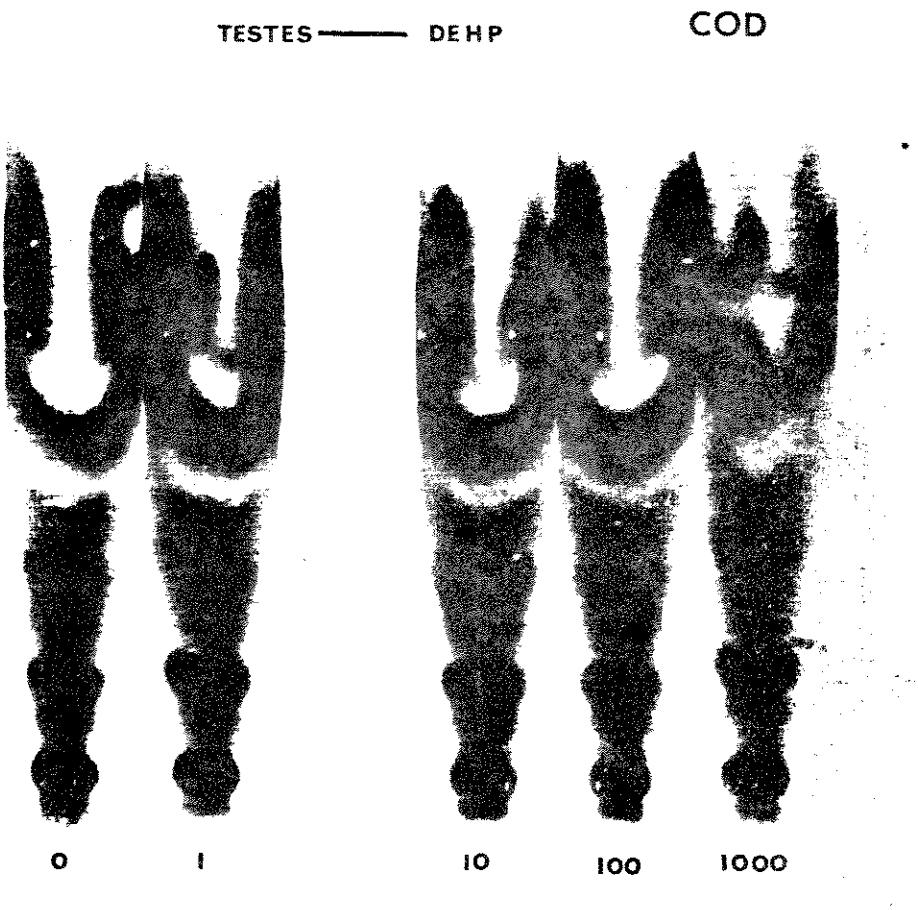
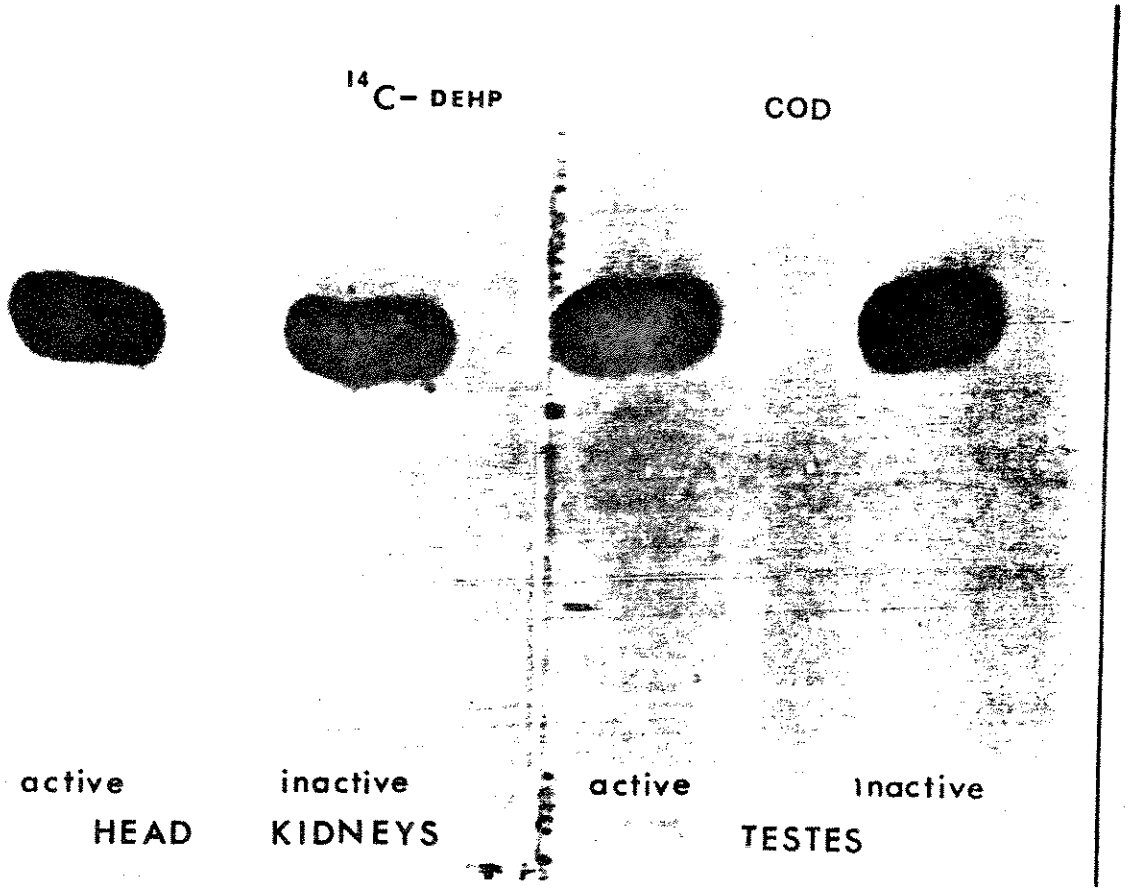


Fig. 3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HEAD
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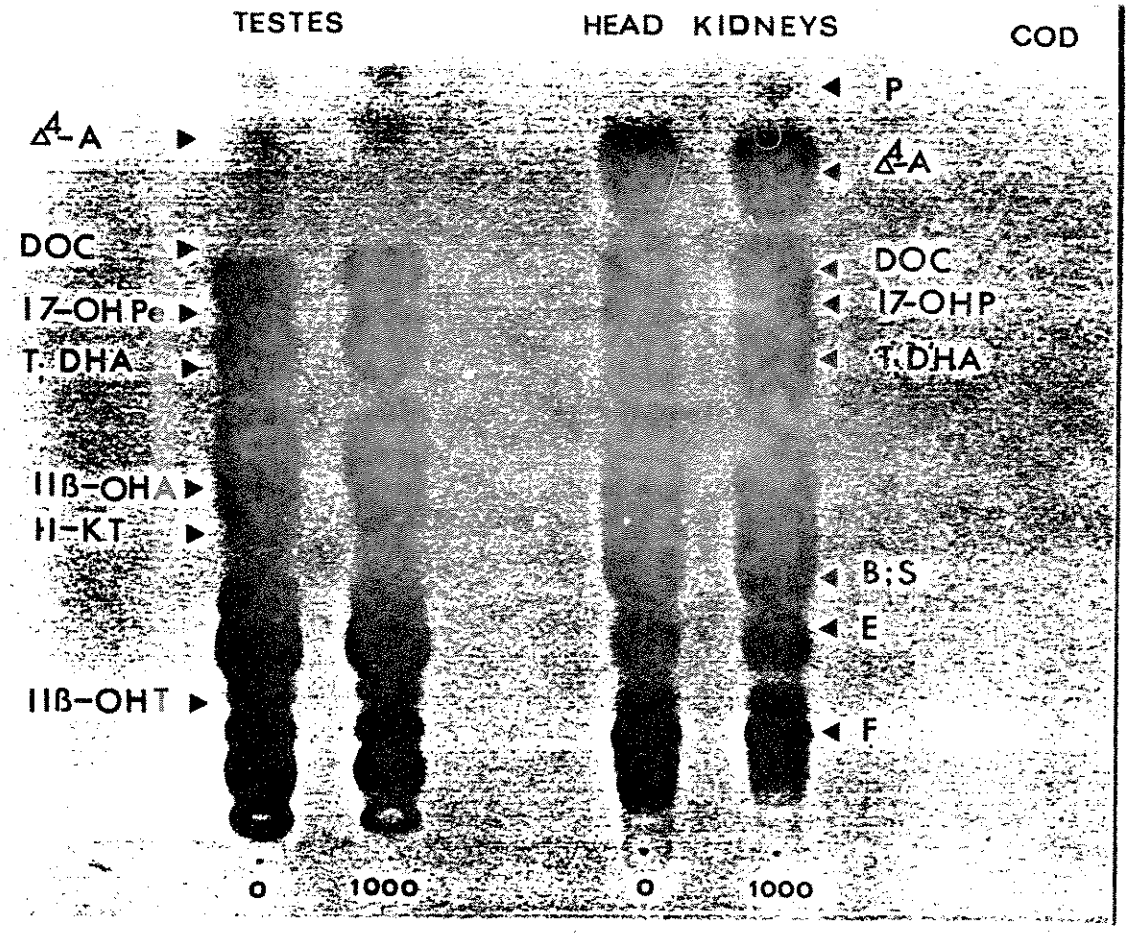
TESTES

Fig. 4


TESTES
HEAD KIDNEYS
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11-KT
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Fig. 5

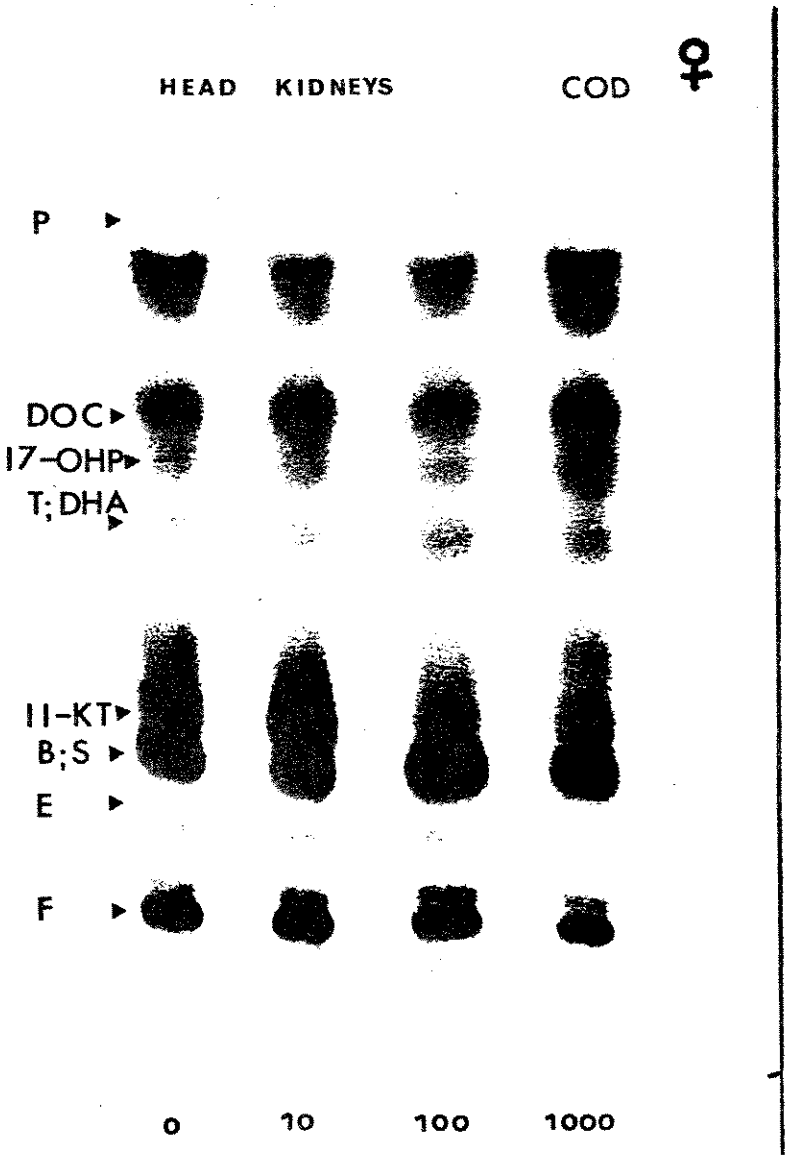

Fig. 6

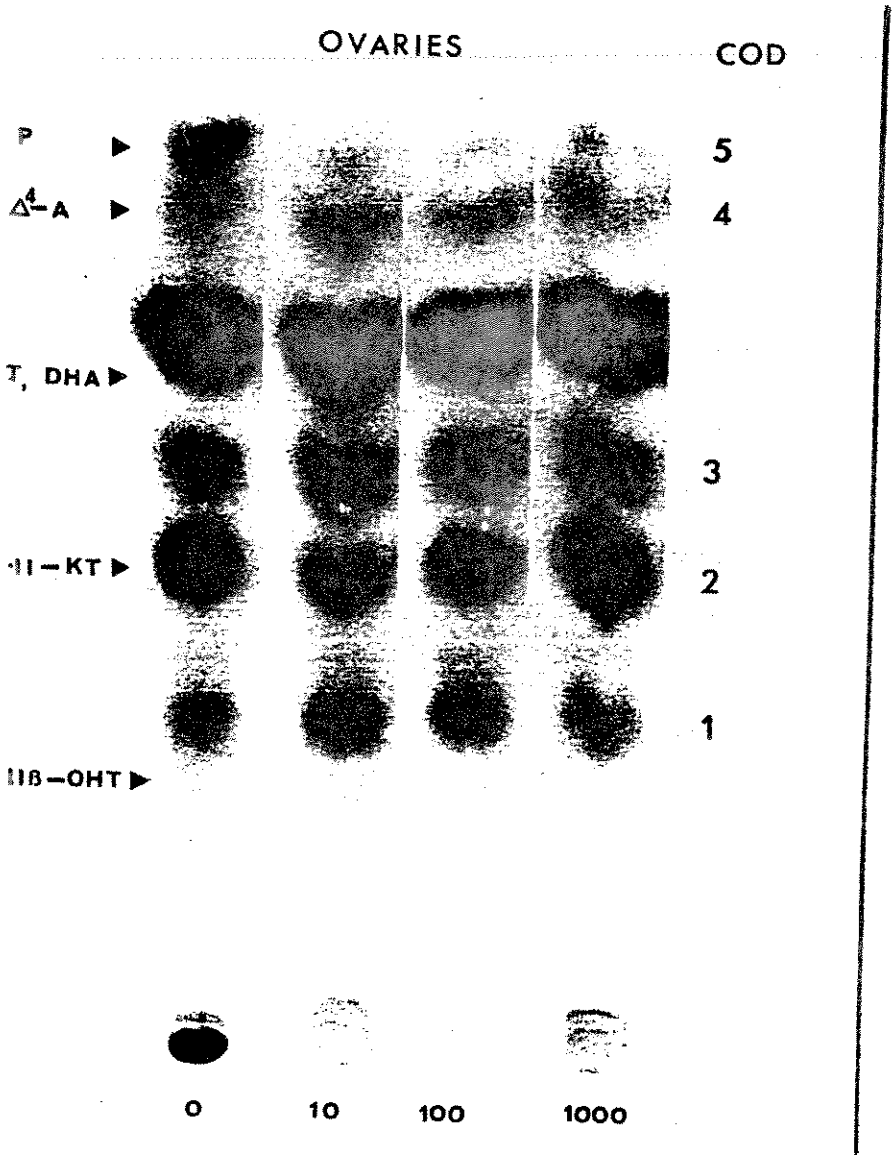

TABLE 1. The ($^{3}H/^{14}C$) ratios of steroids biosynthesized (in vitro) from [^{3}H]-Pregnenolone and [^{14}C]-Progesterone by head kidneys and testes of male Atlantic cod (Gadus morhua).

DEHP µg/g Tissue	($^{3}H/^{14}C$) RATIOS				
	Head Kidney Steroids			Testicular Steroids	
	F	E	T	11β-OHT	11KT
0	7.60	7.47	3.53	4.27	2.78
1	7.93	6.66	2.86	4.31	2.52
10	7.14	6.81	3.17	4.59	-
100	7.53	6.97	3.05	4.40	2.24
1000	7.53	6.88	3.39	4.44	3.14
$\bar{x}$ (± s.d.)	7.55 (0.28)	6.96 (0.31)	3.20 (0.27)	4.44 (0.13)	3.14 (0.38)

TABLE 2. Isotope ($^{3}H/^{14}C$) ratios of steroids biosynthesized from [^{3}H]-Pregnenolone and [^{14}C]-Progesterone *in vitro*, by testes and head kidneys of male cod maintained on a herring diet containing encapsulated DEHP at 0 (control) and 1000 µgDEHP/g for 121 days

Steroid	Testes		Head Kidneys	
	Control	DEHP	Control	DEHP
T	N.D.[a]	N.D.	-	-
11KT	N.D.	N.D.	-	-
11β-OH-T	22.3	24.0	-	-
F	-	-	4.14	4.14
E	-	-	N.D.	N.D.
B	-	-	0.34	0.34
S	-	-	9.68	10.86
DOC	-	-	0.38	0.38

a, Not detectable

TABLE 3. Isotope ratios ($^{3}H/^{14}C$) of steroids biosynthesized from [^{3}H]-Pregnenolone + [^{14}C]-Progesterone. *in vitro*, by head kidneys of female cod maintained on an herring diet containing DEHP for 121 days.

Steroid	µg DEHP/g			
	0	10	100	1000
F	3.29	3.80	5.34	3.96
E	N.D.[a]	N.D.	N.D.	N.D.
S	7.41	6.14	17.39	15.35
DOC	0.10	0.13	0.13	0.12

a, not detectable

EFFETS DE LA CAPTIVITÉ LORS D'EXPÉRIENCES SUR LA TOXICITÉ

D.G. Walton*, W.R. Penrose**, J.W. Kicenwik*, G.M. Green* and L.L. Dowe*

*Research and Resource Service and **Argone National Laboratory

RÉSUMÉ - Deux groupes de plies canadiennes (Tautogolabrus adspersus) ont été gardés pendant neuf semaines dans des bassins d'eau de mer à circulation d'eau. Un groupe a été exposé pendant toute cette période à 0.3-0.8 ppm de pétrole brut afin de voir les effets de ce traitement sur le cycle reproductif. La teneur en arylhydroxylase du foie, le poids du foie, le poids des gonades, la maturité et l'état de ces dernières ont été pris en note. Avant et après l'expérience, la population source a été étudiée sur le terrain. Quand les deux populations captives seulement sont comparées, les résultats sont consistants: l'intoxication au pétrole causait une régression prématurée des gonades chez les femelles. Cependant, les effets de la captivité étaient beaucoup plus graves et complexes: inhibition de la maturation et la détérioration de la condition des sujets ont été observés chez les deux sexes des groupes captifs. L'exposition à de faibles concentrations de pétrole a provoqué une anorexie, la léthargie, et éventuellement la mort des sujets; apparemment, les sujets seraient morts de faim, mais c'est une conclusion qu'on ne peut pas appliquer aux sujets sur le terrain à cause des effets de la captivité.

EFFECTS OF CAPTIVITY IN A TOXICITY EXPERIMENT

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SUMMARY - Two groups of Atlantic cunners (Tautogōlabrus ādspersus) were kept for nine weeks in flowing seawater tanks. One group was treated throughout the period with 0.3-0.8 ppm crude oil, in an attempt to observe effects on the reproductive cycle. Liver aryl hydroxylase, liver weight, gonad weight, maturity and condition were measured. Before and during the experiment, the source population in the field was monitored. If the two captive populations only are compared, the results are internally consistent: oil intoxication caused premature regression of female gonads. The effects of captivity, however, were much more severe and complex: inhibition of maturation and deteriorated condition were observed in both sexes of the captive groups. Low-level oil exposure caused anorexia, lethargy, and eventual death, apparently through starvation, but this result cannot be extrapolated to the field because of the confounding effects of captivity.

THEME IV

La Toxicologie, outil de gestion environnementale?

Conférencier invité.

Toxicology, a tool for Environmental management?

Invited speaker.

Dr. GARY SPRULES
University of Toronto

RÉSUMÉ - En contraste avec les études synoptiques antérieures et sur la base d'une foule d'indices trouvés partout dans la littérature, l'hypothèse suivante est avancée, à l'effet que la taille et le mode de nutrition sont plus déterminants que l'espèce en ce qui concerne le rôle d'un organisme donné à l'intérieur d'un écosystème lacustre. Le benthos, le plancton et les poissons étant ainsi caractérisés, la distribution de la biomasse à l'intérieur des écosystèmes lacustres de structures trophiques différentes est étudiée dans le temps et dans l'espace, de même que sont surveillés les effets sur la structure des communautés de divers facteurs perturbateurs, dont l'apport variable des matières nutritives, les précipitations acides et le stress dû aux pêches. Un modèle informatique d'équilibre d'oxydo-réduction permet de traduire des concentrations analytiques normalisées de certains composants chimiques d'un système donné en termes d'abondance des différentes espèces de chaque élément. Comme pour les écosystèmes marins, les résultats montrent que la biomasse des lacs serait normalement répartie à peu près uniformément suivant des intervalles granulométriques logarithmiques variant entre 1 et 2 200 µm, tandis que le rapport du phytoplancton au zooplancton, en termes de stock actuel, est de 1.24. On peut imaginer qu'il y a régulation parallèle de la transformation de l'énergie dans ces deux écosystèmes aquatiques. Compte tenu de la façon dont la biomasse est répartie entre les différentes formes du plancton, il est possible de regrouper les lacs en types qui différeraient présumément par certains aspects fondamentaux de la transformation de l'énergie. Ce point de vue est appuyé par un classement parallèle

des lacs suivant l'efficacité d'utilisation des matières nutritives. Une partie considérable des variations d'un lac à l'autre de la biomasse phytoplanctonique comestible s'expliquait par des observations de pH, du pourcentage de la zone littorale et de la faible biomasse zooplanctonique, alors que les variations de la biomasse herbivore étaient en relation avec la profondeur du lac et le zooplancton carnivore. La biomasse benthique est en rapport direct avec un indice de productivité primaire et en rapport inverse avec un indice de biomasse des poissons se nourissant de benthos.

SUMMARY - In contrast with previous synoptic studies, and on the basis of considerable literature evidence, it is maintained that size and mode of feeding are more important determinants than species identity of an organism's role in lake ecosystem functioning. With benthos, plankton and fish thus characterized, the apportionment of biomass within lake ecosystems of differing trophic structure is investigated over space and time along with the effects on community structure of such perturbations as nutrient flux, acid precipitation and fishing pressure. A computer redox equilibrium model converts standard analytical concentrations of system chemical components into the abundances of various speciation states of each element. Results show that, as for marine ecosystems, there is a tendency in lakes for biomass to be roughly equally distributed over logarithmic particle size intervals from 1 - 2200 m with a phytoplankton to zooplankton standing crop ratio of 1.24. This suggests parallel regulation of energy processing in these two aquatic ecosystems. According to how biomass is apportioned among plankton components, lakes tend to fall into types which presumably differ in fundamental aspects of energy processing. This is supported by a parallel division of lakes on the basis of nutrient utilization efficiencies. A major portion of the variation in edible phytoplankton biomass over the lakes was explained by pH, percent littoral zone and small zooplankton biomass while variation in herbivorous biomass was related to lake depth and carnivorous zooplankton. Benthic biomass is positively correlated with an index of primary productivity and negatively correlated with an index of benthivorous fish biomass.

Lake management based on functional representations of trophic components

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Introduction

As a result of a number of recent studies it has become increasingly apparent that energy flow within aquatic ecosystems is much more dependent on the body size and feeding ecology of organisms than on their taxonomic status (Kajak and Hillbricht-Ilkowska 1972, Kerr 1974, Sheldon et al 1977, Steele and Frost 1977). This is particularly true of pelagic freshwater communities (Brooks and Dodson 1965, Hall et al 1976, Hillbricht-Ilkowska 1977) and hence variations in their size structure should reflect how energy flows through them. For instance in lakes where grazing zooplankters are large and nannoplankton predominate, energy tends to flow directly from algae to zooplankton, whereas in those where zooplankton grazers are small and net plankton predominate, energy flows more indirectly from algae to zooplankton through bacteria which break down the large inedible algae (Gliwicz 1969). Other things being equal, zooplankton communities in which predacious zooplankters are common will generally produce less biomass for planktivorous fish consumption than will those which lack this additional trophic link (Kajak and Hillbricht-Ilkowska 1972, Petrova et al 1975). On the other hand, in lakes dominated by relatively large-bodied zooplankters, planktivorous fish growth efficiency is expected to be greater than in those lakes dominated by comparatively small-bodied forms (Kerr 1971, Galbraith 1975, Northcote 1972, Goldman et al 1979).

Observations in both marine and freshwater pelagic systems confirm the importance of particle size information as a major ecological tool. Sheldon et al (1972) showed that patterns in the standing stock of organisms in the marine pelagic can be described in terms of the sizes of predators and prey and of the efficiencies of their interactions. This means that the standing stock of any sized particle can be predicted from any other size, a powerful technique for the study of energy flow in complex ecosystems. In my laboratory we have shown that the variance in freshwater community structure assignable to lake properties is greater for a size characterization of the zooplankton than a taxonomic one and that the size groupings reflect gradients in pH and lake turnover time which taxonomy does not (Sprules and Holtby 1979).

My recent research is premised on these ideas - namely that in aquatic ecosystems the best way to study patterns of energy flow and their susceptibility to the addition of toxins and nutrients is to investigate patterns in the distribution of biomass within lakes over a variety of lake types at different points in time. The objective of this paper is to a) present biomass distributions for a variety of small, inland lakes of Ontario and b) to show some effects of lake acidification on the distribution of biomass and hence the trophic structure of these lakes.

I wish to acknowledge the productive interactions I have had with fellow members of the Lake Ecosystem Working Group (LEWG) at the University of Toronto and the financial support of the Natural Sciences and Engineering Research Council.

Methods

Although data are available for the summers of 1978 to 1980, only those for the first year have been used in this analysis. Thirty-seven lakes from central Ontario varying in major morphometric and chemical characteristics and incorporating a diversity of anthropogenic stresses such as cottage development and acidification were selected for fortnightly sampling from late April to early September and once in late October. On each visit to a lake integrated phytoplankton and zooplankton samples were taken at mid-basin with a tube sampler and conical townet respectively while dissolved oxygen, temperature, conductivity and pH profiles were taken with a Hydrolab Surveyor. Water samples taken on two midsummer visits were sent to the Ontario Ministry of Environment, Rexdale for determination of analytical concentrations of key ions, nutrients, trace elements and metals. Each lake was extensively netted once during the year to determine fish species composition, relative abundance and length/weight relations. Limited benthic invertebrate data (expanded in later years) comprising direct SCUBA diver counts and counts

from underwater core samples were obtained for some of the lakes.

In the laboratory phytoplankton and zooplankton data were obtained by enumerating organisms in various size classes, the boundaries of which were chosen on the basis of known constraints on algal sizes available to grazers, fish feeding selectivity etc. Zooplankters were further divided into herbivores and carnivores. For zooplankton, numerical densities were converted to biomass densities from published length/weight relations (Hillbricht-Ilkowska and Patalas 1967) and for phytoplankton by using formulae for appropriate geometric solids and assuming unit specific gravity.

Data analyses were done on an IBM 3033 computer at the University of Toronto Computing Centre.

Results and Discussion

Plotting plankton size on a logarithmic scale and expressing average biomass over all lakes and seasons for each of these sizes on the common scale of micrograms per liter leads to a bimodal "biomass spectrum" for the LEWG lakes (Fig. 1). The standing crop of phytoplankton (first five size classes) is on average higher than that for zooplankton in these lakes. This is similar to the pattern in marine pelagic ecosystems in which biomass is roughly equally distributed over logarithmic particle size intervals

with a ratio of standing crop between adjacent tropnic levels of 1.24 (Sheldon et al 1972). For the LEWG lakes tne ratio of phytoplankton to zooplankton standing crop is 1.70. Whether this difference in the ratios indicates primary differences in the two ecosystems or simply reflects slight differences in data presentation is not yet clear. Tne lower biomass in the size range of 200μm (Fig. 1) is fairly consistent from one lake to the next. This may represent protozoan biomass which was not evaluated in our study or could be a unique characteristic of freshwater systems. Further analyses are required to evaluate tne significance of this pattern. Insofar as biomass spectra reflect patterns of energy flow, the results suggest that in a general sense energy flow is similarly constrained in marine and freshwater systems.

Of greater interest is the pattern of variation in these biomass spectra from one lake to the next. Averaging planktonic biomass for each lake over the eight seasonal samples produces four relatively distinct biomass spectra (Fig. 2) ranging from those dominated by phytoplankton through those with roughly equal biomass of algae and zooplankton to those dominated by zooplankton. Clearly there is considerable variation in the distribution of biomass suggesting concomittant variation in major paths of energy flow. Questions arise such as why biomass in a particular size range is unusually high or low? What features of a lake affect these spectra and hence, by

implication, energy flow? Possibilities include lake morphometry ameliorating predation intensities or rate of nutrient circulation, or chemical features favouring, say, detritus production with associated effects on grazers. Future research plans are to use statistical analyses to identify these sorts of relations in our data set. To the extent that biomass variations are related to gradients of anthropogenic stress such as acid precipitation and nutrient loading incorporated in our lake set, we can begin to understand how lakes respond to such perturbations and possibly to develop management strategies.

In fact there is evidence from these spectra of acidification effects in the LEWG lakes. Two of the four acid-stressed lakes in our system are dominated by small zooplankters (Fig. 2, bottom right panel) with phytoplankton standing crops considerably lower than in other lakes. This pattern is also manifest as decreasing algal biomass with decreasing pH (Fig. 3) and by an increasing percentage of small zooplankters with decreasing pH (Fig. 4). The question thus arises - how can this relatively high standing crop of zooplankton persist given the comparatively low biomass of algae? Perhaps the low algal biomass is actually turning over at quite a high rate thus providing sufficient production for the herbivores. This hypothesis could clearly be evaluated with in situ measures of phytoplankton productivity. It could be that

algal production is really low and that detrital and bacterial biomass, upon which small zooplankters feed more efficiently than large ones (Gliwicz 1969), is higher in these acid lakes. Again these data are not now available but could easily be obtained.

Other explanations could account for the shift to small zooplankters in acid lakes. I have observed elesewhere (Sprules 1975) that large zooplankters disappear from lakes at higher pH than do small ones suggesting the possibility that in acid lakes small grazers are relatively free of invertebrate predation. The decreased mite densities in acid lakes noted by Dr. Collins of our group could further reduce predation on small grazers. Small plankters could be more resistant to direct effects of low pH and associated chemical changes although Hall's (1979) results on nickel uptake by Daphnia point to the importance of surface exchange phenomena which should give large species (high surface to volume ratio) an advantage.

This trend in changing size structure of the zooplankton community is even more strongly related to manganese concentration (Fig. 5). The correlation is influenced strongly by the four lakes in the upper right quadrant but these are the most acid lakes in our system. It is most likely that this highly reactive metal is merely an early indicator of the whole host of chemical changes that accompany increasing hydrogen ion concentration,

although there is the remote possibility of direct toxic effects on certain plankters.

Conclusions

Whichever is the correct explanation for the inverse relation between zooplankton size and pH the fact is that our use of biomass spectra leads quickly to the identification of major variation of possible significance in ecosystem function. These spectra are whole system, functional representations of interacting organisms which provide a new perspective on aquatic ecosystem structure. Attempts to explain deviations in biomass patterns from the norm leads ultimately to testable hypotheses about factors governing major features of energy flow in these communities. It is our definite intent to go back to the field to seek,for instance, the hypothesiszed detrital and bacterial biomass in acid lakes or to test hypotneses about algal turnover rates with actual measurements. Examination of statistical relations between lake type and functional characterizations of organisms clearly leads to insights generating a rigorous and directed experimental approach to the study of lakes.

In addition to providing a basis for studying purely ecological aspects of lakes, this biomass approach could be adopted as a tool for sensible lake management. These functional representations of ecosystem components have the potential not only for more rapid appreciation of the nature

of structural change induced by perturbation but also for insight into the concomittant changes in trophic structure of the ecosystem. In other words our approach provides information on the ultimate distribution of biomass (e.g. into coarse fish, filamentous algae) resulting from the addition to lakes of toxins or nutrients as well as an appreciation for the dynamic interactions which have been affected. This is the sort of baseline understanding required for sensible management of freshwater resources.

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Figure Captions

Figure 1. Biomass spectrum averaged over 37 lakes at 8 time periods for phytoplankton (0 - 100um) and zooplankton.

Figure 2. Representative biomass spectra resulting from seasonal averaging for each lake.

Figure 3. Relation between seasonal averages in algal biomass and midsummer epilimnion pH.

Figure 4. Relation between seasonal average in % small zooplankters (< 600μm) and midsummer epilimnion pH.

Figure 5. Relation between % small zooplankters and midsummer epilimnion manganese concentration.

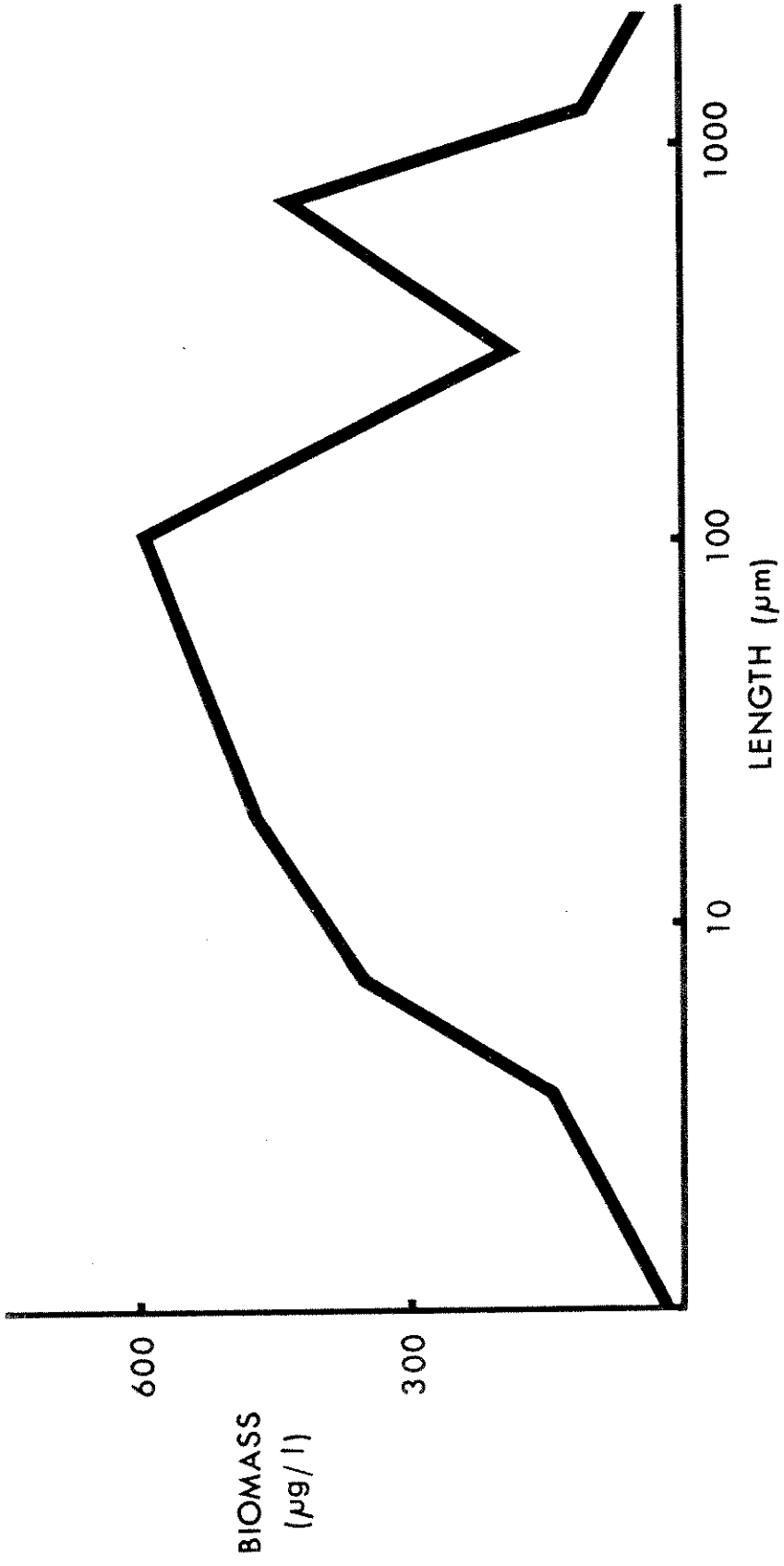

Figure 1.

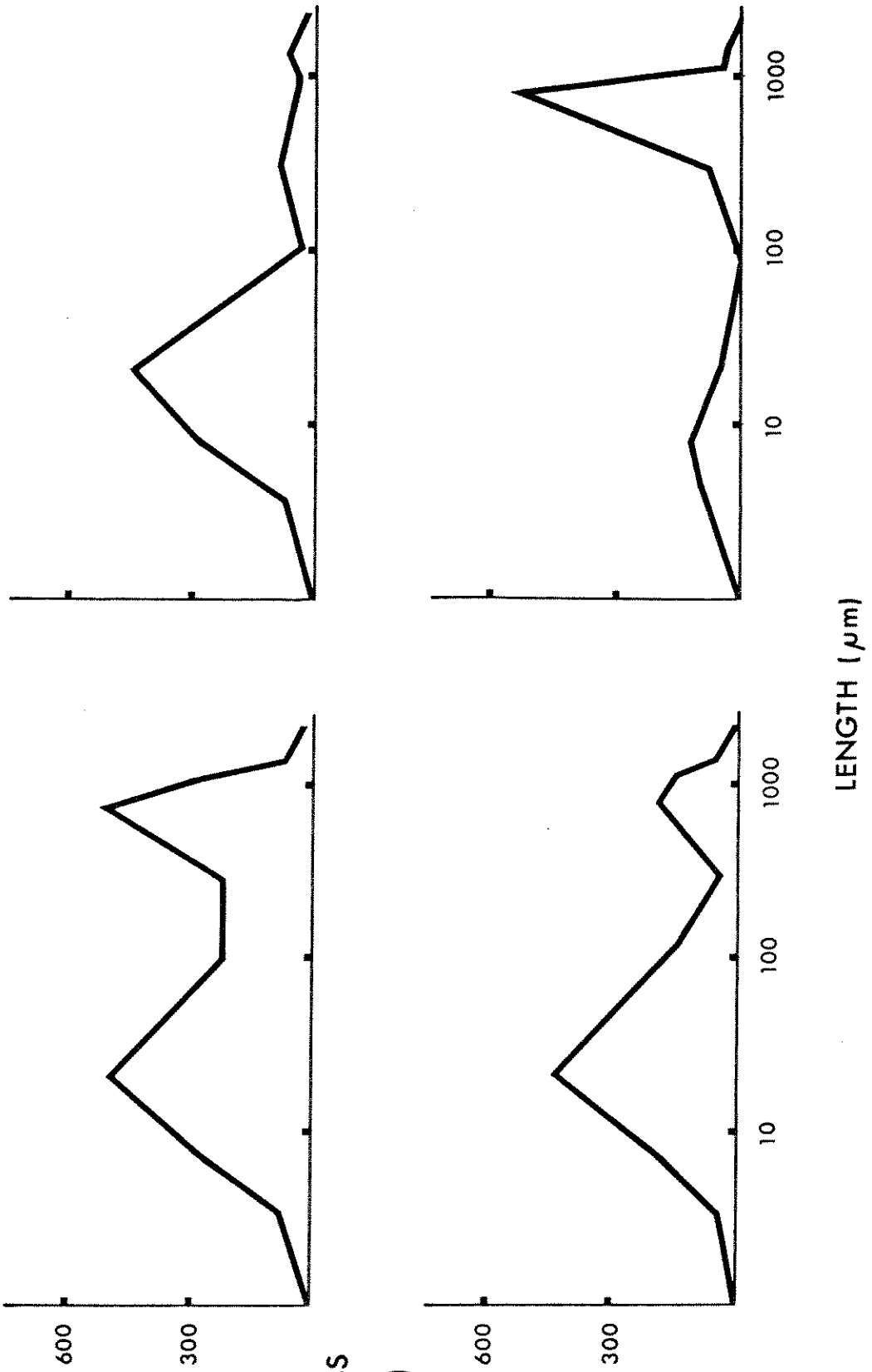

Figure 2.

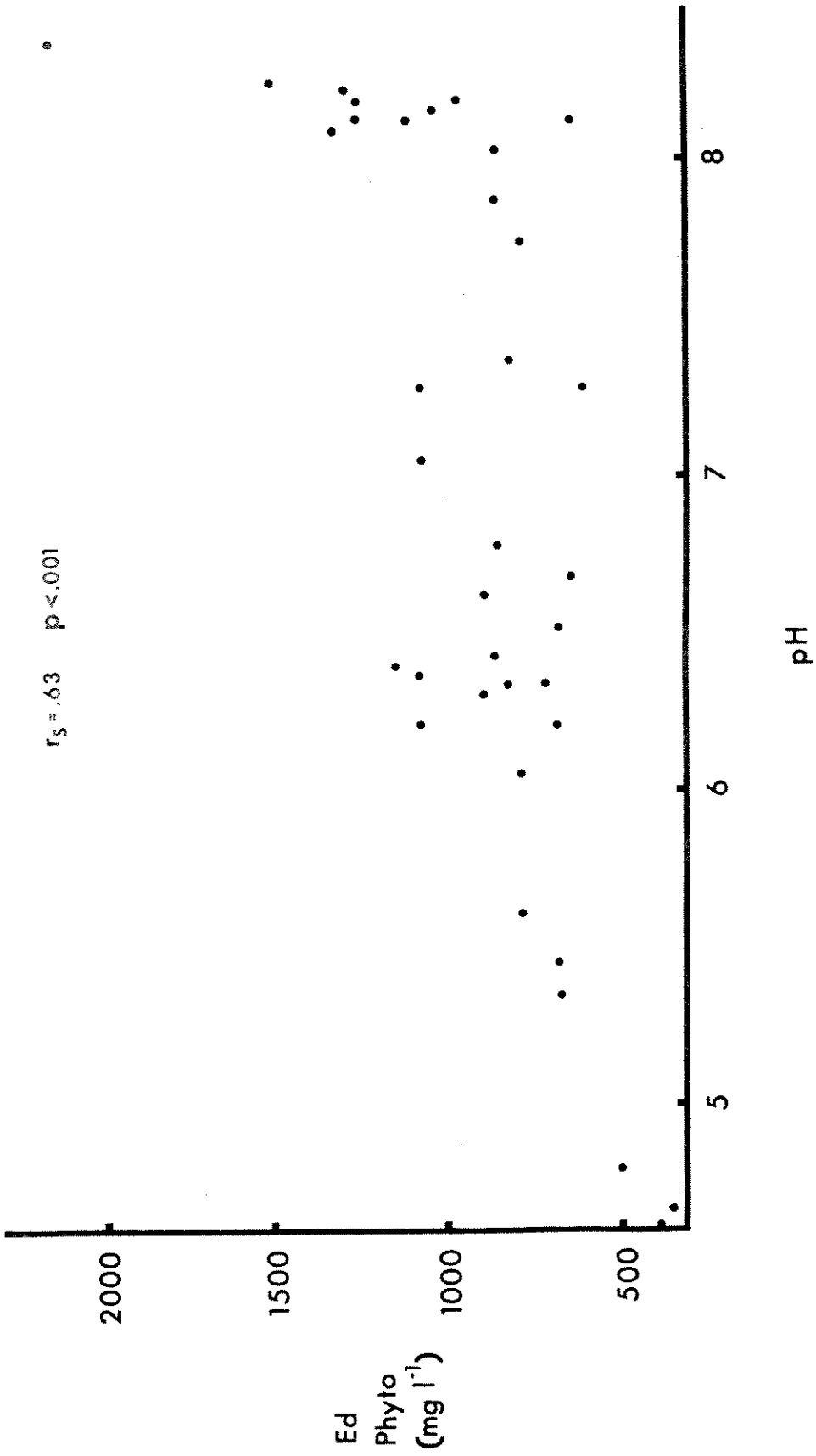

Figure 3.

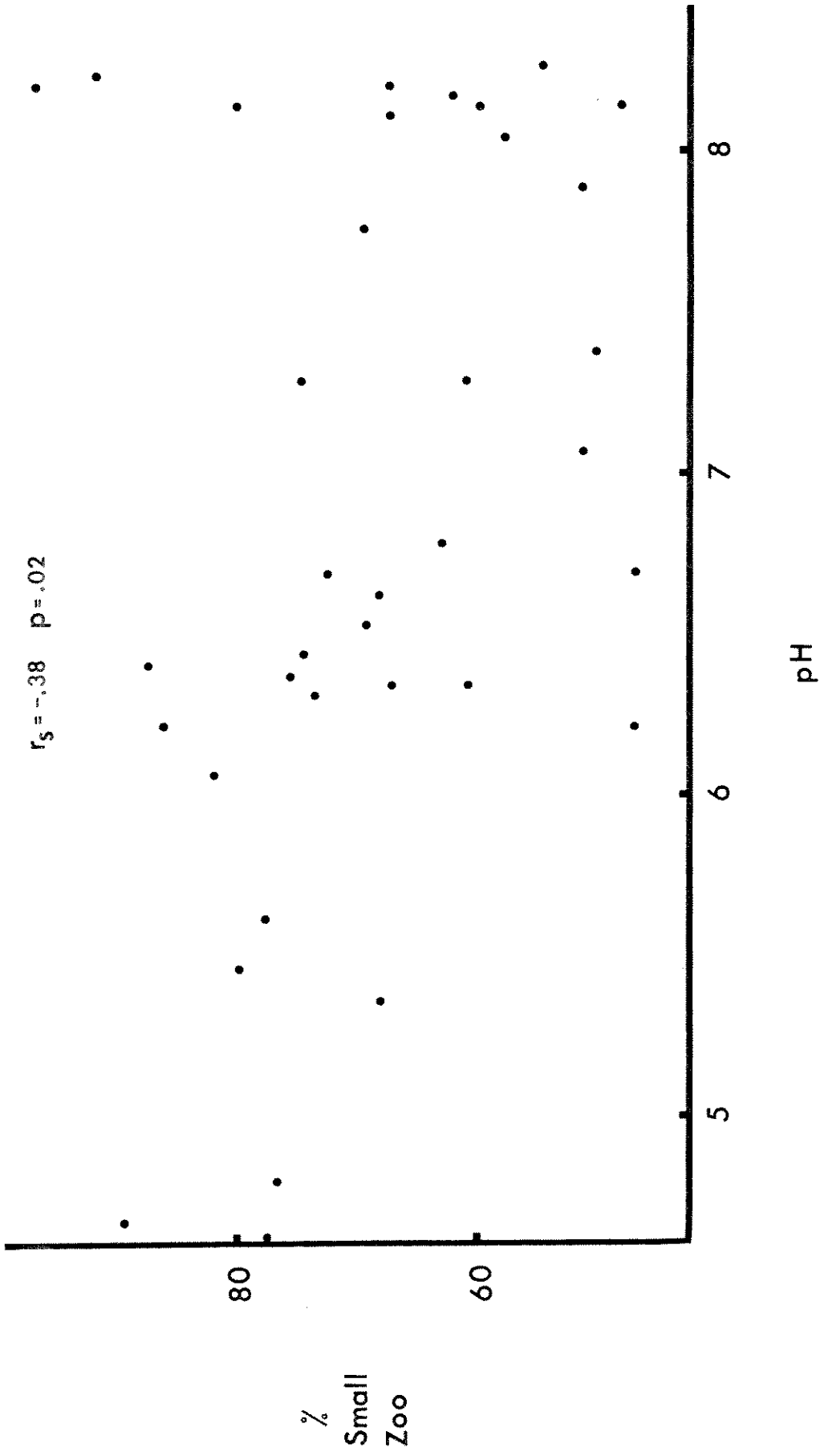

Figure 4.

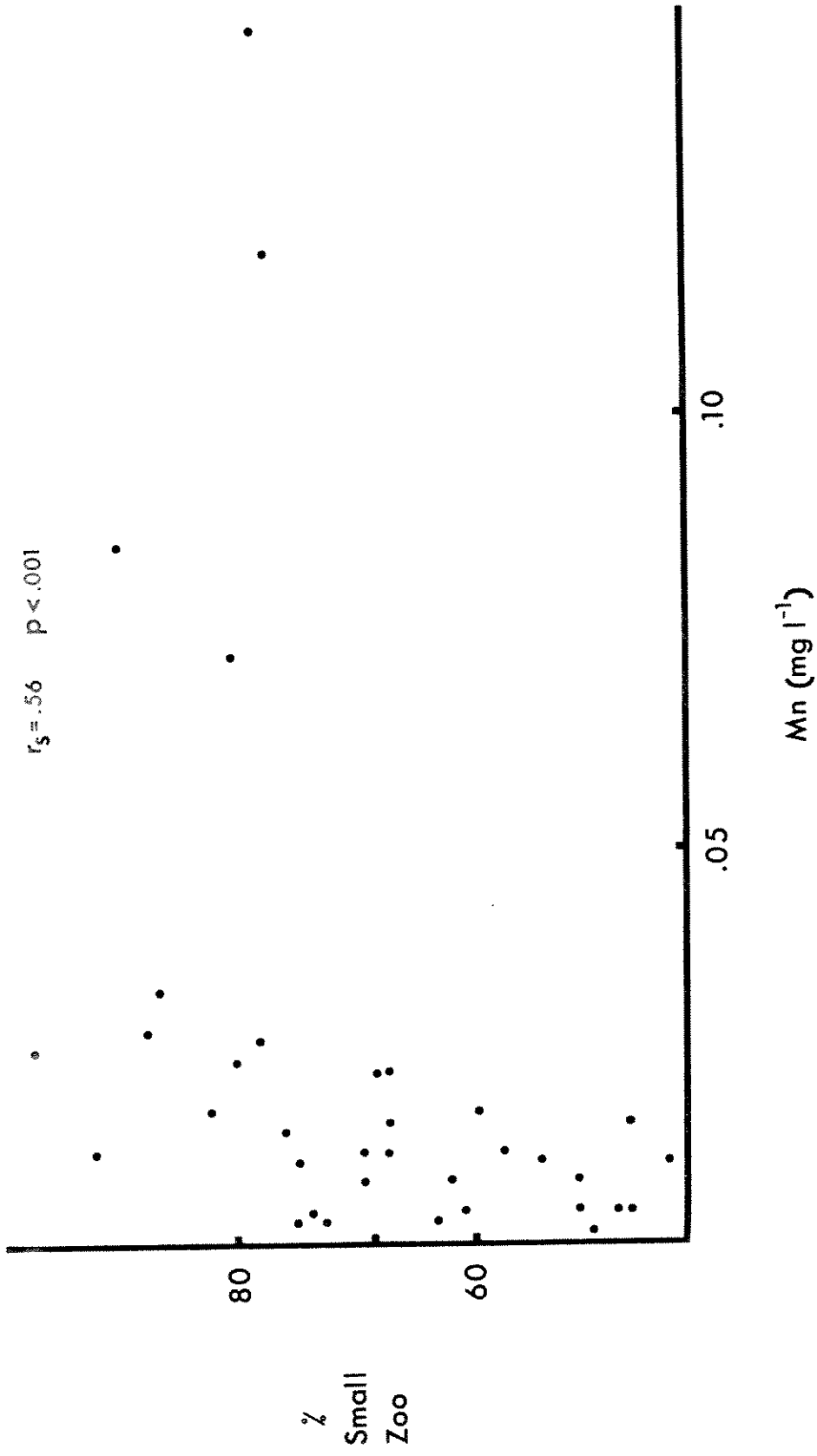

Figure 5.

ÉTUDE COMPARATIVE DES RÉACTIONS À LA TOXICITÉ ENTRE LA TRUITE *SALMO GAIRDNERI* ET QUATRE AUTRES INTÉGRATEURS BIOLOGIQUES SUR 36 CAS DE BIOESSAIS STATIQUES

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RÉSUMÉ - Cette étude a consisté à déterminer la sensibilité relative de plusieurs organismes utilisés dans des bioessais. Les analyses ont été effectuées sur des échantillons d'effluents industriels sur lesquels des tests avec *Salmo gairdneri* venaient d'être réalisés. Les autres organismes utilisés furent l'algue *Selenastrum capricornutum*, l'algue mobile *Chlamydomonas variabilis*, la daphnie *Daphnia magna* et une bactérie de type *Photobacterium phosphoreum* (Microtox® de Beckman). Les résultats sont exprimés en unités toxiques. Pour la fraction brute, la sensibilité relative des organismes utilisés est la suivante: *Selenastrum* > *Salmo* > *Daphnia* > Microtox® > *Chlamydomonas*. On retrouve à peu près le même ordre de sensibilité relative pour les échantillons de la fraction autoclavée; cependant dans ces derniers, une baisse évidente de la sensibilité a été observée, par rapport à la fraction brute.

COMPARATIVE STUDY OF REACTIONS TO TOXICITY BETWEEN THE *SALMO GAIRDNERI* TROUT AND FOUR OTHER BIOLOGICAL INTEGRATORS IN 36 CASES OF STATIC BIOASSAYS

GÉRALD JOUBERT

SUMMARY - The aim of this study was to determine the relative sensitivity level of several organisms used in bioassays. Thus, industrial effluent samples on which tests had just been run with *Salmo gairdneri* were analyzed. The other organisms used were *Selenastrum capricornutum* algae, moving *Chlamydomonas variabilis* algae, *Daphnia magna* and *Photobacterium phosphoreum* (Beckman Microtox®) type bacteria. The results were noted in toxic units. For the rough fraction, the relative sensitivity level of the organisms used was as follows: *Selenastrum* > *Salmo* > *Daphnia* > Microtox® > *Chlamydomonas*. The relative sensitivity sequence is approximately the same for the samples of the autoclaved fraction. However, in the latter, a significant drop in the sensitivity level was noted in comparison with the rough fraction.

INTRODUCTION

Depuis les premières méthodes de bioessais décrites par Hart *et al* (1945), Doudoroff *et al* (1951) et Sprague (1969, 1973), un grand nombre de tests furent développés pour évaluer et mesurer la toxicité à l'aide d'organismes biologiques vivant dans différents milieux et représentant divers maillons de la chaîne trophique (APHA-Standard Methods, 1975).

Ces dernières années, les besoins sans cesse grandissant d'outils efficaces, combinés à une augmentation exhaustive des coûts des analyses utilisant les poissons ont obligé le gestionnaire à développer d'autres tests capables de mesurer la toxicité sur un grand nombre d'échantillons à volumes restreints. Cependant, leur emploi sur une base routinière s'avère difficile étant donné que les tests employant les poissons sont devenus des outils légaux en Amérique du Nord; de plus, très peu d'études comparatives ont été réalisées dans le but de connaître leur sensibilité relative et d'en arriver ainsi à les standardiser.

La présente étude a pour but de déterminer le degré de sensibilité relative de divers organismes tests. Les réactifs biologiques étudiés dans cette étude sont: la truite arc-en-ciel *Salmo gairdneri*, l'algue *Selenastrum capricornutum*, l'algue mobile *Chlamydomonas variabilis*, la daphnie *Daphnia magna* et une bactérie de type *Photobacterium phosphoreum* (Microtox® de Beckman).

INTRODUCTION

Since the first bioassay methods described by Hart *et al* (1945), Doudoroff *et al* (1951), and Sprague (1969, 1973), a large number of tests have been developed to evaluate and measure toxicity using biological organisms living in different mediums and constituting different links in the food chain (APHA-Standard Methods, 1975).

In the past few years, the growing need for effective instruments, and the spiralling cost of analyses using fish, have obliged researchers to develop other tests to measure toxicity in a large number of limited-volume samples. Regular use of these other tests is not without its problems, however, as the tests using fish are now recognized legal instruments in North America. Very few comparative studies have been carried out on the newer tests to determine their relative sensitivity and thus enable them to be standardized.

The aim of this study is to establish the level of relative sensitivity of a number of test organisms. The biological reactors studied are: the *Salmo gairdneri* rainbow trout, the *Selenastrum capricornutum* algae, the *Chlamydomonas variabilis* moving algae, the *Daphnia magna* daphnid, and a bacteria of the *Photobacterium phosphoreum* group (Beckmans' Microtox®).

MÉTHODES

Les tests utilisant la truite arc-en-ciel sont basés sur la méthode décrite par Doudoroff *et al* (1951) et Sprague (1969, 1973), avec certaines modifications adoptées par le Comité technique national d'Environnement Canada sur la toxicité. Ces modifications sont: le rapport litre/g/jour qui a été abaissé de 2 à 1, la nécessité d'une aération à un taux de 5,0 à 7,5 cc/min./litre et le poids des poissons qui peut se situer entre 0,5 et 10 grammes.

La méthode utilisée avec l'algue *Selenastrum capricornutum* est telle que décrite par Joubert (1980). Toutefois, nous avons ici évalué la toxicité à différents temps d'incubation, soit 7 jours, 8 jours et 14 jours (EPA 1971, 1978; Standard Methods, APHA, 1975), afin de définir à quel moment le test semblait le plus sensible.

Dans le cas de l'algue *Chlamydomonas variabilis*, le protocole expérimental de l'Institut national de recherche chimique appliquée (IRCHA) de France a été suivi, avec des modifications concernant la préparation de l'inoculum. Ces changements concernent l'élimination de l'étape de la centrifugation qui contribue à l'agglomération des cellules et au bris des flagelles des algues. Pour favoriser la croissance des cultures, on a utilisé le milieu synthétique recommandé par l'IRCHA, tout en faisant barboter dans les cultures du CO_2 (2 %). La photopériode est de 14 heures

METHODS

Tests using the rainbow trout are based on the method described by Doudoroff *et al* (1951) and Sprague (1969, 1973), with certain modifications adopted by Environment Canada's National Technical Coordinating Committee on toxicity. These modifications are as follows: the litre/g/day ratio is reduced from 2 to 1, aeration must be at a rate of between 5.0 and 7.5 cc/min./litre, and fish weight should be between .5 and 10 grams.

The method using the *Selenastrum capricornutum* algae is that described by Joubert (1980). However, in the present case, we evaluated toxicity after incubation periods of 7, 8 and 14 days (EPA 1971, 1978; Standard Methods, APHA, 1975), so as to determine for which period the test was most sensitive.

In the case of the *Chlamydomonas variabilis* algae, we followed the experimental method of the Institut national de recherche chimique appliquée (IRCHA), with modifications as regards preparation of the inoculum. These changes involved eliminating the centrifuging step which serves to concentrate the cells and break up algae flagellae. To encourage culture growth, we used the synthetic medium recommanded by the IRCHA, and bubbled CO_2 (2 %) trough the cultures. The photoperiod was 14 hours of light at an intensity of 1 435 lux, followed by 10 hours

de lumière suivie de 10 heures d'obscurité à une intensité de 1 435 lux. La concentration d'algues ainsi obtenue après quelques jours est suffisante pour permettre leur utilisation directement à partir de la culture. La durée d'exposition choisie pour le test est de 24 heures.

La méthodologie utilisée pour les daphnies est celle qui a été publiée par l'Association française de normalisation (AFNOR, 1974), avec des modifications dans le cas de l'élevage des daphnies. L'élevage est ici effectué dans des aquariums de 15 litres. Au fond de l'aquarium, on place 200 grammes de terreau autoclavé puis l'aquarium est rempli avec de l'eau du robinet. On laisse reposer pendant une semaine et les pertes dues à l'évaporation sont compensées avec de l'eau déminéralisée. Les aquariums sont placés à 20° C, avec une photopériode de 16 heures suivie de 8 heures d'obscurité. Un faible barbotage d'air assure une oxygénation suffisante du milieu tout en favorisant une certaine homogénéité. On introduit ensuite des femelles daphnies adultes, que l'on nourrit 3 fois/semaine avec deux solutions différentes. La première contient 15 g/l d'extrait de boeuf et 15 g/l de dextrose (AFNOR, 1974) et la seconde contient 12 g/l de nourriture à truite pulvérisée, 3 g/l de luzerne puvérisée et 6 g/l de levure (Metikosh, 1979). Cette dernière solution est passée au mélangeur puis décantée pendant 10 minutes dans un bécher. Par la suite, on prélève 800 ml

of darkness. The concentration of algae obtained after a few days was sufficient to allow their use directly from the culture. An exposure time of 24 hours was chosen for the tests.

Methodology used for the daphnia was that published by AFNOR, the French standards association (AFNOR, 1974), with modifications regarding the conditions in which they were raised. We used 15 litre tanks into which we placed 200 grams of autoclaved soil before filling them with tap water. They were left for one week, and losses due to evaporation were replaced using demineralized water. The aquariums were kept at 20° C, with a photoperiod of 16 hours, followed by 8 hours of darkness. A limited air bubbling system provided sufficient oxygenation while maintaining a certain homogeneity. Adult female daphnia were then placed in the tank and fed three times a week with two different solutions. The first contained 15 g/l of beef extract and 15 g/l dextrose (AFNOR, 1974), and the second contained 12 g/l of powdered trout food, 3 g/l of powdered alfalfa, and 6 g/l of yeast (Metikosh, 1979). This second solution was mixed in a blender and then separated for ten minutes in a beaker, after which 800 ml of the supernatant substance was removed for use as the feed solution.

du surnageant, qui constitue la seconde solution nutritive.

Pour les tests avec la bactérie de type *Photobacterium phosphoreum*, l'appareil Microtox® de Beckman a été utilisé, avec la méthodologie recommandée par la compagnie, y compris pour les échantillons colorés.

Les différentes analyses pré-mentionnées ont été réalisées sur des effluents industriels, prélevés par des équipes des laboratoires régionaux d'Environnement Canada, où des tests de toxicité ont été effectués avec la truite arc-en-ciel. Seuls les échantillons qui provoquaient la mortalité des truites étaient expédiés au laboratoire de bioessai du ministère de l'Environnement du Québec. L'expédition s'effectuait dans des galcières en présence de "ice-pak" et les analyses biologiques étaient généralement réalisées au plus tard 48 heures après leur réception.

Afin de vérifier les effets de l'autoclavage sur la toxicité des échantillons, chaque analyse fut effectuée sur une fraction brute et sur une fraction autoclavée, sauf pour le test avec la truite. Le second but visé par cette pratique était de connaître laquelle des deux fractions apparaissait la plus toxique.

RÉSULTATS

Pour fin de comparaison, les valeurs de

For tests with the *Photobacterium phosphoreum* bacteria, we used Beckman's Microtox® kit, and observed the methodology recommanded by the company, including that recommanded for the couloured samples.

The analyses mentioned above were carried out on industrial effluent samples collected by teams from Environment Canada's regional laboratories, where toxicity tests were executed using rainbow trout. Only the samples which provoked the death of the trout were sent to the bioassay laboratory of the ministère de l'Environnement du Québec. Samples were sent in coolers filled with ice packs, and biological analyses were, in most cases, carried out within 48 hours of their reception.

As a check on the effects of autoclaving on the toxicity of samples, each analysis was carried out on an untreated fraction and an autoclaved fraction, except for the test with the trout. The other purpose of this approach was to determine which of the two fractions appeared to be more toxic.

RESULTS

For comparative purposes, the values of

CL_{50} ou de CE_{50} et CI_{50} ont été transformées en nombre absolu, soit en unités toxiques, tel que défini par Sprague & Ramsay (1965). Ceci est effectué en appliquant l'équation ci-après:

$$UT = \frac{100\ \%}{CL_{50}}$$

où UT = unités toxiques

100% = concentration de l'échantillon

CL_{50} = concentration létale 50 en % V/V

Cette valeur représente le nombre de fois que la CL_{50} est contenue dans l'échantillon initial.

Dans la Table 1, représentant l'ensemble des résultats obtenus sur la portion brute*, les échantillons ont été groupés selon le type d'effluents auxquels ils appartenaient.

Si l'on compare les trois séries de résultats obtenus avec l'algue *Selenastrum*, afin de connaître quel serait le temps d'incubation donnant la meilleure sensibilité au test, on obtient, en faisant l'addition des unités toxiques pour chaque période, les résultats suivants:

7 jours: 1681 UT

8 jours: 1052 UT

14 jours: 546 UT

LC_{50}, or of EC_{50} and IC_{50} were expressed in absolute numbers, as toxic units, as defined by Sprague & Ramsay (1965). This was obtained by applying the equation given below:

$$TU = \frac{100\ \%}{LC_{50}}$$

where TU = Toxic Units

100% = sample concentration

LC_{50} = lethal concentration 50 as a % V/V

This value represents the number of times the LC_{50} is present in the initial sample

In Table 1, which gives overall results obtained from the untreated fraction*, samples have been grouped according to the type of effluent they belong to.

If a comparison is made of the three series of results obtained using the *Selenastrum* algae, to determine the incubation period which would provide the best sensitivity for the test, we arrive at the following results by adding together the toxic units for each period:

7 days: 1681 TU

8 days: 1052 TU

14 days: 546 TU

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* Dans le cas de l'algue *Selenastrum capricornutum*, la portion brute a été filtrée sur filtre Millipore® de 0.45 µ, afin d'éviter des interférences dans la mesure de la biomasse.

* In the case of the algae *Selenastrum capricornutum*, the untreated fraction was filtered through a .45 µ Millipore® filter, to avoid interferences in the measurement of the biomass.

Table 1. Toxicité* de la fraction brute

Table 1. Toxicity* of the untreated fraction

Types d'effluents	No labo.	*Salmo* (96 h.)	*Selenastrum* 7 j.	*Selenastrum* 8 j.	*Selenastrum* 14 j.	*Chlamydomonas* (24 h.)	*Daphnia* (24 h.)	Microtox®	Type of effluent
Alimentaires	80-38	4.2	3.9	2.7	1.6	1.1	24	nd	Food
	80-39	13	6.7	4.2	2.6	nd	1.0	nd	
	80-43	2.9	13	5.6	1.3	nd	1.4	nd	
Bois et produits	79-256	14	32	21	7.7	nd	3.0	8.3	Wood and wood products
	79-262	1.7	nd	nd	nd	nd	nd	nd	
	79-263	22	3.4	2.9	nd	nd	1.8	nd	
	80-1	2.7	nd	nd	nd	nd	nd	nd	
	80-10	1.8	9.1	5.3	3.0	2.0	nd	nd	
	80-15	20	230	180	26	nd	10	59	
	80-16	2.9	18	15	11	nd	5.0	0.14	
	80-17	2.9	33	22	19	nd	6.3	3.6	
	80-29	3.6	17	17	3.1	1.2	1.4	1.8	
	80-32	1.5	6.3	4.8	5	nd	nd	nd	
	80-33	17	23	11	5	1.3	1.9	9.1	
Cuir et produits	80-2	3.3	100	50	55	3.3	3.3	3.2	Leather
Métallurgie	80-14	5.3	20	10	3.8	1.7	34	nd	Metallurgy
	80-34	1.0	1.4	1.4	1.1	1.4	7.7	nd	
Mines	79-247	1.3	240	130	100	nd	1.3	nd	Mines
	80-11	1.9	5.0	4.5	3.4	nd	nd	nd	
	80-30	6.5	17	6.7	3.7	nd	3.1	0.29	
	80-31	1.1	1.4	nd	nd	nd	nd	nd	
Produits chim. inorganiques	80-37	>31	nd	nd	nd	nd	1.1	nd	Inorganic chemical products
	80-44	4.2	220	48	9.3	12	9.1	nd	
Produits chim. organiques	79-264	21	120	130	69	38	2.1	19	Organic chemical products
	80-3	-	51	43	16	3.1	5.7	2.1	
	80-4	1.9	7.3	10	9.1	nd	1.4	nd	
	80-8	-	170	44	21	1.4	nd	nd	
	80-9	-	100	87	47	9.1	20	5.6	
	80-12	1.3	23	17	8.3	nd	6.5	nd	
	80-13	1.2	19	15	9.7	nd	5.3	nd	
	80-19	-	5.3	3.7	2.3	5.6	2.4	nd	
	80-20	-	94	77	33	13	14	13	
	80-23	3.1	19	19	17	nd	3.9	nd	
	80-24	3.0	20	19	18	nd	21	0.4	
	80-67	1.3	21	17	14	nd	nd	nd	
	80-68	1.2	31	29	20	nd	nd	nd	

* La toxicité est exprimée en unités toxiques

nd = Non détectable

* Toxicity is expressed in Toxic Units

nd = Not detectable

Afin de déterminer la sensibilité relative des différents organismes impliqués dans cette étude, on a défini une cote de 1 à 7 pour chacun des échantillons d'effluents, selon le nombre d'unités toxiques obtenus avec chaque organisme. La cote 1 a été donnée à l'organisme ayant démontré la plus fai-

To establish the relative sensitivity of the different organisms used in this study, we used a notation of 1 to 7 for each of the effluent samples, according to the number of toxic units obtained with each organism. The value 1 was given to the organism which demonstrated the lowest level

ble sensibilité*. En faisant la sommation des cotes, les résultats obtenus sont les suivants:

Selenastrum 7 jours days	>	*Selenastrum* 8 jours days	>	*Selenastrum* 14 jours days	>	*Salmo* 96 h.	>	*Daphnia* 24 h.	>	Microtox®	>	*Chlamydomonas* 24 h.
(157)		(139)		(100)		(97)		(81)		(50)		(49)

La Table 2 représente l'ensemble des résultats obtenus sur la fraction autoclavée des échantillons.

En comparant les trois séries de résultats obtenus avec l'algue *Selenastrum* pour les différentes durées d'incubation, nous obtenons les valeurs suivantes:

7 jours: 1505 UT

8 jours: 860 UT

14 jours: 415 UT

L'ordre numérique s'accorde avec celui obtenu avec les échantillons bruts. De plus, le degré de sensibilité relative des organismes varie peu:

Selenastrum 7 jours days	>	*Selenastrum* 8 jours days	>	*Selenastrum* 14 jours days	>	*Daphnia* 24 h.	>	*Chlamydomonas* 24 h.	>	Microtox®
(144)		(119)		(87)		(47)		(43)		(35)

* Pour cette compilation, les quelques échantillons comportant une donnée manquante n'ont pas été inclus dans les calculs.

of sensitivity*. After adding the notations, we obtain the following results:

Table 2 gives overall results obtained from the autoclaved fraction of the samples.

If a comparison is made of the three series of results obtained using the *Selenastrum* algae for the different incubation periods, we arrive at the following results:

7 days: 1505 TU

8 days: 860 TU

14 days: 415 TU

The numerical order is in line with that obtained with the untreated fractions and, furthermore, there is little variation in the levels of relative sensitivity of the organisms:

* For the purposes of this compilation, those few samples for which some data was missing were excluded from the calculations.

Table 2. Toxicité*de la fraction autoclavée

Table 2. Toxicity* of the autoclaved fraction

Types d'effluents	No labo.	*Selenastrum* 7 j.	*Selenastrum* 8 j.	*Selenastrum* 14 j.	*Chlamydomonas* (24 h.)	*Daphnia* (24 h.)	Microtox®	Type of effluent
Alimentaires	80-38	12	4.6	2.2	1.7	-	-	Food
	80-39	8.3	7.7	4.5	nd	nd	0.13	
	80-43	19	12	1.7	2.0	-	nd	
Bois et produits	79-256	13	10	3.4	nd	1.8	nd	Wood and wood products
	79-262	nd	nd	nd	nd	nd	nd	
	79-263	5.8	5.0	1.9	1.2	3.0	1.3	
	80-1	2.6	1.7	nd	nd	nd	nd	
	80-10	5.6	4.1	1.8	nd	nd	nd	
	80-15	290	240	65	2.0	5.3	nd	
	80-16	8.4	4.4	2.0	nd	nd	nd	
	80-17	6.7	4.5	3.2	2.1	2.4	nd	
	80-29	11	6.7	2.5	1.4	nd	1.3	
	80-32	6.3	4.3	2.1	nd	nd	0.53	
	80-33	59	47	8.3	nd	1.1	7.7	
Cuir et produits	80-2	5.0	3.7	3.6	1.8	nd	nd	Leather
Métallurgie	80-14	38	21	4.2	1.6	1.1	nd	Metallurgy
	80-34	1.6	1.7	1.4	1.5	0.7	nd	
Mines	79-247	440	120	75	7.7	2.4	nd	Mines
	80-11	5.0	4.2	2.6	nd	nd	nd	
	80-30	9.5	11	6.7	nd	1.8	nd	
	80-31	1.8	1.2	nd	1.3	nd	0.11	
Produits chim. inorganiques	80-37	2.0	1.1	nd	nd	2.1	nd	Inorganic chemical products
	80-44	83	35	7.2	13	9.1	-	
Produits chim. organiques	79-264	100	81	56	17	1.6	13	Organic chemical products
	80-3	10	6.6	4.0	3.6	nd	0.77	
	80-4	12	11	8.3	nd	1.6	nd	
	80-8	50	34	31	2.6	nd	nd	
	80-9	35	33	17	6.3	9.3	5.0	
	80-12	13	11	6.5	nd	4.2	nd	
	80-13	16	15	10	nd	4.0	nd	
	80-19	7.0	6.4	4.2	4.0	2.7	nd	
	80-20	16	14	7.4	5.0	3.4	6.5	
	80-23	22	13	13	nd	nd	nd	
	80-24	17	14	14	nd	nd	nd	
	80-67	26	17	16	nd	nd	nd	
	80-68	147	53	28	nd	nd	nd	

* La toxicité est exprimée en unités toxiques
nd = Non détectable

* Toxicity is expressed in Toxic Units
nd = Not detectable

Pour connaître laquelle des deux fractions, brute ou autoclavée, est la plus toxique, on effectue respectivement sur ces deux fractions la sommation des unités toxiques. Les résultats apparaissent à la Table 3.

To determine which of the two fractions, the untreated or the autoclaved, is the most toxic, the sum of the toxic units is calculated respectively for each of the fractions. The results appear in Table 3.

Table 3. Comparaison entre les unités toxiques des fractions brutes et autoclavées

Table 3. Comparison between toxic units of untreated and autoclaved fractions

Organismes Organism	Total des unités toxiques Total number of toxic units	
	Fraction brute Untreated fraction	Fraction autoclavée Autoclaved fraction
Selenastrum 8 jours (days)	1053	849
Chlamydomonas (24 h.)	97	76
Daphnia (24 h.)	156	58
Microtox®	148	29

DISCUSSION ET CONCLUSION

Selon les sommes d'unités toxiques obtenues pour les trois différents temps d'incubation dans le cas du tests avec l'algue *Selenastrum* et ce, autant pour les échantillons bruts que pour les échantillons autoclavés, nous sommes portés à conclure que la période d'incubation de 7 jours semble présenter, au premier abord, la meilleure sensibilité du test. Cependant, une augmentation sensible du nombre de cellules entre les jours 7 et 8, combinée à un manque de résolution du test au jour 7 et à des points beaucoup mieux alignés sur la droite de toxicité pour le test de 8 jours, sont les principales raisons pour abandonner la période d'incubation de 7 jours. Entre les 8e et 14e jours d'incubation, aucune augmentation

DISCUSSION AND CONCLUSION

According to the sum of the toxic units obtained for the three different periods of incubation in the case of the test using the *Selenastrum* algae, whether it is for the untreated or the autoclaved samples, we would be tempted to conclude that, on first appearances, the 7-day incubation affords the best sensitivity in the test. However, the noticeable increase in the number of cells between day 7 and day 8, as well as a lack of resolution in the test on day 7, and points which are much better positioned along the toxicity line for the 8-day test, are the main reasons which have led us to abandon the 7-day incubation period. No significant increase in the number of cells was observed between the 8th and the 14th days of incuba-

significative du nombre de cellule a été observée dans les standards, de même que dans les différentes dilutions de la majorité des tests. Cependant, dans quelques rares tests on a observé une faible augmentation du nombre de cellules; comme ceci ne s'est jamais produit dans les standards, on suppose un léger retard de croissance qui serait relié à un effet toxique. Toutefois, il a été constaté que malgré la stabilité du nombre de cellules entre les 8e et 14e jours, on remarquait une augmentation significative de la biomasse correspondante. Ce phénomène peut s'expliquer par une accumulation de réserves nutritives dans la cellule, suite au blocage des processus de division cellulaire. En effet, Samson (1980) a observé une augmentation des glucides, des lipides et du carbone cellulaires au cours de la phase stationnaire de croissance, sur 9 espèces d'algues endogènes. Compte tenu de ces observations, la durée de 8 jours telle qu'établi par Joubert (1980), serait la plus propice pour la détection de la toxicité avec *Selenastrum capricornutum*.

tion either in standard concentration or in the different dilutions in the majority of tests. We did however observe a slight increase in the number of cells in a very limited number of tests, but as this never occured in standard concentrations, we can only presume that this slight delay in growth was due to a toxic effect. We also noted that in spite of the stability of the number of cells between the 8th and the 14th days, there was a sizeable increase in the corresponding biomass. This phenomenon can be explained by the accumulation of nutritional reserves in the cells, after the process of cellular division has been stopped. Samson (1980) observed an increase in glucides, lipides and cellular carbon during the stationary growth stage in 9 species of endogenous algae. On the basis of these observations, the 8-day incubation period established by Joubert (1980), would seem to be the most appropriate for the detection of toxicity using *Selenastrum capricornutum*.

En comparant la sensibilité relative des cinq organismes utilisés, on constate que le test avec l'algue *Selenastrum* s'est avéré le plus sensible, alors que celui avec l'algue *Chlamydomonas* l'a été le moins. Cette différence réside probablement au niveau du temps de contact des substances avec les algues. De plus, les mécanismes mis en cause sont différents puisque le test avec *Selenastrum*

A comparison of the relative sensitivity of the five organisms used indicates that the test using the *Selenastrum* algae is the most sensitive, and that using the *Chlamydomonas* algae, the least sensitive. This difference is probably due to differences in contact time between the substances and the algae. In addition, the mechanisms affected are different, as the test using

implique un blocage de division cellulaire, sur une période de 8 jours, alors que celui avec *Chlamydomonas* est lié à un arrêt de la mobilité, après 24 heures.

La Table 3 démontre une chute de sensibilité sur la portion autoclavée pour tous les organismes et cette chute est particulièrement marquée chez *Daphnia* et le Microtox®. De ces observations, on peut déduire que l'autoclavage change considérablement la nature des échantillons et par le fait même, elle serait à déconseiller, même pour le test avec *Selenastrum*.

Des corrélations statiques ont été tentées entre les résultats des différents organismes biologiques mais aucune corrélation valable n'a été obtenue. Dans certain cas, il semblait y avoir corrélation mais la variance expliquée (r^2) était trop faible pour les considérer avec certitude. Ceci est explicable par le fait que chacun des organismes a une sensibilité différente et que le mode d'action des substances toxiques sur chacun d'eux est différent.

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* INRS-Eau: Institut national de la recherche scientifique-Eau.

Selenastrum stops cellular division for a period of 8 days, while the test with *Chlamydomonas* arrests movement after 24 hours.

Table 3 shows a drop in sensitivity in the autoclaved fraction for all the organism; this drop is particularly noticeable in the case of *Daphnia* and the Microtox®. These observations clearly show that autoclaving brings about considerable change in the nature of the samples, and therefore should not be used, even for the test with *Selenastrum*.

We attempted to make statistical correlations between the results of the different biological organisms, but no valid correlations were obtained. In some cases there did seem to be a correlation, but the determination coefficient (r^2) was too weak to be accepted without question. Obviously, each organism has a different level of sensitivity, and toxic substances act on each of them in a different way.

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CONSEQUENCES ECOLOGIQUES DE LA PRESENCE D'EFFLUENTS LIQUIDES DE MINE DANS LE BASSIN DE LA RIVIERE ONAPING, ONTARIO

P.M. Bolger. Inco Metals Co.

RESUME - La localité minière de Levack près de Sudbury, Ontario, déverse ses effluents liquides dans la rivière Onaping. La présente étude a été entreprise en 1977 afin de découvrir les sources de déversement et les caractéristiques des effluents, et pour étudier les effets de ces derniers sur les organismes qui habitent les cours d'eau de ce réseau hydrographique.

Dix études physicochimiques ont été réalisées en 1977 dans 18 stations d'échantillonnage sur tout le bassin de la rivière Onaping. Des échantillons de macro-invertébrés benthiques ont été prélevés à deux reprises, en juin et en septembre, des poissons ont été capturés en juillet et des macrophytes aquatiques ont été cueillis en septembre. Les macro-invertébrés ont été prélevés au moyen d'un échantillonnage ("Surber") et d'une puise, les poissons ont été capturés au moyen d'une seine et de matériel électrique, et les macrophytes aquatiques ont été cueillis le long des rives.

Les examens physicochimiques ont montré que des métaux et des composés soufrés et partiellement oxydés sont les principaux contaminants contenus dans les effluents. En aval de Levack, nous avons observé des concentrations de nickel atteignant 1.08 mg/L, et des concentrations de cuivre atteignant 0.18 mg/L dans la rivière Onaping. L'oxydation en sulfates des composés soufrés provoquait un important abaissement du pH et de la teneur en oxygène dissous dans la rivière Oanping. Le cas le plus spectaculaire est survenu en juillet 1977, quand le pH de la rivière Onaping est tombé de 6.8 à 3.5 et la teneur en oxygène dissous, de 8.9 mg/L à 3.0 mg/L.

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Les examens biologiques ont montré que les organismes aquatiques de la rivière Onaping étaient considérablement affectés par le déversement des effluents de mine. La distribution des macro-invertébrés benthiques, des macrophytes aquatiques et des poissons a été complètement perturbée à cause de l'abaissement du pH et de la forte concentration de cuivre et de nickel.

Les résultats sont analysés en vue de la protection de la rivière Onaping. Des méthodes concrètes de traitement des eaux usées sont proposées. Des indications de la présente étude laissent à penser qu'un circuit de traitement à bassins multiples constituerait une méthode efficace de traitement des eaux usées, qui contiennent de fortes concentrations de métaux et de composés soufrés partiellement oxydés.

ECOLOGICAL EFFECTS OF LIQUID MINING EFFLUENTS ON THE ONAPING RIVER SYSTEM IN ONTARIO

P.M. Bolger. Inco Metals Co.

Summary - The Onaping River, located near Sudbury, Ontario, receives liquid effluents from the mining community at Levack. The present study was undertaken in 1977 to identify the sources and characteristics of effluent discharges and to discover the effects of these effluents on organisms inhabiting the river system.

Ten physicochemical studies were carried out during 1977 at 18 sampling locations along the Onaping River system and its tributary streams. Benthic macroinvertebrates were collected on two occasions in June and in September, fish were collected in July, and the aquatic macrophytes were collected in September. The macroinvertebrates were collected with a surber sampler and a hand held dip net, fish specimens were collected using a seine net and electro-shocking equipment, and aquatic macrophytes were collected by wading along the shoreline.

Results of physicochemical surveys showed that metals and partially-oxidized sulphur compounds were the major contaminants in mining effluents. Nickel levels as high as 1.08 mg/l and copper as high as 0.18 mg/l were found in the Onaping River downstream of Levack. The oxidation of sulphur compounds to sulphates caused severe reductions in levels of pH and dissolved oxygen in the Onaping River. In the most severe instance, in July 1977, the pH of the Onaping River was lowered from 6.8 to 3.5 and the dissolved oxygen from 8.9 mg/l to 3.0 mg/l.

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Results of biological surveys showed that the aquatic life in the Onaping River was severely affected by mining effluent discharges. The distribution of benthic macroinvertebrates, aquatic macrophytes and fish was completely altered by conditions of low pH and elevated levels of copper and nickel.

Results are discussed in regard to minimizing future impairment of the Onaping River. Practical methods of wastewater treatment are presented. Evidence from the present study suggests that a "multi-pond" treatment system is an effective way of treating wastewaters which contain elevated levels of partially-oxidized sulphur compounds and metals.

ECOLOGICAL EFFECTS OF LIQUID MINING EFFLUENTS ON THE ONAPING RIVER SYSTEM IN ONTARIO

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1.0 INTRODUCTION

Considerable conflict exists between the development of mineral resources and the preservation of the nearby aquatic environment. During the early development stages of mining centres in Canada, a very low priority was given to environmental protection. Today, these attitudes have changed. Government, industry, and the public have become more aware of the potential harmful effects of mining activities, and a greater priority is being placed on minimizing the environmental impact.

In the Levack area, attitudes and priorities have also changed. Exploration and development of mineral resources at Levack began nearly a century ago, and for many years untreated mining effluents were discharged to the Onaping River. Treatment systems for the Levack effluents were built during the 1950's and 1960's, but they did not totally eliminate contamination of the Onaping River.

During the 1970's, it was recognized that improved wastewater treatment systems were required at Levack. Before adequate treatment could be developed, it was essential that the mining companies obtain a good understanding of the problems. Thus, the present study was undertaken in 1977 to define the existing conditions of water quality in the Onaping River as well as the sources and effects of contaminants. The project was sponsored by Inco Metals Company and was carried out as a Masters thesis project through Laurentian University of Sudbury (1). Assistance was obtained from Falconbridge Nickel Mines Limited, which also has operations in the Levack area, and from the Ontario Ministry of the Environment.

2.0 METHODS

2.1 Description of the Study Area

The Onaping River is located in northern Ontario, approximately 25 km northwest of the City of Sudbury. The Onaping is a fast flowing river which has a mean flow of 8.9 m^3/sec and a watershed area of 697 km^2 (2). The flow of water is continuous for 67 km, and is not interrupted by lakes.

The river flows past the nickel-copper mining community of Levack where nine mines and four concentrator plants have been developed. Operations are carried out by Inco Metals Company and Falconbridge Nickel Mines Limited.

2.2 Sampling Locations

During 1977, 18 sampling stations were established. Six of the stations were located along the Onaping River (O_1 and O_2 were upstream of Levack and O_3, O_4, O_5 and O_6 were downstream). The other twelve stations were located along major tributary streams in the Levack area.

2.3 Physicochemical Surveys

Ten physicochemical surveys of the Onaping River systems were carried out between February and November of 1977. Measurements were taken in the field for oxygen, temperature and pH, and water samples were collected for analyses by Inco's Environmental Laboratory. Many experiments were carried out to identify problems caused by acid-generating substances.

2.4 Benthic Macroinvertebrate Surveys

Benthic macroinvertebrate samples were collected in the Onaping River and its tributary streams in June and in September. Organisms were collected in riffle area habitats using a Surber square-foot stream-bottom sampler. Organisms were also collected in a wide variety of other habitats using a hand-held dip net (grab sample). A total of 115 macro-invertebrate samples were collected during 1977.

2.5 Aquatic Macrophytes

Aquatic macrophytes were collected from the Onaping River and its tributary streams in September, in areas which had a depth of less than one metre.

2.6 Fish

Fish were collected from the Onaping River in July using a seine net and electro-shocking equipment.

3.0 RESULTS AND DISCUSSION

3.1 Physicochemical Surveys

The Onaping River contained very high levels of contaminants as a result of mining effluent discharges from Levack. The major contaminants were metals and acidity (nickel and copper levels were as high as 1.08 and 0.18 mg/l respectively; pH levels were as low as 3.5). Elevated levels of sulphates, chlorides, hardness, dissolved solids and conductivity were also observed in the river downstream of Levack (Table 1). The typical pattern of contamination in the river is illustrated for nickel in Figure 1.

The increase in acid levels in the Onaping River did not occur immediately downstream of Levack (Figure 2). Instead, the pH of the river decreased gradually at increasing distances downstream (Figure 3). Depression of pH in the Onaping River occurred as a result of the discharge of partially-oxidized sulphur compounds from Levack. A distance of a few kilometres was required for the complete oxidation of the compounds to sulphuric acid. The specific source of the partially-oxidized sulphur compounds was the effluent from the Levack Tailings Pond. In July, this effluent had partially-oxidized sulphur levels of 590 mg/l and a chemical oxygen demand of 348 mg/l. The effluent, when stored in the laboratory, underwent a pH change from 6.4 to 2.2, which showed that it was capable of approximately a 16,000 times increase in acidity.

Depressed oxygen levels were found in the Onaping River as a result of the tremendous demand for oxygen caused by the sulphur-oxidation processes. For example, in July, the oxygen level in the river was lowered from 8.9 to 3.0 mg/l.

Table 1. Physicochemical conditions of water quality in the Onaping River at locations upstream and downstream of Levack.

Physicochemical Aspects of Water Quality	ONAPING RIVER UPSTREAM OF LEVACK			ONAPING RIVER DOWNSTREAM OF LEVACK		
	Maximum	Mean ± S.E.	Number of Determin-ations	Maximum	Mean ± S.E.	Number of Determin-ations
Field H^+ µeq/l (pH)	1.00 (6.0)	0.45 ±0.07 (6.3)	15	316.00 (3.5)	27.5 ±11.7 (4.6)	40
Nickel mg/l	0.009	0.004±0.001	15	1.08	0.286±0.040	40
Copper mg/l	0.014	0.004±0.001	15	0.18	0.029±0.006	40
Iron mg/l	0.27	0.172±0.04	6	1.15	0.458±0.073	16
Zinc mg/l	0.012	0.01 -	4	0.026	0.015±0.001	16
Chlorides mg/l	8	4±1	6	38	15±2	16
Dissolved Sulphates mg/l	16	14±1	15	140	67±5	40
Conductivity µmhos/cm or µS/cm	52	41±2	14	358	162±13	40
Dissolved Solids mg/l	116	70±10	6	240	183±10	16
Hardness mg/l	60	33±6	6	230	110±12	16

± S.E. = Standard Error

Figure 1. Nickel levels in the Onaping River upstream and downstream of the Levack Mining Centre. Results are mean levels ± the standard error.

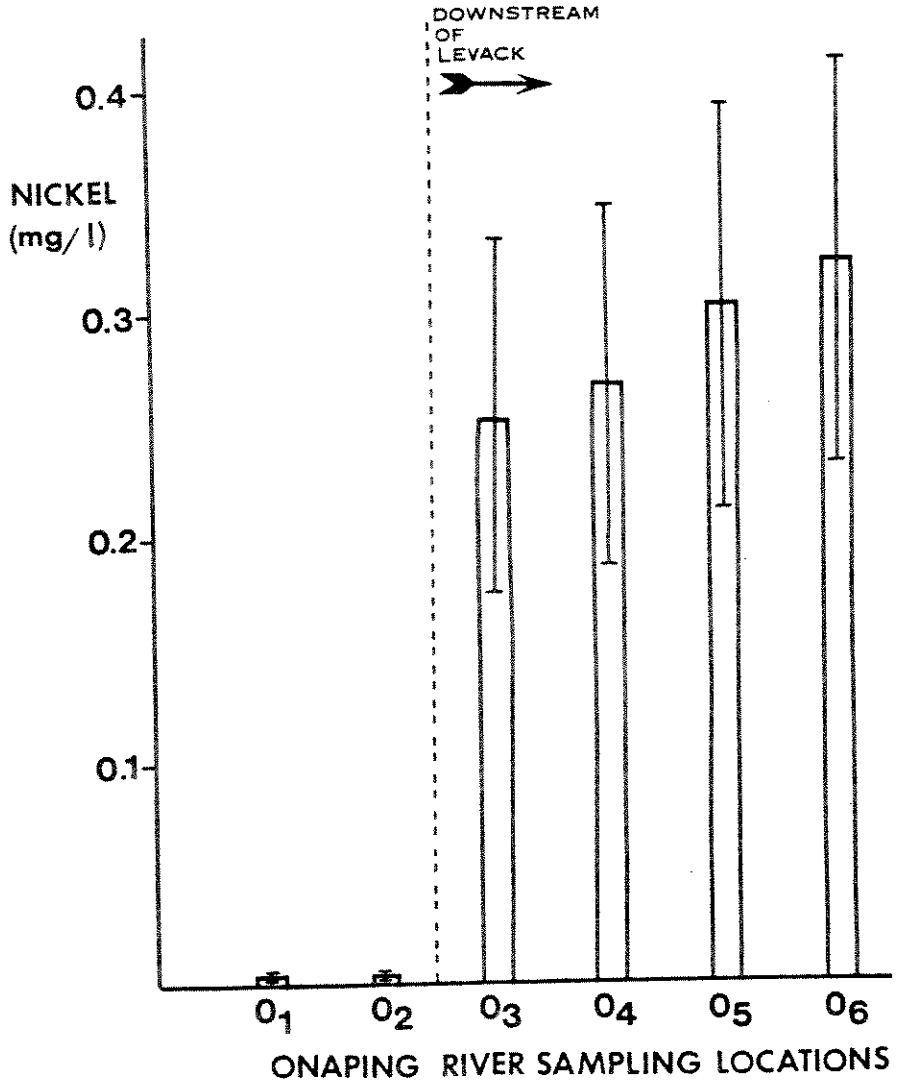


Figure 2. Hydrogen-ion activity and pH results in the Onaping River upstream and downstream of the Levack Mining Centre. Bars show mean levels of hydrogen-ion activity ± the standard error. Numbers in brackets refer to the pH.

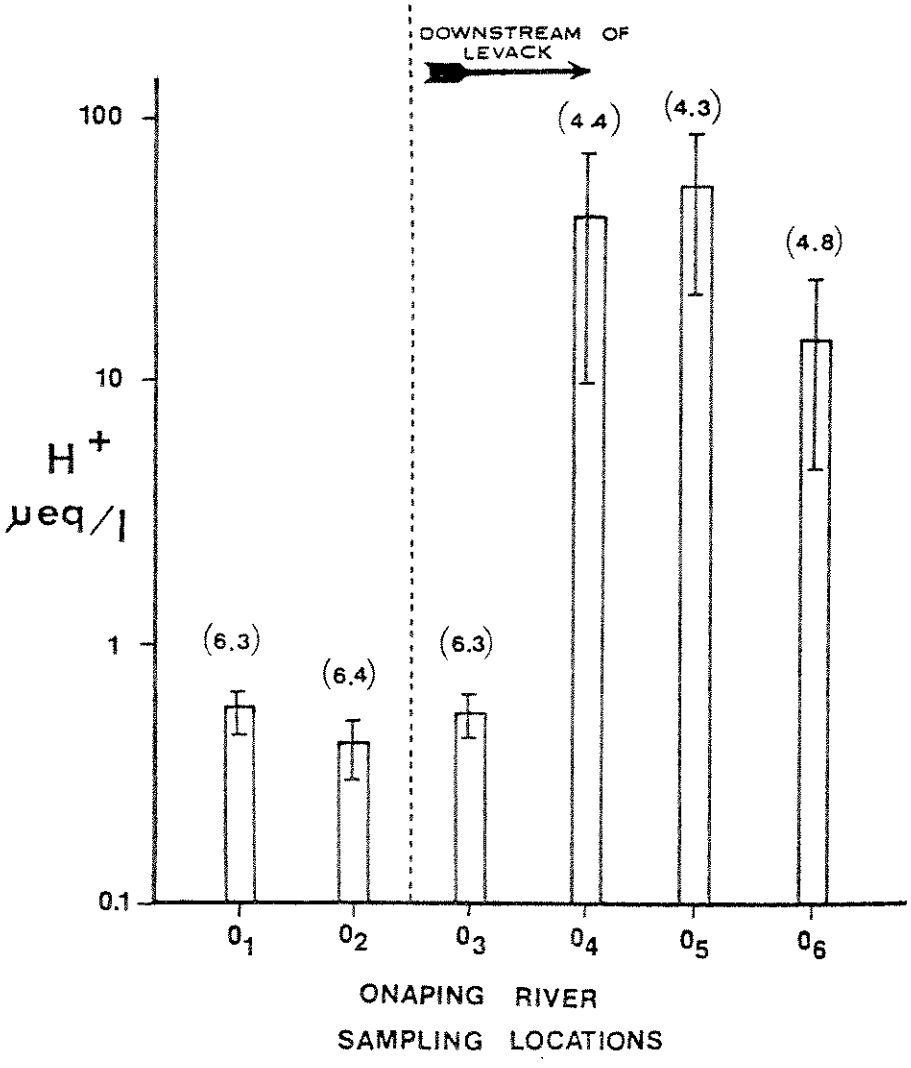
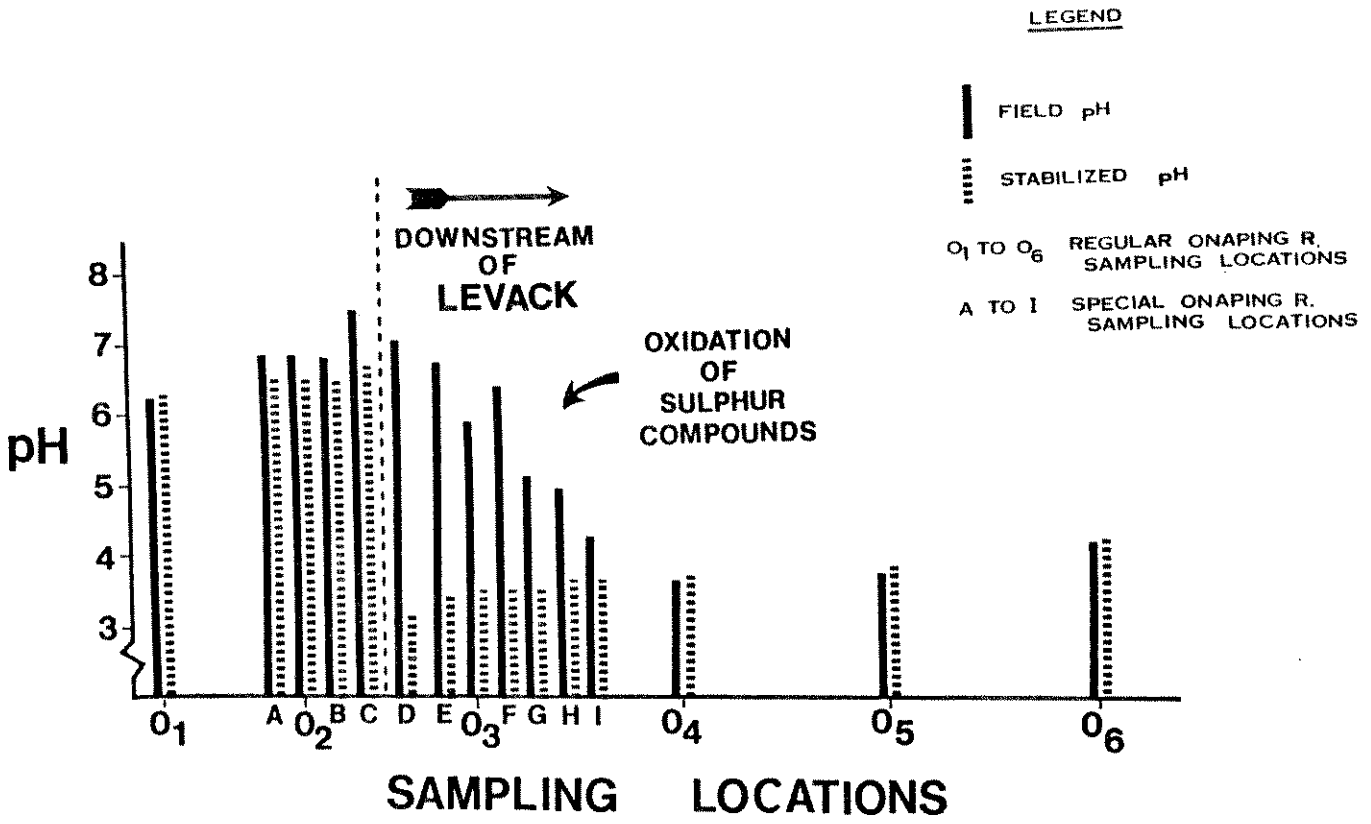


Figure 3. Field and stabilized pH for water samples collected from the Onaping River on July 18, 1977.



3.2 Benthic Macroinvertebrate Surveys

Mining effluents caused many changes in the community structure of benthic macroinvertebrate organisms. A distinct reduction in diversity and numbers of taxa was found in the areas of the Onaping River downstream of Levack (Figures 4 and 5). Sampling stations affected by mining effluents were dominated by dipteran and oligochaete organisms, whereas the unaffected locations were dominated by molluscs, caddisflies and mayflies.

Three distinct types of communities were found from the surveys of benthic macroinvertebrates, i.e.,

Community #1 (Natural Locations)

-A natural community in the Onaping River which developed in the absence of mining effluents.

Community #2 (Moderately Contaminated Locations)

-A community which developed in the Onaping River in the presence of elevated levels of nickel and copper and reduced levels of pH and oxygen.

Community #3 (Severely Contaminated Locations)

-A community which developed in effluent streams in the presence of elevated levels of nickel and copper, and reduced levels of pH and oxygen.

Results in Table 2 show that organisms which were sensitive to acid or metal contamination were partially or completely eliminated at the contaminated stream locations. Table 3 lists the extreme levels of pH, oxygen, nickel and copper which were responsible for the differences in community types.

3.3 Aquatic Macrophytes

Mining effluents severely affected the distribution of aquatic macrophytes. The numbers of macrophyte species found at sampling stations along the Onaping River and its tributary streams are shown

Figure 4. Diversity of benthic macroinvertebrates found in the Onaping River during June of 1977.

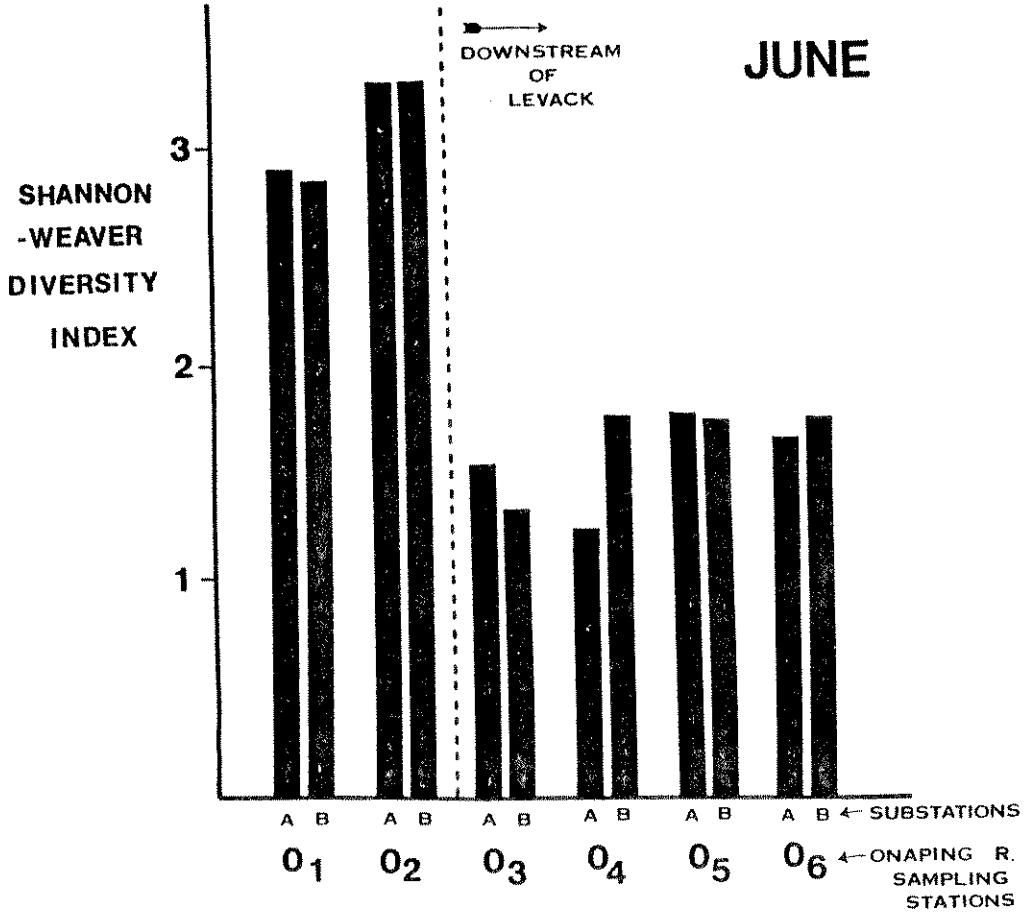


Figure 5. Total number of taxa of benthic macroinvertebrates found in the Onaping River during June of 1977. (Results are from 3 Surber samples and 1 grab sample/substation.)

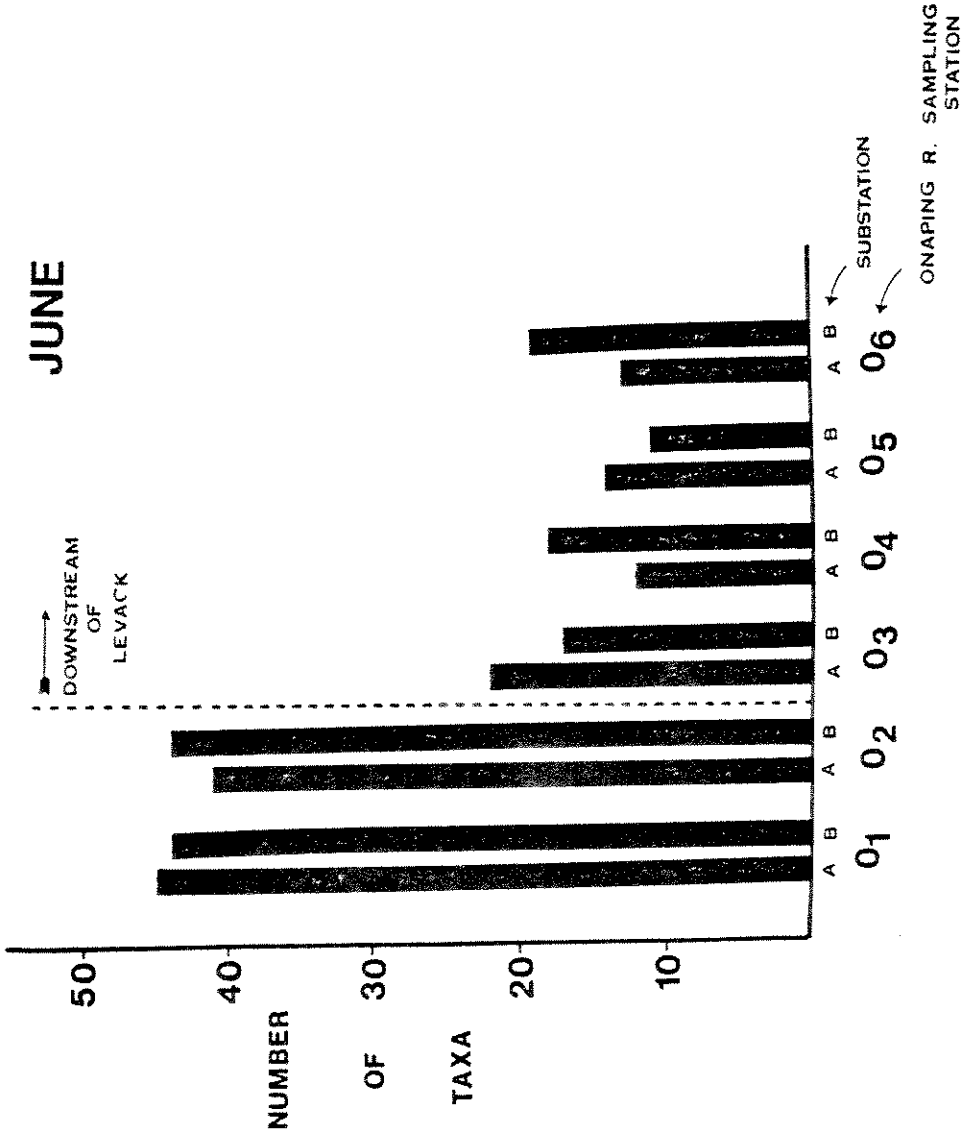


Table 2. The three community types of benthic macroinvertebrates found throughout the Onaping River and tributary streams showing the numbers of sensitive taxa.

COMMUNITY TYPE	NUMBER OF TAXA OF ORGANISMS BELONGING TO GROUPS SENSITIVE TO pH AND METAL CONTAMINATION					
	MOLLUSCA	HIRUDINEA	EPHEMEROPTERA	PLECOPTERA	TRICHOPTERA	TOTAL
Community #1 (Natural Locations)	6	6	15	5	17	49
Community #2 (Moderately Contaminated Locations)	Absent	Absent	3	1	9	13
Community #3 (Severely Contaminated Locations)	Absent	Absent	Absent	Absent	Absent	Absent

Table 3. Extreme levels of pH, oxygen, nickel, and copper at locations supporting the different community types found in the Onaping River system.

COMMUNITY TYPE	Minimum pH	Minimum Oxygen Saturation	Maximum Nickel (mg/l)	Maximum Copper (mg/l)
Community #1 (Natural Locations)	6.0	89%	0.009	0.014
Community #2 (Moderately Contaminated Locations)	3.5	38%	1.01	0.18
Community #3 (Severely Contaminated Locations)	3.9	43%	19.9	1.09

in Figure 6. Only five species of macrophytes were found in the areas of the river contaminated by metals and low pH, compared to 13 in the uncontaminated waters upstream of Levack.

3.4 Fish

Seven species of fish were found from surveys of the Onaping River. Two of these species, the white sucker (Catostomus commersoni) and the brook stickleback (Culaea inconstans) were very abundant at the contaminated river stations (Figure 7), and appeared to be attracted there because of the increased hardness and different invertebrate food source.

A fish kill was observed in July when the pH of the river was lowered to 3.5 and the oxygen level to 3.0 mg/l.

4.0 REMEDIAL MEASURES

The 1977 aquatic monitoring program identified the sources and nature of mining-effluent contamination in the Levack area as well as the impact of these effluents on the aquatic biota. To minimize contamination in the future, a different approach to wastewater treatment is required.

A 'single' tailings pond has traditionally been used to treat mill wastewater. Unfortunately, the pH conditions required to efficiently oxidize sulphur compounds (<4) conflict with the pH conditions required to precipitate metals (>10). Thus, adequate treatment of tailings wastewaters is difficult in a 'one-pond' treatment system.

A 'multi-pond' system can provide effective treatment of tailings wastewaters by providing optimum conditions for all aspects of treatment. A series of at least three ponds are required (Figure 8). The first pond in the series (The Tailings Pond) should have a short but adequate retention time for removal of the suspended solids. The second pond (The Oxidation Pond) should have a low pH in the range of 3 to 4, and a large retention time of approximately one year. The one-year retention time is required because of the very slow rate of sulphur oxidation which occurs during

Figure 6. Number of aquatic macrophyte species found in the Onaping River and its tributary streams during 1977.

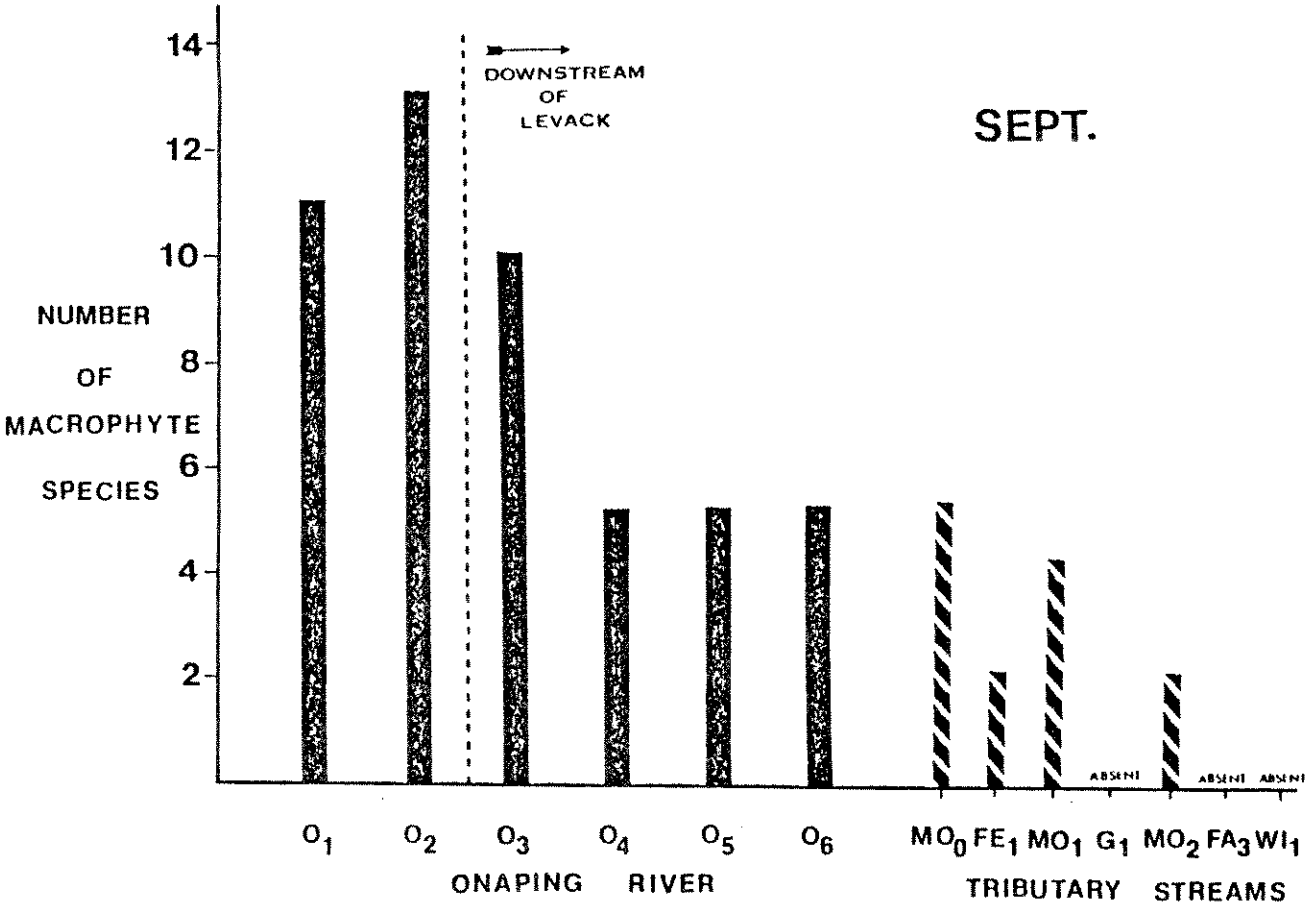
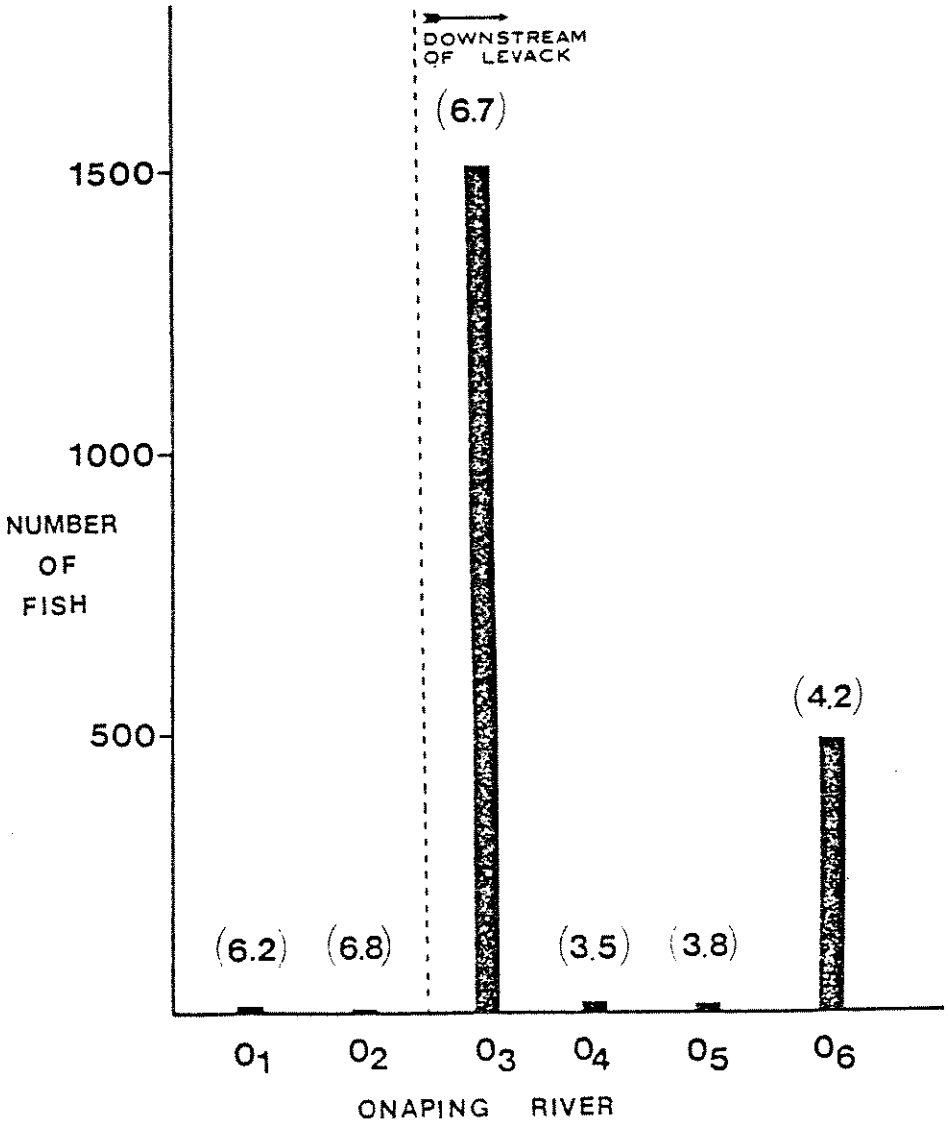


Figure 7. Numbers of fish and pH results collected at the six Onaping River sampling stations during July of 1977. Numbers in brackets refer to the pH.



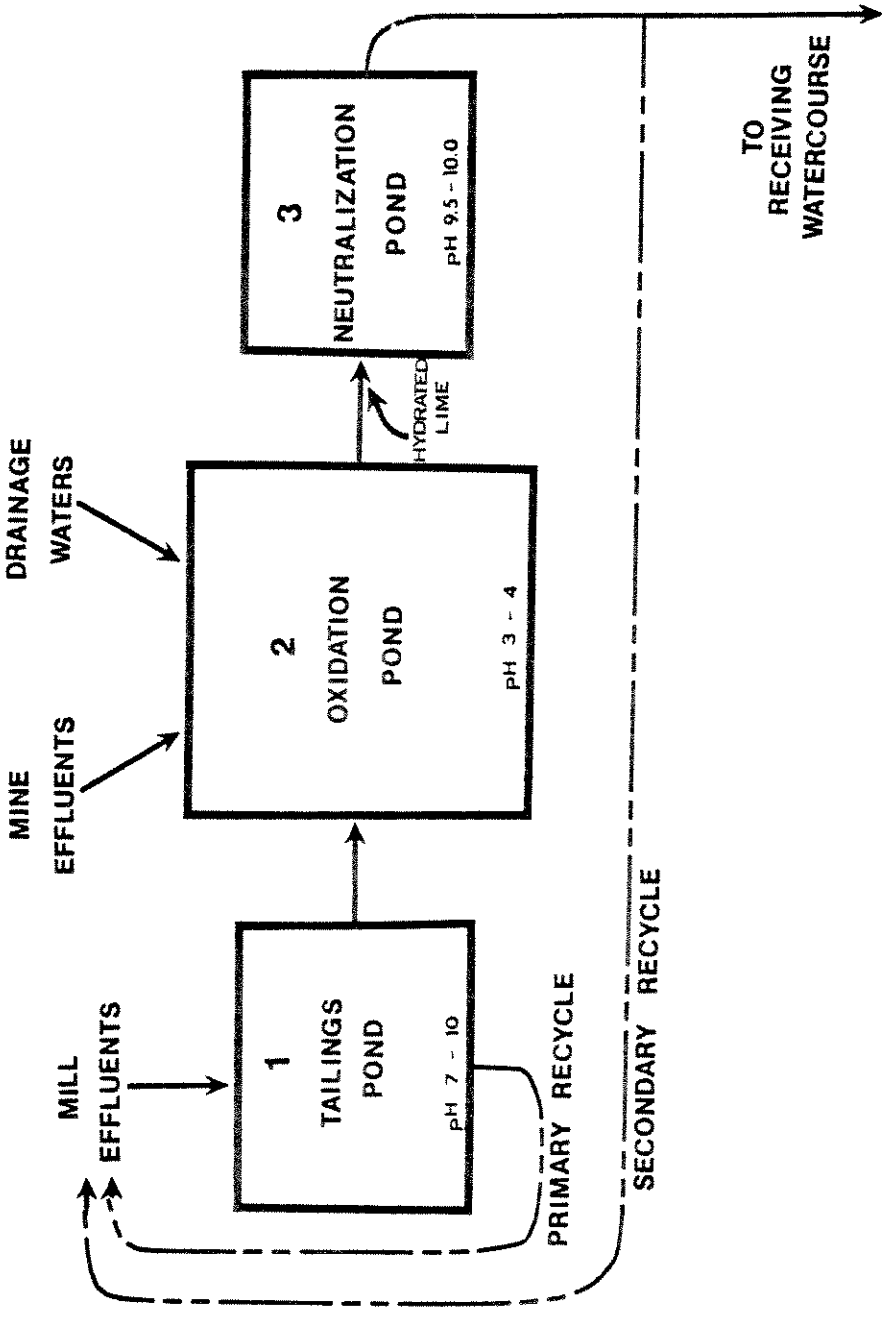


Figure 8. A multi-pond system for treating tailings wastewaters containing elevated levels of partially-oxidized sulphur compounds and metals.

winter months. In the third pond (The Neutralization Pond), hydrated lime must be added to raise the pH to approximately 10 for precipitation of metals.

A 'multi-pond' treatment system similar to that described above, has been successfully operated in the Levack area since 1970[3].

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1 Bolger, P.M. 1980. Ecological Effects of Liquid Mining Effluents on the Onaping River System in Ontario. Master Thesis Project, Laurentian University, Sudbury, Ontario.

2 Water Surveys of Canada, 1976. Historical Stream Flow Summary - Ontario. Publication of Fisheries and Environment Canada.

3 Rivett, L. S. and U. M. Oko. 1971. Tailings disposal, generation of acidity from pyrrhotite and limestone neutralization of wastewater at Falconbridge's Onaping Mines. Can. Mining and Met. Bull. Aug.: 108-113.

ÉVALUATION DU MICROTOX® COMME MOYEN DE DÉTECTION D'AGENTS TOXIQUES INDUSTRIELS DANS L'EAU

Q.M. Samak and R. Noiseux
Union Carbide Canada Limited

RÉSUMÉ - L'exposé va porter sur la réaction de bactéries luminescentes, employées selon la technique Microtox, avec des substances toxiques dans l'eau. Les points suivants seront considérés:

1) La réponse bactérienne à des substances toxiques organiques, inorganiques ou du type des hydrocarbures dans des solutions à un seul soluté, de même que la reproductivité des résultats.

2) La réponse bactérienne à des effluents liquides réels.

3) Parallèle entre les réponses bactériennes et celles des truites et des dards-perches (CL_{50}).

4) Quelques propositions concernant la réduction des données obtenues selon la technique Microtox.

EVALUATION OF THE MICROTOX® AS A TOOL FOR MONITORING AQUATIC TOXICITY OF INDUSTRIAL ORIGIN

Q.M. Samak and R. Noiseux
Union Carbide Canada Limited

SUMMARY - The communication will address the response of luminiscent bacteria, used in the Microtox technique, to chemical toxicants in water. The presentation will cover the following aspect:

1) The bacterial response to toxicants of hydrocarbon, inorganic and organic nature contained in single component solutions as well as the reproducibility of results.

2) Bacterial response to actual wastewater effluents.

3) Correlation of the bacterial response and those obtained from trout and zebra fish expressed as LC_{50}.

4) Suggestions on data reduction for the Microtox technique.

ACUTE AQUATIC TOXICITY MEASUREMENT BY THE BECKMAN MICROTOX

BY:

Q.M. SAMAK

R. NOISEUX

UNION CARBIDE CANADA LIMITED

PLASTICS AND CHEMICALS

MONTREAL, QUEBEC

PRESENTED AT THE SEVENTH ANNUAL AQUATIC TOXICITY WORKSHOP IN
MONTREAL, CANADA
NOVEMBER 1980

BY: L. HAMEL

INTRODUCTION

The Microtox is an analytical apparatus developed by Beckman Instrument Inc. to offer a rapid method for assaying toxicity of aquatic samples either of wastewater or of general chemical origin. The principle of operation used in this method is the inhibitory effect experienced by certain strains of luminescent marine bacteria when exposed to a toxic medium. This inhibition is manifested in the attenuation of their light emission which is positively correlated with the concentration of toxicants present in the tested solution (1), (2). The degree of this attenuation is used to establish a scale of toxicity for the tested assays. This scale could presumably be correlated with established toxicity scales and parameters using higher organisms and approved by the regulatory authorities such as the LC_{50} parameter based on trout or fathead minnows.

The superiority of this method, if established, resides in the fact that while traditional toxicity tests on fish require as much as 96 hours to establish the statutory lethal concentration of an effluent, with the Microtox an equivalent parameter could be established in 20 or 30 minutes.

The Microtox has been under evaluation in the Environmental Laboratories of Union Carbide Canada for the last 9 months. This communication is reporting on the results of this evaluation.

EXPERIMENTAL

Following the testing procedure recommended by the manufacturer (3) and using the bacterium photobacterium phosphoreum supplied with the apparatus' reagents, an experimental plan composed of two stages was developed, namely:

1/ Testing the effect of simple solutions of common chemicals and toxicants on the Microtox.

2/ Examining the Microtox response to actual waste water samples of petrochemical origin and comparing this response with that obtained from testing the same waste water toxicity with fish. In the second stage of the experimental plan zebrafish (Branchydanio rerio) was used as a testing organism since it is the fish used for in-house monitoring of waste water quality by U.C.C. Correlation of the Microtox response with that of trout is currently in progress.

RESULTS

Fig. 1 shows a typical pattern of the characteristic light emission of Photobacterium phosphorium upon reconstitution from a freeze-dried state into a 2% saline solution. Fig. 2 shows the attentuation of light emission as the bacteria are challenged with a toxic medium with the degree of attentuation being dependent upon the toxicant's concentration. Fig. 3 shows plots of the Microtox response as a function of concentration for the shown toxicant according to the scheme of data reduction suggested by the manufacturer. In this scheme a parameter referred to as EC_{50} (analogous to LC_{50} in fish toxicity) is defined as the solution concentration which result in a 50% reduction in light emission in reference to a standard emission obtained from a toxicity-free saline solution. From Fig. 3 it is shown that EC_{50} for cyanide, is about 6.5 ppm; that of phenol is about 42 ppm while EC_{50} for sulphide is 13.5 ppm. Fig. 4 shows similar plots for some hydrocarbons and diethanolamine where naphthalene proves to be the most toxic with an EC_{50} of about 2 ppm obtained by extrapolation. Table 1 shows the EC_{50} values obtained for some of the simple solutions tested. In these results the EC_{50} values are evaluated at 15^0C and after 5 minutes from challenging the bacteria with the toxic medium.

The Microtox response was also tested using actual industrial waste water. The effluent tested, which has been found toxic most of the time to fish, is contributed to mainly by a hydrocarbon cracking unit. Two representative results of the Microtox's response to samples of that effluent are shown on Fig. 5 and 6.

Simultaneously, the same samples were tested with zebrafish to determine the toxicity level as expressed by LC_{50}. After 72 hours of fish testing during which the test solution is moderately aerated, the solution was tested again by the Microtox. Fig. 5 and 6 show the Microtox response to the effluent as obtained and after 72-hour aeration period during fish testing, the corresponding pollution parameters are also shown on the graphs together with the EC_{50} values as well as the corresponding LC_{50} obtained by testing with zebra fish.

DISCUSSION AND ANALYSIS

DATA REDUCTION:

Examination of Fig. 3 - 6 suggests that the semilogarithmic plots of concentration vs % light loss proposed by the Microtox manufacturers give reasonably linear plots. These lines would be represented by the general model:

$$\log Y = ax + b$$

where
- y = solution concentration
- x = % of light loss
- a = slope
- b = intercept

In these lines both constants "a" and "b" are inversely proportional to toxicity. Thus in Fig. 3 cyanide is the most toxic followed by sulphide then by phenol. Similarly in Fig. 5 and 6 the samples tested after aeration are less toxic than as collected. This reduction in toxicity is graphically represented by the increase in the intercept of the response with the concentration logarithmic scale; physically this reduced toxicity is attributed to the partial stripping and/or oxidation of some of the toxic components in the sample tested during an extended period of aeration.

When the response of the Microtox is treated mathematically as such and plotted on the vertical logarithmic scale against concentration as an independent variable on the linear horizontal scale, a different type of plot is obtained. Example of this plot is shown on Fig. 7 where a curvilinear response asymptotically approaching a limiting light loss value is obtained. This type of response is common in microbial inhibition

reactions, which is the case with the Microtox, as well as in surface phenomena such as adsorption. By analogy with adsorption a linearizable model was found to depict the non-linear response of the Microtox adequately; moreover it requires linear rather than logarthmic coordinate for linear graphical representation which renders data reduction simpler and more accurate. This model is an analogue of the Langmuir model of adsorption isotherms:

$$Y = \frac{K_1 C}{1 - K_2 C}$$

where Y = Surface concentration of an absorbate on a certain absorbent

C = Concentration of the absorbate in the solution in thermadynamic equilibrum with the absorbant

K_1 and K_2 = Constants particular to the system

If Y is taken as representing % light loss and C as the effluent concentration in ppm or percent and upon inverting the model the following linear relation is obtained:

$$\frac{1}{Y} = \frac{1}{K_1} \cdot \frac{1}{C} + \frac{K2}{K_1}$$

The data shown on Fig. 7 were replotted according to this model in Fig. 8 on linear coordinates. As shown accurate straight lines are obtained relating the reciprocal of % light to the reciprocal of the effluent concentration.

EFFECT OF pH:

The tested effluent was adjusted at different pH levels to study the effect of pH variation on the bacterial response of the Microtox; the range examined was PH 5 to 9. The obtained EC_{50} for the same effluent at the 5 values of pH covered by that range are shown in Fig. 9. According to this plot it is evident that the response of the Microtox is more or less stable between pH 5.5 and pH 8 for the effluent tested.

DEPENDENCE OF THE MICROTOX RESPONSE ON POLLUTION PARAMETER

The results of measurements of toxicity by the Microtox expressed as EC_{50} were analyzed in terms of pollution parameters by means of modelling with the multiple regression method. The parameters used were phenol concentration, TOC and ammonia. The analyzed data as well as corresponding results of toxicity testing on zebra fish expressed as LC_{50}, when available, are presented in Table 2.

Mathematical analysis shows the highest dependency of EC_{50} to be on phenol with a correlation coeff. of -0.714 followed by TOC with a weak dependency reflected in a correlation coeff. of -0.423. Ammonia, as expected gave a weak positive correlation coefficient of 0.357.

The model obtained from the analysis is as follows:

$$LC_{50} = 45.43 - 5.111 \text{ (phenol conc. in ppm)} - 0.0071 \text{ (TOC in ppm)} + 1.348 \text{ (ammonia conc. in ppm)}$$

(approx. $\pm$ 20%)

It should be noted that if toxicants like sulphide or cyanide were present in detectable quantities in the tested effluent, their contribution could have been detected by the model.

CORRELATION WITH LC_{50} OBTAINED FROM ZEBRA FISH:

The Microtox EC_{50} were correlated with the LC_{50} obtained from testing toxicity with zebrafish. A plot of the data from Table 2 is found in Fig. 10. The data analysis rendered a reasonable correlation coeff. of 0.884 between EC_{50} and LC_{50} for Zebra. The prediction model obtained is:

$$LC_{50} = 2.1459\ EC_{50} + 12.744 \quad \text{(approx. } \pm \text{ 7\%)}$$

Examination of Fig. 10 suggests that if the Microtox is to be used as a toxicity tool then a fish biassay is not required unless the EC_{50} registered on the Microtox is less than 50%. Naturally this threshold value will vary depending on the origin and nature of the waste water tested.

CONCLUSIONS:

Evaluation of the Microtox toxicity analyzer on real plant effluents has shown that the method is efficient as a toxicity measurement technique and is potentially usable as a screening tool augmented by fish bioassay.

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1 - Senat, F.W., F.E. Budinger, and P.K. Mueller, Journal of Bacteriology, Vol. 90, Sept. 1965, pp. 832-833

2 - Jordon, L., U.S. Patent 3.370.175, Feb. 20, 1968

3 - "Microtox Toxicity Analyzer" Interim Manual No. 110679,

Beckman Instruments Inc. Microbics Operations
Carlsbad, California

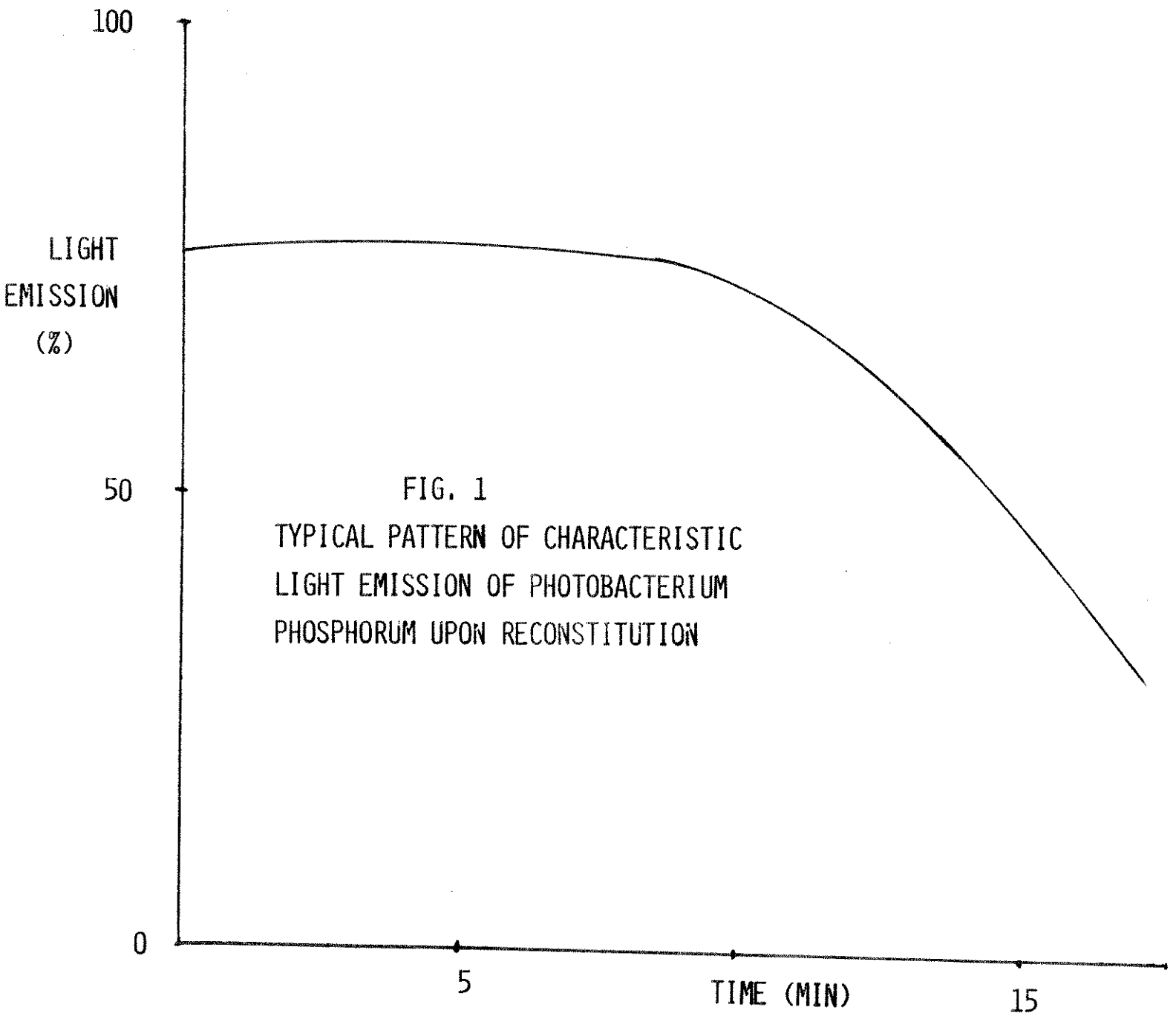
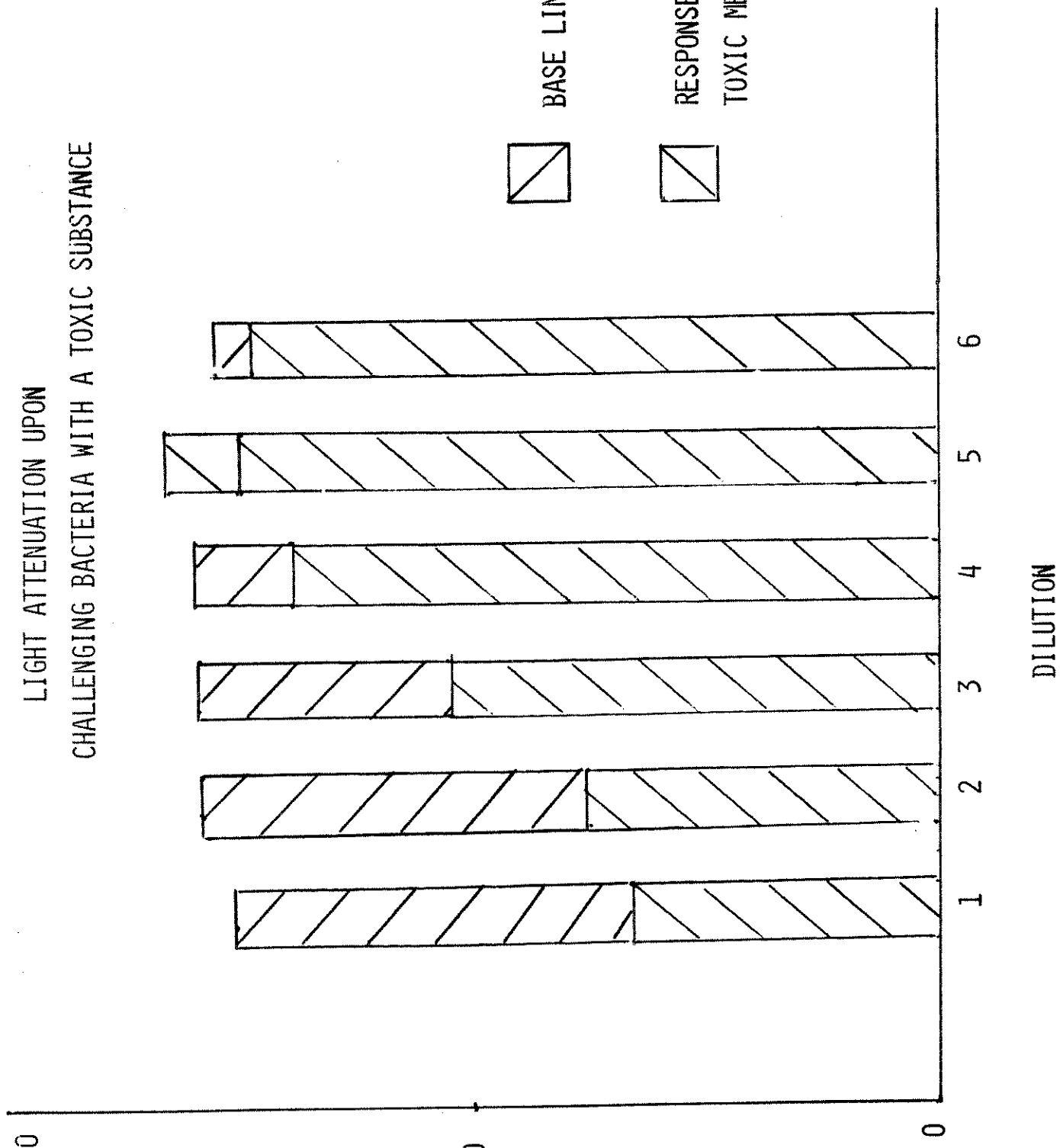


FIG. 1
TYPICAL PATTERN OF CHARACTERISTIC
LIGHT EMISSION OF PHOTOBACTERIUM
PHOSPHORUM UPON RECONSTITUTION

FIG. 2

LIGHT ATTENUATION UPON
CHALLENGING BACTERIA WITH A TOXIC SUBSTANCE



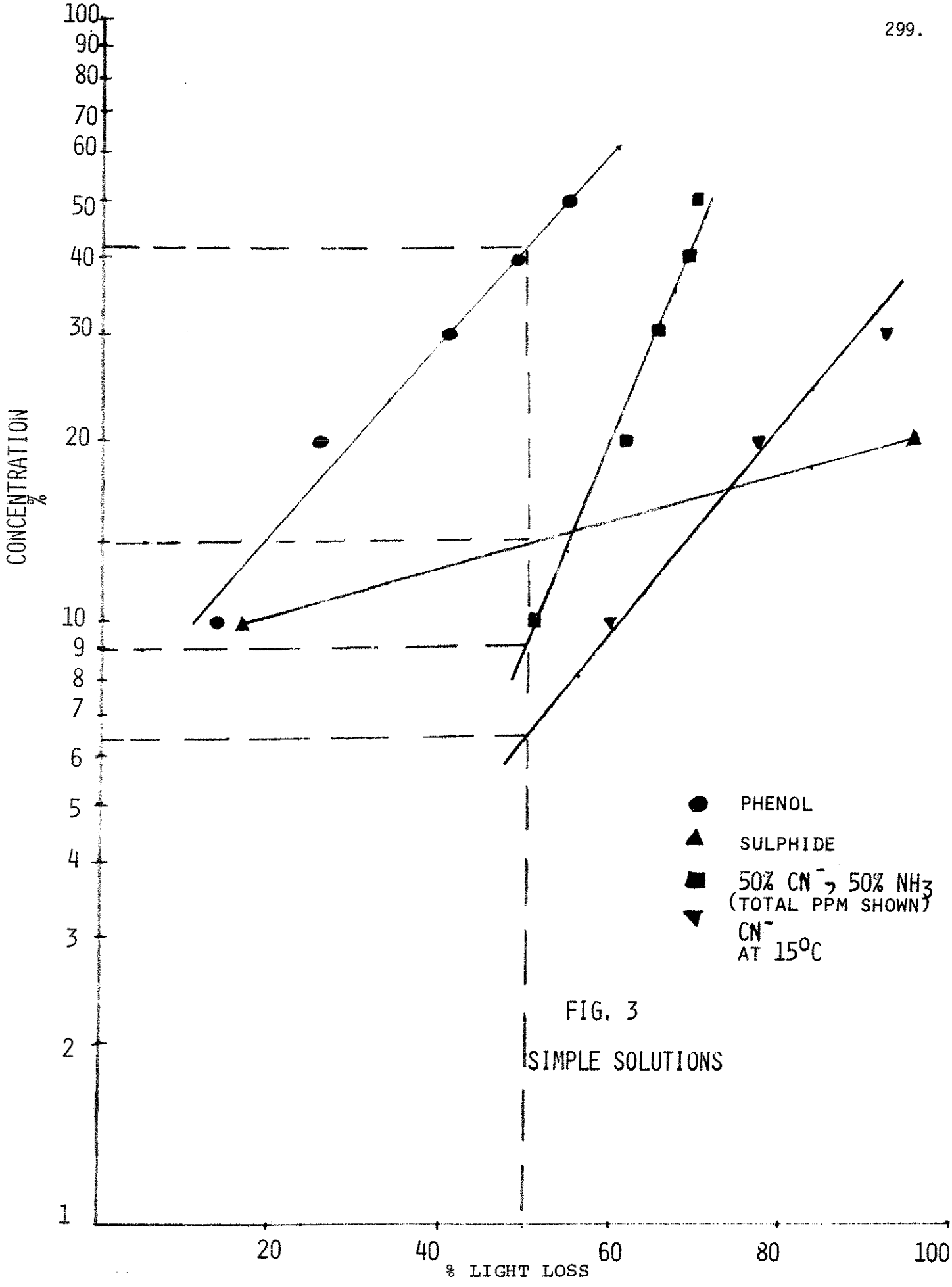


FIG. 3

SIMPLE SOLUTIONS

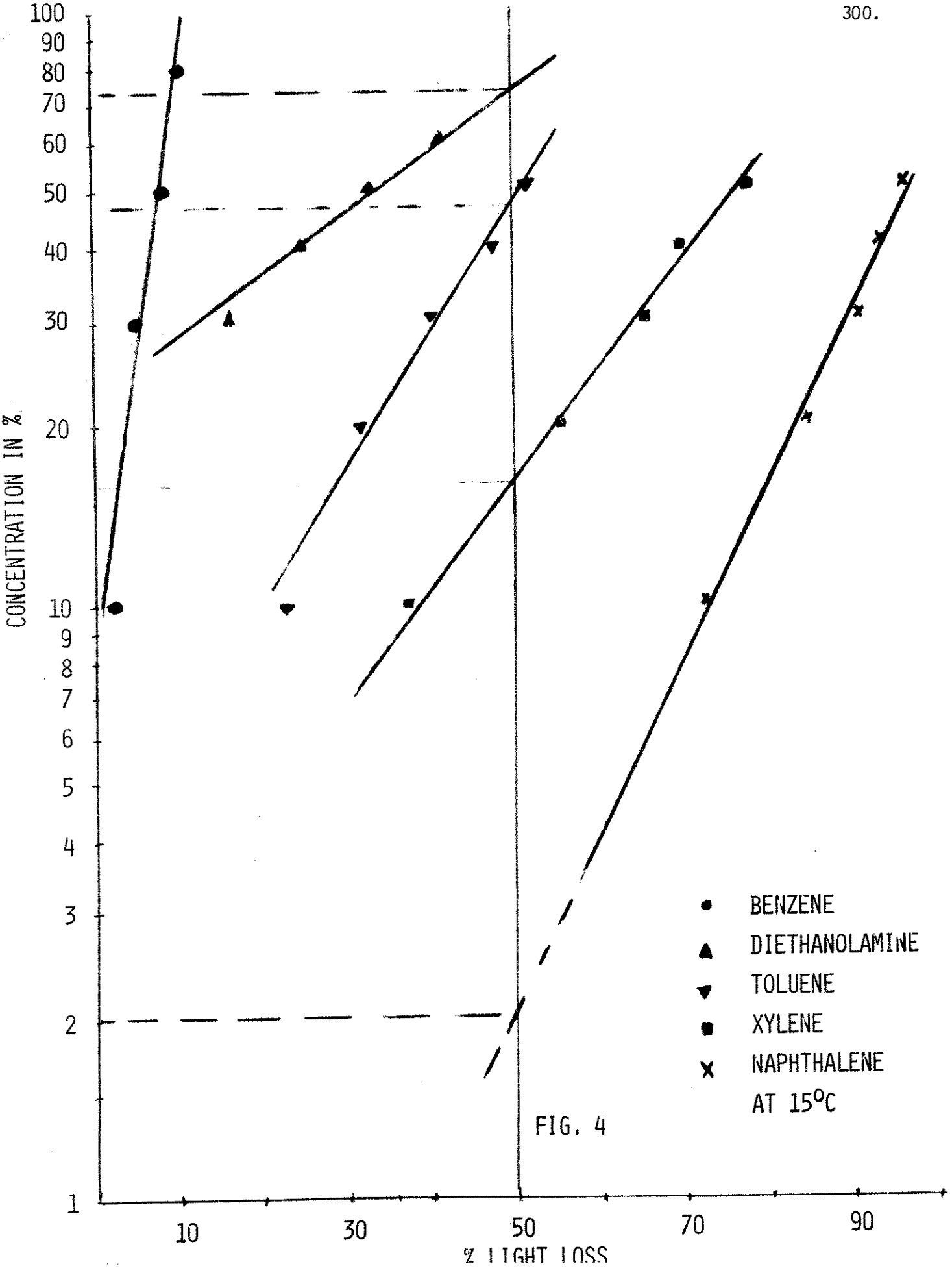

CONCENTRATION IN %
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
10
30
50
70
90
% LIGHT LOSS
BENZENE
DIETHANOLAMINE
TOLUENE
XYLENE
NAPHTHALENE
AT 15°C

FIG. 4

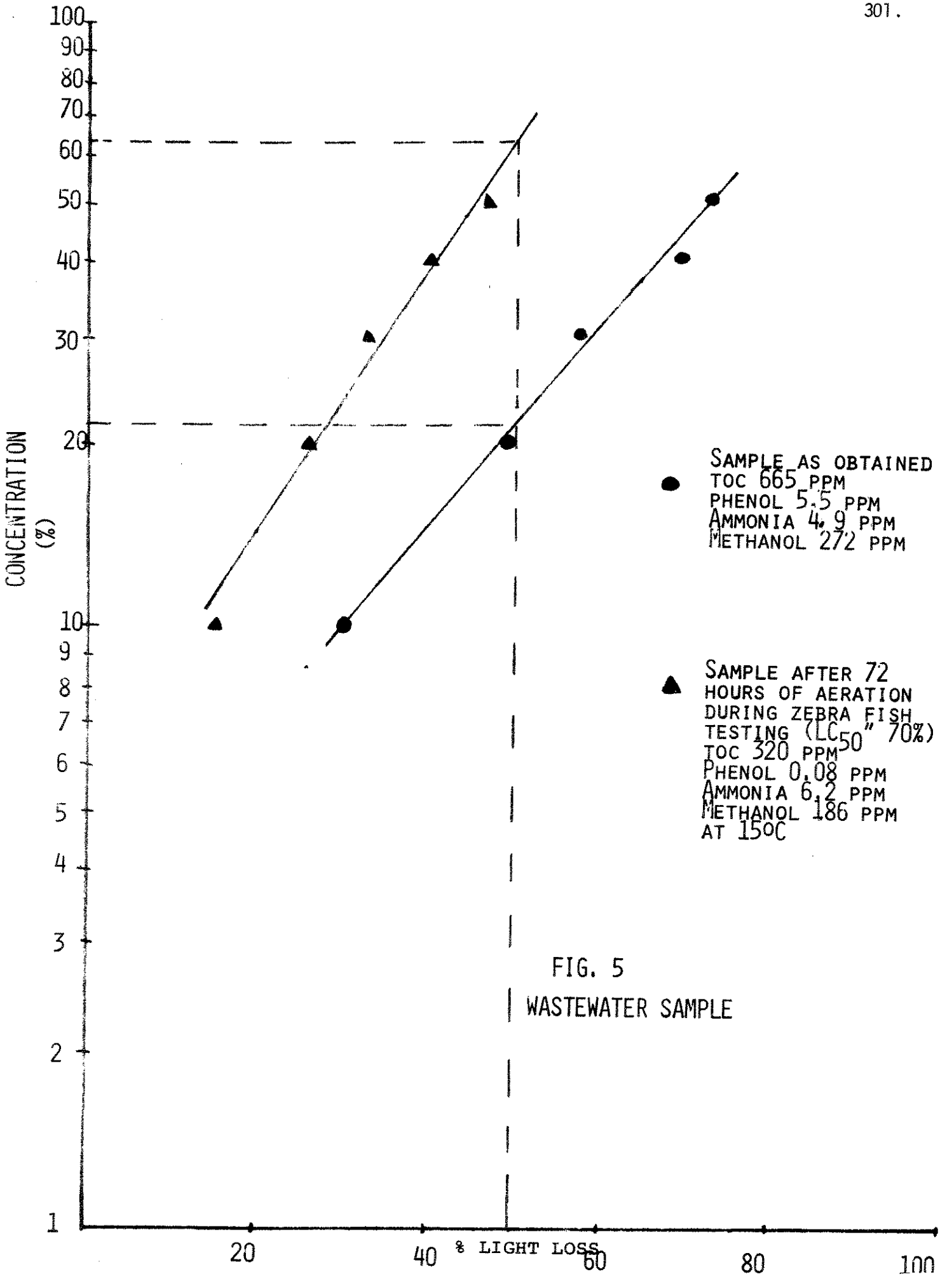


FIG. 5

WASTEWATER SAMPLE

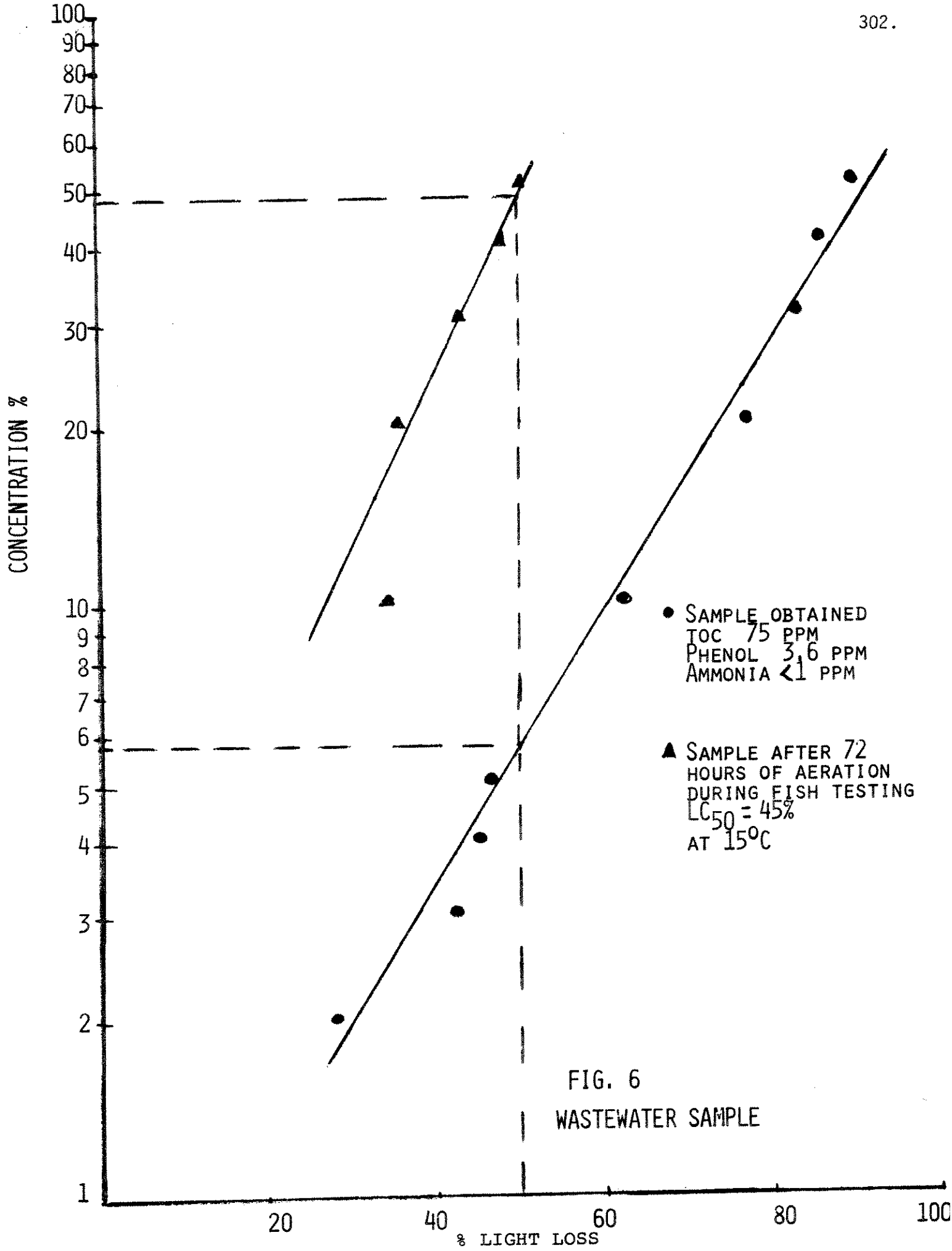


FIG. 6

WASTEWATER SAMPLE

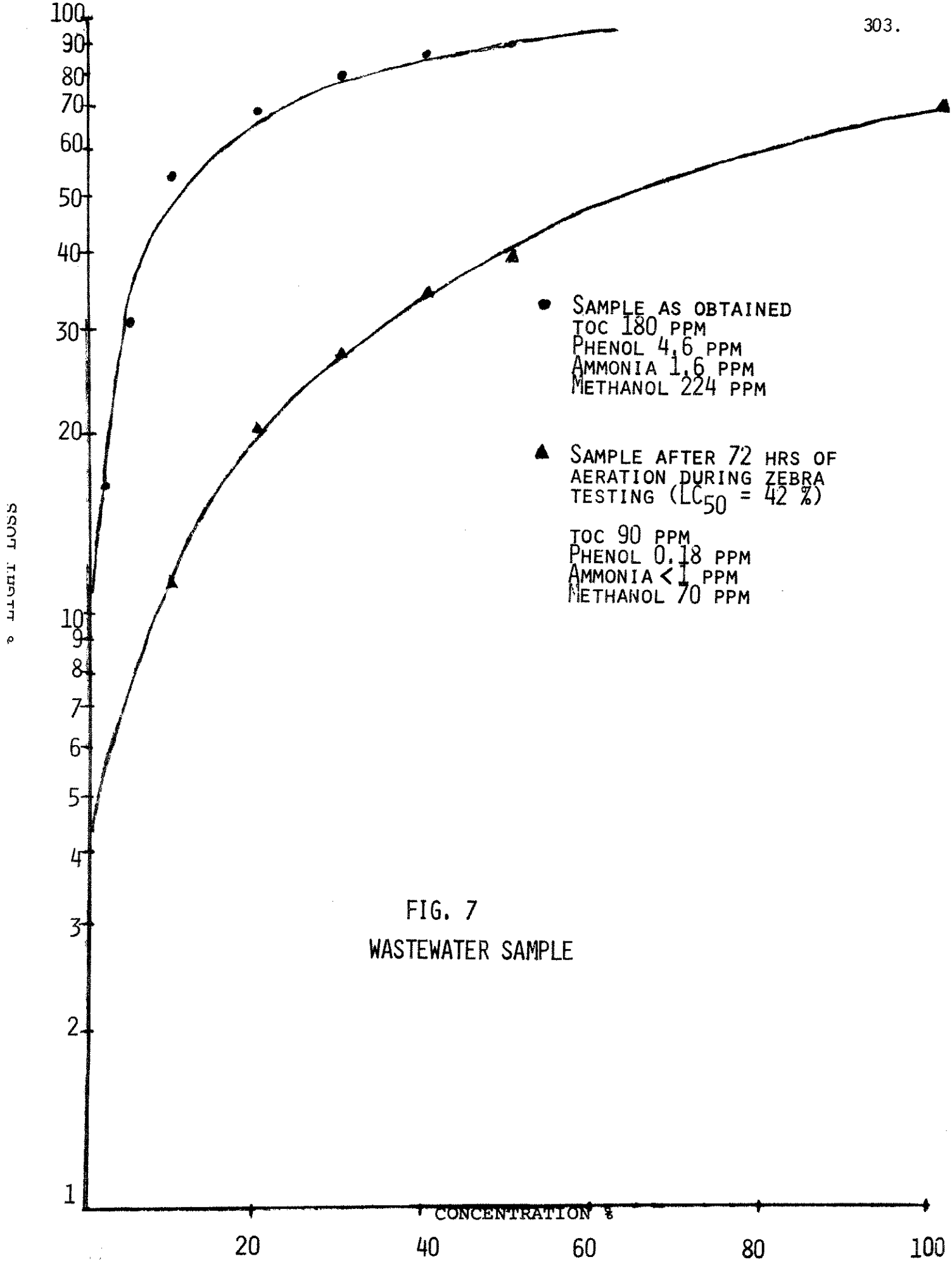


FIG. 7

WASTEWATER SAMPLE

FIG. 8
WASTE WATER SAMPLE

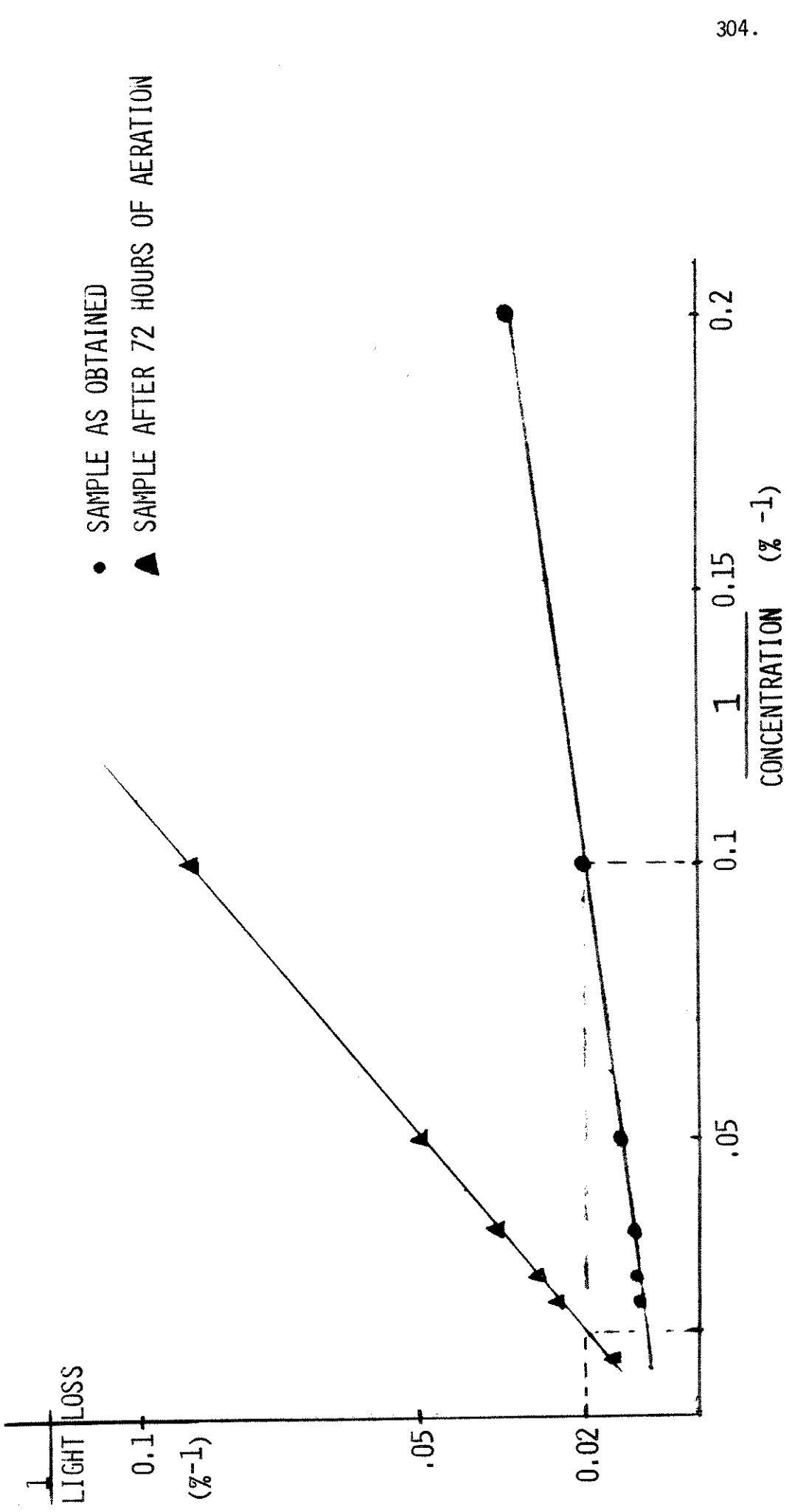
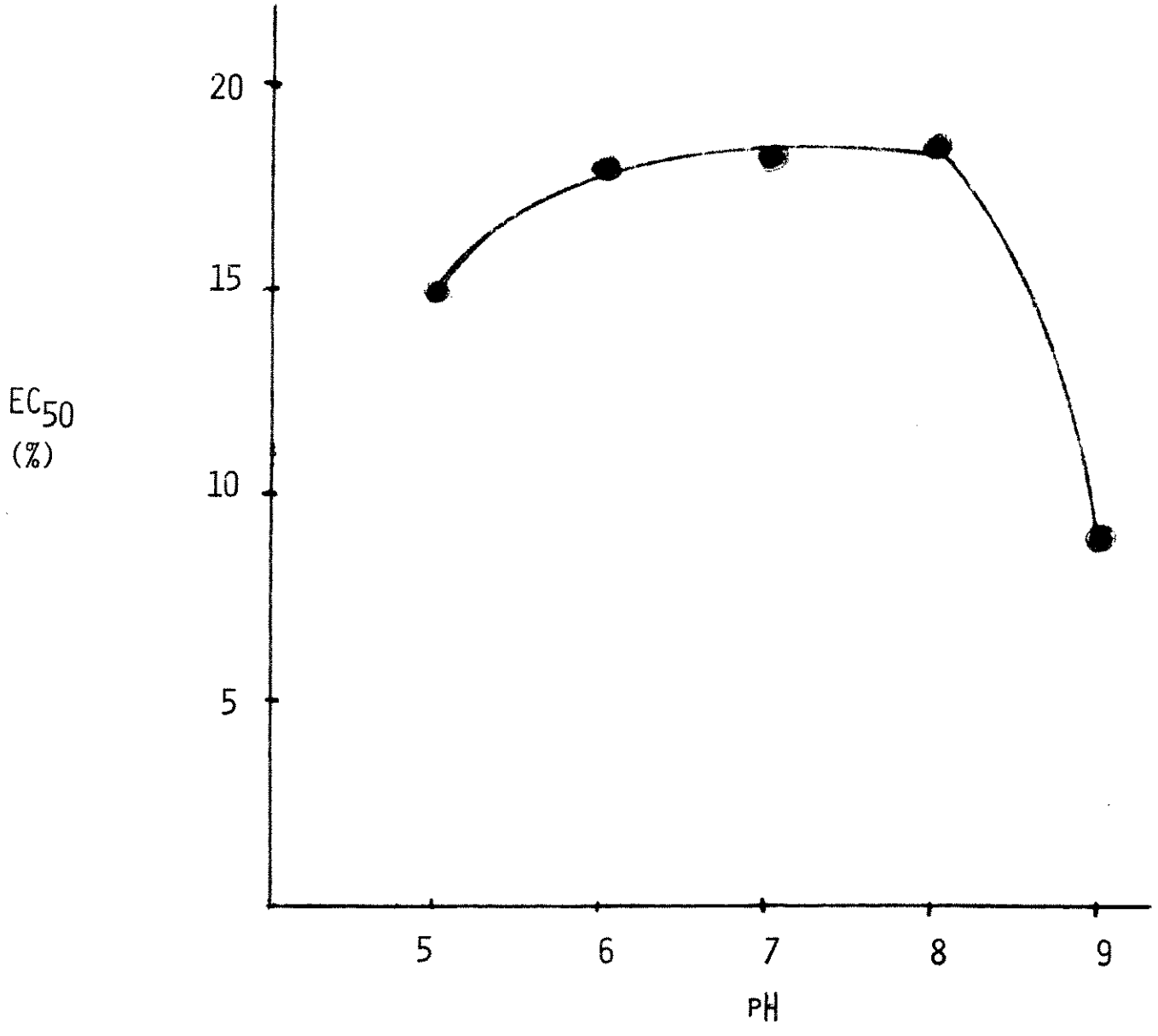


FIG. 9

EC_{50} VS. pH



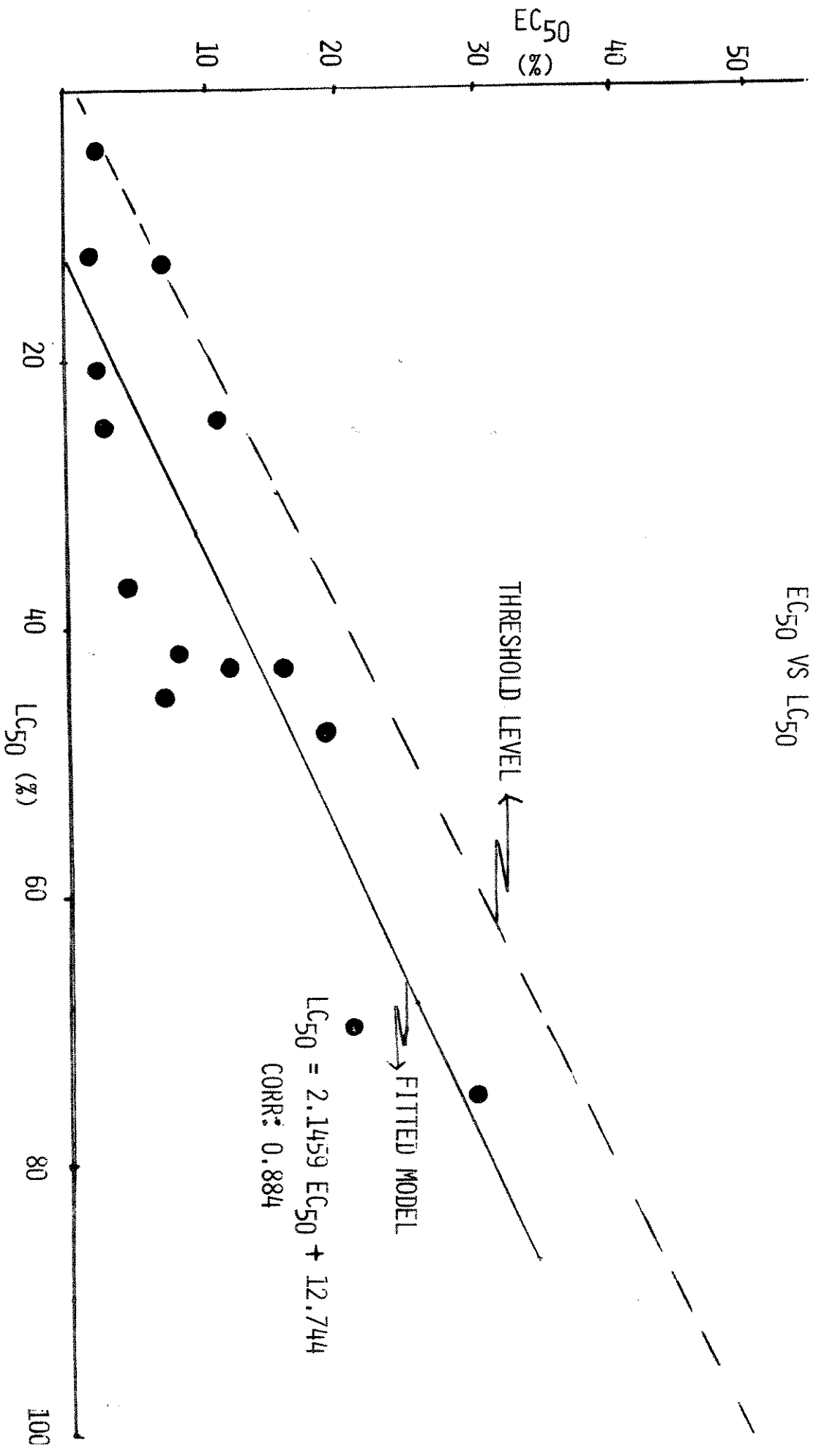


FIG. 10

EC_{50} VS LC_{50}

TABLE I

EC_{50} (5 min, 15°C) FOR COMPOUNDS TESTED

COMPOUND	CONCENTRATION (MAX)	EC_{50}
Isopropyl Alcohol	50 ppm	Non toxic
Methyl alcohol	50 ppm	Non toxic
Ammonia	100 ppm	Stimulatory
Naphthalene	50 ppm	2 ppm (extrapolation)
Xylene	50 ppm	16 ppm
Toluene	50 ppm	48 ppm
Diethanolamine	50 ppm	73 ppm (extrapolation)
Benzene	50 ppm	Non toxic up to 80 ppm
Ethylene Glycol	50 ppm	Non toxic
Triethanolamine	50 ppm	Stimulatory
Cyanide	50 ppm	6.5 ppm (extrapolation)
Sulphide	50 ppm	13 ppm
Phenol	50 ppm	42 ppm

TABLE II

MICROTOX DATA* ON QUENCH BLOWDOWN

TEST	CN^-	Phenol	TOC	NH_3	LC50	EC50
No. 1	.01	6.2	125	1.15	43%	16%
	.01	0.2	70	0	-	70%
No. 2 after aeration	.01	6.0	200	1.2	48%	19%
	.01	0.12	145	1.2	-	65%
No. 3 after aeration	.01	6.2	218	3.8	75%	30%
		.256	95	4.5	-	100 "extrapolation
No. 4	.01	7.0	585	1.0	13%	7%
No. 5	.01	9.8	400	1.0	21%	2%
No. 6	.01	7.2	1400	2.7	12.5%	1.5%
No. 7	.09	5.2	790	1.3	43%	12%
No. 8	.12	5.8	815	1.0	25%	2.5%
No. 9	.01	4.5	180	1.5	42%	8%
No. 10	.01	3.8	215	-	37%	4.5%
No. 11 after aeration	.01	5.5	665	4.9	70%	21%
	.01	0.08	280	0.2	-	53%
No. 12	.01	3.8	270		24.5%	11%
No. 13	.01	3.6	175		45%	7%
No. 14	.01	7.6	470	1.7	4.6%	2.0%

* all concentrations are in ppm

LA POLLUTION TOXIQUE DANS LES EAUX FRANÇAISES: CARACTÉRISATION A L'AIDE DE BIOESSAIS

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RÉSUMÉ - Le renforcement de la réglementation et la mise en place d'un système de redevances en France, ont nécessité de disposer de paramètres de contrôle des effluents et de toute substance chimique pouvant aboutir dans le milieu aquatique.

Il s'est avéré nécessaire de mettre au point des bioessais pour connaître, notamment, les effets toxiques létaux et sublétaux des substances vis-à-vis des organismes des écosystèmes aquatiques.

Actuellement, quatre niveaux trophiques ont été pour le choix des organismes tests:

- les bactéries
- les algues
- les crustacés
- les poissons.

Pour chacun de ces niveaux, plusieurs projets sont à l'étude en ce moment. Nous analyserons successivement les différentes méthodes faisant l'objet d'un projet de norme. Nous discuterons, entre autres, du choix des espèces étudiées ainsi que des différents critères de toxicité analysés.

TOXIC POLLUTION IN FRENCH WATERS: CHARACTERIZATION THROUGH BIOASSAYS

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SUMMARY - Making parameters for the control of effluents and of any chemical substance which might reach the aquatic environment available has become necessary since regulations have been strengthened in France, and a system of fines has been introduced.

It has become important to design bioassays identifying especially the lethal and sublethal effects of substances on organisms in the aquatic ecosystem.

Currently, the choice of test organisms embraces four trophic levels:

- Bacteria
- Algae
- Crustaceans
- Fish

At this time several projects are being investigation for each of these groups. The various methods which are the subject of a standard project will be analyzed successively. Inter alia, the choice of test species and the various analytical criteria of toxicity will be discussed.

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La pollution toxique dans les eaux françaises: caractérisation à l'aide de bio-essais

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INTRODUCTION

Face au développement industriel croissant et à l'augmentation de la population, le gouvernement et le Parlement français ont pris conscience qu'il fallait doter le pays de nouvelles structures para-administratives dans le domaine de la gestion des eaux.

C'est ainsi qu'ont été créées les Agences Financières de Bassin (AFB) par la loi du 16 décembre 1964. Celles-ci ont un double objectif:

- améliorer la répartition des eaux;
- participer à la lutte contre leur pollution.

Parallèlement, la législation a été renforcée visant à instituer une véritable police de la qualité des eaux. Celle-ci se caractérise par 3 dispositions actuellement mises en oeuvre:

- réalisation d'un inventaire de la qualité des eaux;
- autorisations pour les rejets dans tous les milieux;
- réglementation de la vente et la diffusion des produits.

Les AFB, au nombre de six, sont des établissements publics de l'Etat dotés de l'autonomie administrative et financière. Leur fonctionnement est basé sur la distribution de subventions aux industriels et aux communes, pour la réalisation de travaux d'épuration ou d'aménagement des eaux, grâce à la perception de redevances de prélèvements et de pollution.

LE PRINCIPE DE LA REDEVANCE

La redevance de pollution a été fondée sur les quantités de pollution déversées au milieu naturel. Celles-ci sont soit mesurées, soit estimées forfaitairement sur la base de l'activité industrielle, ou de "l'équivalent-habitant" s'il s'agit de rejets domestiques.

Les paramètres de pollution retenus pour la redevance sont:

- matières en suspension (MES);
- matières oxydables (MO) = $\frac{DCO + 2\ (DBO_5)}{3}$;
- sels solubles (conductivité);
- matières inhibitrices (MI).

Ainsi, le renforcement de la réglementation sur l'eau et la mise en place d'un système de redevances ont nécessité de disposer de paramètres de contrôle des effluents eux-mêmes, d'une part, et de toute substance chimique pouvant aboutir dans le milieu aquatique, d'autre part.

LES TESTS BIOLOGIQUES

Il est devenu nécessaire de mettre au point des bio-essais pour connaître, notamment, les effets toxiques létaux et sublétaux des substances vis-à-vis des organismes aquatiques.

Le principe général consiste à mettre en contact une substance chimique seule ou en association avec un réactif biologique dans des conditions de milieu, température, durée d'exposition, concentration définis, et de déterminer la concentration responsable d'un certain effet.

Actuellement, quatre niveaux trophiques ont été retenus pour le choix des organismes tests:

- les bactéries;
- les algues;
- les crustacés (daphnies);
- les poissons.

Pour chacun de ces niveaux, plusieurs projets sont à l'étude en ce moment. Le choix d'une méthode est conditionné par les exigences suivantes, inhérentes à tous les bio-essais:

- reproductibilité;
- sensibilité;
- simplicité dans la mise en oeuvre;
- représentativité des phénomènes naturels mis en jeu.

Nous analyserons plus particulièrement les différentes méthodes faisant l'objet d'une norme ou d'un projet de norme AFNOR (Association Française pour la Normalisation). Dans ce cadre, nous discuterons du choix des espèces, ainsi que des critères de toxicité appliqués, sans faire cependant d'analyse critique de ces méthodes. Notons également que nous ne considérerons ici que la mise en évidence d'effets toxiques à court terme (toxicité aiguë), et en eau douce.

A. Toxicité vis-à-vis des bactéries

Bien que ce soit les organismes les plus simples dans la chaîne alimentaire, les bactéries posent beaucoup de problèmes au laboratoire en tant que réactif biologique. Les critères pris en considération pour mettre en évidence les effets toxiques sont très nombreux.

Trois méthodes sont actuellement proposées et en cours de discussion en France, à l'AFNOR:

1. Détermination de la concentration qui inhibe 50% de la consommation d'oxygène par des micro-organismes d'une boue activée.

2. Détermination de l'inhibition de l'aptitude à la survie et à la multiplication de cinq souches bactériennes testées séparément (effet bactéricide).

3. Détermination de l'inhibition du potentiel hétérotrophe des populations bactériennes naturelles basée sur l'étude de l'assimilation d'un substrat marqué au ^{14}C.

B. Toxicité vis-à-vis des algues

De très nombreux tests ont été décrits pour mettre en évidence le plus souvent des effets inhibiteurs.

Au niveau AFNOR, il existe un projet de norme (T90304) basé sur l'inhibition de la croissance de Scénédesmus subspicatus (effet algistatique). On détermine la CI50-5 jours, c'est-à-dire la concentration en substance qui en 5 jours inhibe 50% de la croissance d'une population de Scénédesmus subspicatus. La croissance est évaluée soit par comptage direct au microscope, soit par mesure de la densité optique à 665 nm.

C. Toxicité vis-à-vis d'un crustacé: la daphnie

Dans ce cas, la sitution est plus avancée. La méthode mise au point fait l'objet d'une norme expérimentale (AFNOR T90301).

Le test est basé sur l'inhibition de la mobilité de Daphnia magna: on détermine la CI50-24h, c'est-à-dire la concentration qui inhibe, en 24h, la mobilité de 50% des daphnies expérimentées.

La méthode, à la fois simple, rapide et fiable, est couramment appliquée pour étudier la toxicité des effluents et servir de base au calcul de la redevance "Matières inhibitrices" actuellement appliquée par les AFB. Elle est également utilisée dans le cas de substances chimiques.

D. Toxicité vis-à-vis des poissons

Le poisson est considéré comme l'un des principaux organismes des milieux aquatiques. Dans les tests biologiques, on utilise soit des poissons réellement présents dans les milieux aquatiques, soit des poissons dits d'aquariums, non représentatifs des milieux naturels mais se prêtant mieux à l'élevage et la réalisation d'essais en séries.

La méthode française (norme expérimentale T90303) utilise le poisson zèbre Brachydanio rerio. Le test est basé sur la détermination de la concentration qui, en 24h et 48h, est responsable de la mortalité de 50% des poissons expérimentés (CI50-24h et CI50-48h).

CONCLUSION

Nous n'avons abordé ici que les bio-essais mesurant des effets toxiques aigus, et destinés à être normalisés à l'échelon national dans le but de développer un contrôle structuré et coordonné de la qualité des eaux en France. Dans le même objectif, un effort de standardisation est également entrepris au niveau international.

En ce qui concerne les mesures d'effets chroniques (toxicité à long terme), plusieurs méthodes sont à l'étude. Celles-ci mettent en jeu principalement les daphnies et les poissons.

La mise au point d'un test standardisé permet une comparaison valable et satisfaisante des effluents ou de toute substance chimique dans des conditions définies afin d'établir un ordre de priorité relatif au danger potentiel dont ils peuvent être responsables vis-à-vis du milieu aquatique.

LES IMPACTS SUR LES EAUX RECEPTRICES DE LA POLLUTION DIFFUSE URBAINE

D. Couillard* et P. Lavallée**
*INRS-Eau et **ME Québec

RESUME - Malgré les efforts entrepris pour épurer les eaux usées municipales, les eaux de la pollution diffuse urbaine, négligées dans l'évaluation des sources majeures de contamination des eaux réceptrices, sont rejetées au cours d'eau sans traitement. Ces eaux usées, rejets intermittents reliés au ruissellement de surface en milieu urbain, transportent de grandes quantités de métaux et de solides en suspension à partir des contaminants déposés sur les surfaces imperméables des municipalités, et des nutriments, de la matière organique, des bactéries et virus provenant des eaux usées domestiques. Par exemple, les sources diffuses de pollution urbaine rejettent annuellement 45 fois plus de plomb, et 15 fois plus de solides en suspension qu'un effluent d'usine d'épuration biologique.

Quoiqu'intermittents, ces déversements ne peuvent être considérés négligeables; puisque des phénomènes d'accumulation dans les sédiments, de bioaccumulation, et l'observation d'un effet de choc ("shock load") sont autant de modifications du milieu qui peuvent être causées par la pollution diffuse urbaine. Des augmentations des concentrations de métaux dans les sédiments d'une rivière, juste en aval d'une municipalité, par des facteurs variant de 2 à 5, tendent à prouver l'incapacité du cours d'eau à disperser efficacement tous les contaminants déversés. Des tests biologiques sur Selenastrum Capricornutum confirment la toxicité des eaux de ruissellement urbain pour divers organismes aquatiques.

Dans le cadre d'une évaluation de synthèse des impacts des eaux usées de la pollution diffuse urbaine, nous avons démontré que ces eaux usées dépassent des normes d'effluent, établies pour préserver une certaine qualité du milieu en aval de déversements. De plus, ces seuls déversements diminuent la qualité de l'eau du milieu récepteur en dessous du seuil minimum acceptable pour bénéficier du plein usage du cours d'eau.

THE IMPACT OF DIFFUSE URBAN POLLUTION ON RECEIVING WATERS

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SUMMARY - In spite of all efforts to purify used municipal waters, waste waters containing ample urban pollution, neglected in an evaluation of the major sources of contamination of receiving waters, are dumped untreated into waterways. These used waters, intermittent waste together with surface run-off in an urban milieu, transport large quantities of metals and solids in suspension, originating from contaminants settled on impermeable municipal surfaces, and the foods, organic matter, bacteria and viruses from domestic waste waters. For example, diffuse sources of urban pollution annually eject 45 times more lead, and 15 times more solids in suspension than the effluent from a biological purification plant.

Though intermittent, these outflows cannot be regarded as negligible since phenomena of accumulation in sediments, bioaccumulation and shock ("shock load") are some of many environmental changes which may be caused by such diffuse urban pollution. The 2 - 5-fold increase of metal concentrations in the sediment of a river just downstream from a municipality seems to prove that the watercourse is incapable of dispersing all the inflowing contaminants effectively. Biological tests on Selenastrum capricornutum confirm the toxicity of urban run-off waters for various aquatic organisms.

In an overall evaluation of the impacts of used water from diffuse urban pollution, we have demonstrated that these waste-waters exceed the standards of effluents established to preserve a certain quality of the environment downstream from effluent outlets. Moreover, the influx of these run-offs alone reduces the water quality of the receiving stream below the acceptable minimum to allow for full use of the watercourse.

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Les impacts sur les eaux réceptrices de la pollution diffuse urbaine

Présentation* au
Septième Atelier Annuel sur la Toxicité Aquatique

Montréal
6 novembre 1980

Thème: la toxicologie, outil de gestion environnementale?

par

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* Ce texte de la présentation ne doit servir que pour familiariser les traducteurs

INTRODUCTION

Dans la plupart des pays industrialisés, beaucoup d'efforts sont actuellement entrepris pour diminuer les quantités de polluants, déversés sans traitement au milieu naturel. Dans l'évaluation des charges rejetées en fonction du type de milieu, le territoire urbain est considéré comme l'une des principales sources de détérioration de nos cours d'eau. Divers programmes d'assainissement mis en vigueur au Québec et ailleurs au Canada, ont comme objectifs principaux de réduire les quantités de contaminants rejetées au cours d'eau et de redonner aux citoyens le plein usage d'un cours propre et sain. Dans ce but, des ouvrages de traitement ont été installés sur le réseau d'égout principal des municipalités afin de traiter les eaux usées domestiques. Les eaux usées ainsi traitées ne sont cependant pas la seule source de contamination reliée au réseau d'égout des villes. En temps de pluie, les eaux de ruissellement urbain sont maintenant considérées comme étant d'importantes sources de détérioration du milieu naturel, lorsqu'elles sont rejetées sans traitement malgré leur fort potentiel contaminant.

LA POLLUTION DIFFUSE URBAINE

En milieu urbain, les eaux de ruissellement de surface sont canalisées le plus possible pour éviter des problèmes de circulation sur les artères de la municipalité lors de forts orages. Outre le ruissellement urbain directement aux cours d'eau, l'exutoire d'un réseau de type séparatif ne transportant que les eaux de ruissellement de surface (réseau pluvial) et les exutoires, autres que l'exutoire principal[1] d'un réseau unitaire, que sont les débordements et les dérivations avant traitement ("by-pass") (figure 1) sont classés comme sources diffuses de pollution. Ces sources sont qualifiées de diffuses puisqu'elles sont intermittentes, reliées aux précipitations, à la qualité et à la quantité des eaux de ruissellement qui drainent le territoire urbain. Pour procéder à une évaluation de la qualité des eaux de débordements et de dérivation avant traitement, il faut ajouter aux contaminants présents dans les eaux usées domestiques, ceux des eaux de ruissellement urbain.

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[1] Normalement relié à une usine d'épuration des eaux usées.

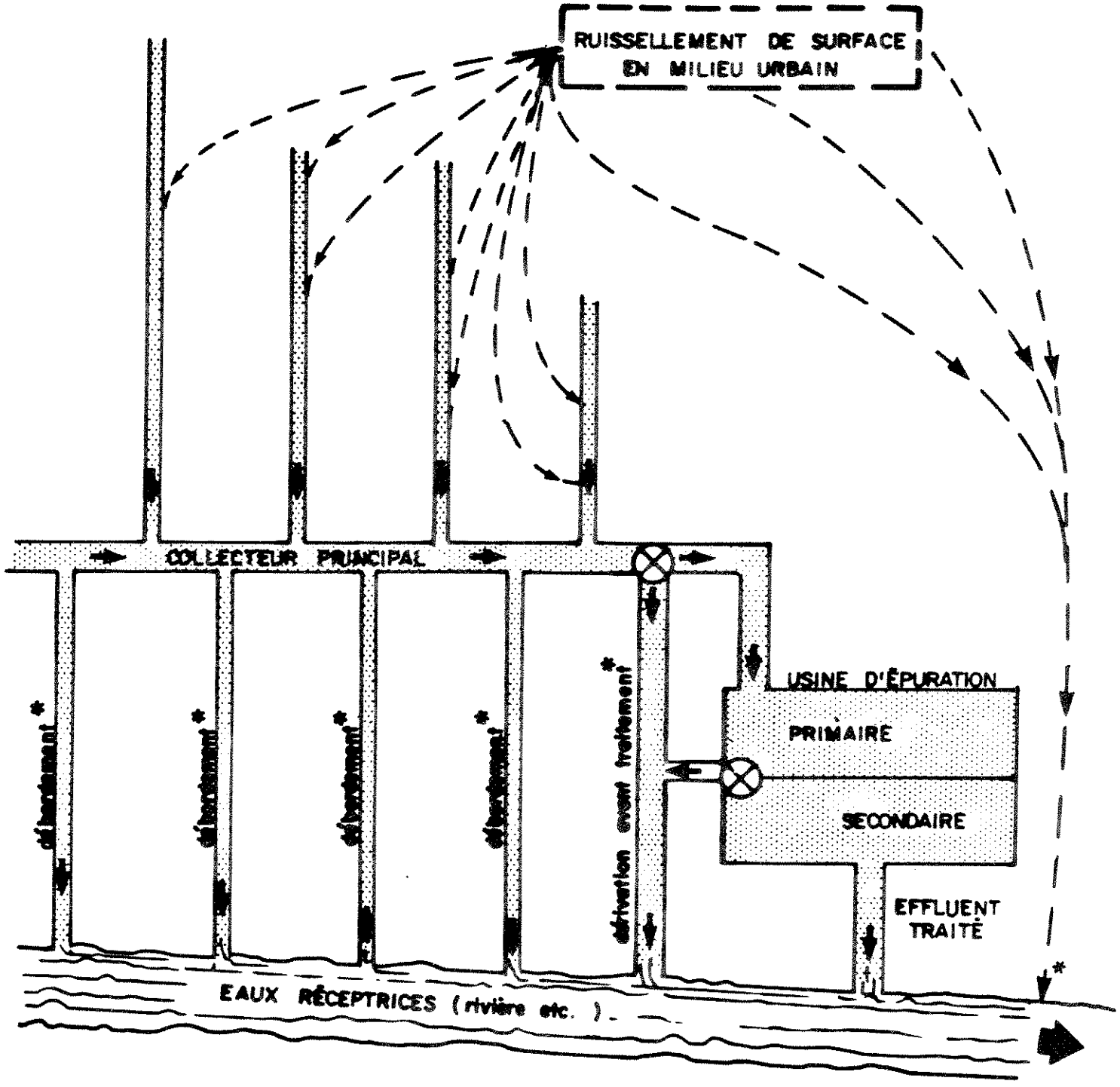

Figure 1. Les sources diffuses de pollution urbaine (municipalité avec un réseau unitaire) .

La qualité des eaux de ruissellement urbain, source primaire de la pollution diffuse urbaine, est influencée par plusieurs facteurs. Beaucoup d'activités en territoire urbain peuvent être sources de rejets contaminants sur le sol: construction, dépôts atmosphériques[1], véhicules automobiles, produits chimiques, etc... (figure 2) (Dick et Marsalek, 1979). Ajoutons que plus le milieu sera centralisé[2], plus les charges de contaminants seront importantes dues à une plus grande circulation et à une augmentation des surfaces imperméables.

La création, en milieu urbain, d'un grand nombre d'aires imperméables (stationnements, rues, troits d'édifices, etc...) a contribué à augmenter considérablement le volume des eaux de ruissellement qui devront être évacuées par le réseau d'égout. Malheureusement, le réseau de type unitaire[3] n'est pas conçu pour transporter toutes les eaux de ruissellement. Sa capacité est prévue pour pouvoir transporter les pointes de débit des eaux usées domestiques[4] (Tétrault, 1980) ce qui ne correspond pas aux volumes du mélange eaux domestiques-eaux de ruissellement de surface qui sont à transporter durant un épisode pluvieux. Les débordements deviennent donc nécessaires pour évacuer les surplus d'eaux usées, afin d'éviter le refoulement jusqu'aux résidences raccordées au réseau.

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[1] Les dépôts atmosphériques sont les particules émises dans l'atmosphère par les usines et qui retombent au sol, soit sous forme de poussière sèche ou par les précipitations.

[2] Centralisé: beaucoup d'édifices à bureau, édifices commerciaux, peu d'espaces à vocation résidentielle.

[3] 80% de la population de la province de Québec est desservie par des réseaux unitaires.

[4] Pointes reliées à l'utilisation non uniforme dans le temps des eaux de consommation.

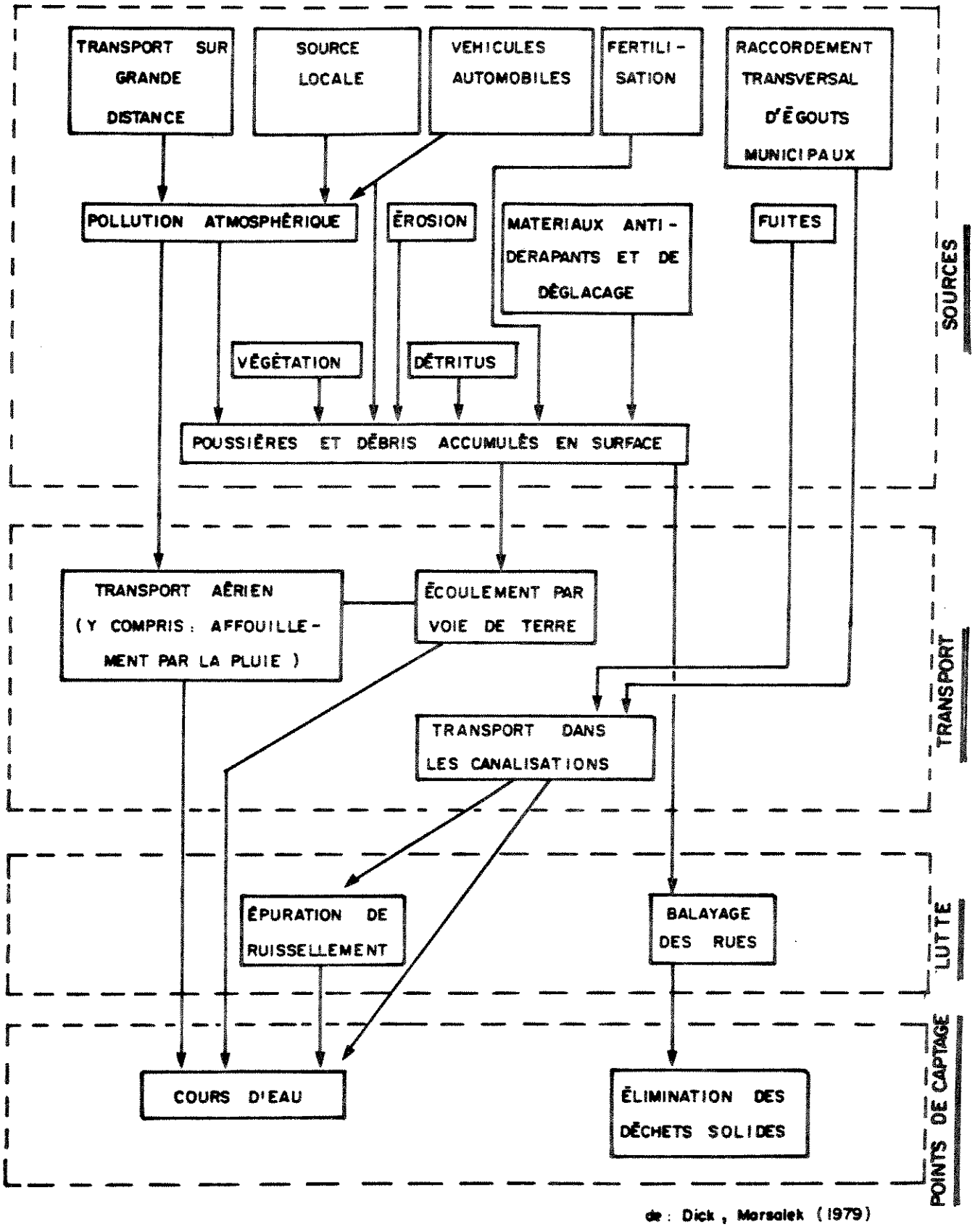

Figure 2 . Facteurs influencant la qualité du ruissellement urbain .

Dans le cas de municipalités desservies par un réseau unitaire, l'accroissement du débit transporté au-delà de la capacité de conception du système n'est pas un phénomène exceptionnel. Une étude de Lager et al. (1977) a dénombré 100 débordements par année pour les villes de New York et Chicago. En Ontario (Sullivan et al., 1978), les débordements se produisent 36% du temps.

L'étude d'un événement en particulier permet d'évaluer le pourcentage du volume de ruissellement qui ne pourra être acheminé vers l'usine d'épuration par le réseau d'égout. Pour des précipitations de 12.5 mm en 24 heures, précipitations de période de retour mensuelle pour la région de Montréal (Ministère des Richesses naturelles, 1974), qui couvrirait de façon uniforme un territoire de 200 kilomètres carrés[1] ayant un coefficient de ruissellement de 50%[2], nous aurions un volume total de 1.25 millard (1.25×10^9) de litres d'eaux usées de ruissellement de surface à évacuer. Si ce territoire, desservi par un réseau unitaire de capacité égale à deux fois le débit moyen de temps sec (D.M.T.S.)[3] est occupé par une population de un million d'habitants (5 000 h/km^2), ce réseau pourra évacuer 0.44 (0.44×10^9) milliard de litres par jour. Lors de l'évacuation de ce volume total (volume de ruissellement de surface et le débit sanitaire théorique de un million d'habitants), la majeure partie, soit 70%, débordera.

CARACTERISTIQUES DES EAUX USEES DE LA POLLUTION DIFFUSE URBAINE

De l'examen des caractéristiques des divers types d'eaux usées urbaines, nous pouvons conclure que les eaux de ruissellement de surface et de débordements de réseau unitaire ont les plus fortes concentrations de solides en suspension et de métaux que tous les autres types d'eaux usées urbaines. Il s'avère également que ces deux types de déversements sont responsables sur une base annuelle de la majorité des solides et des métaux rejetés au cours d'eau.

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[1] L'équivalent des 2/5 de l'Ile de Montréal

[2] Coefficient de ruissellement accepté pour un territoire ayant une très faible proportion à vocation résidentielle.

[3] Pour protéger une usine d'épuration biologique d'une surcharge hydraulique, la capacité du réseau à l'entrée de l'usine est réduite à deux fois le D.M.T.S. (Trudel, 1979).

Les caractéristiques, en charges annuelles déversées, des eaux usées de débordement de réseau unitaire et des eaux de ruissellement de surface (réseau pluvial) ont été comparées aux effluents traités d'une municipalité. Cette analyse démontre que dans le cas où les eaux usées municipales, de temps sec, seront traitées, la principale source de contamination en provenance de milieu urbain serait la pollution diffuse. En effet, le ruissellement urbain rejette annuellement au cours d'eau jusqu'à 100 fois plus de solides en suspension qu'un effluent de traitement secondaire. Les débordements déverseront dans le milieu six fois plus de phosphore qu'un effluent de traitement physico-chimique (tableau 1).

DESCRIPTION DES IMPACTS POTENTIELS SUR LE MILIEU RECEPTEUR

Les principaux contaminants provenant de la pollution diffuse urbaine (bactéries, virus, solides en suspension, métaux, produits chimiques tels les hydrocarbures, les détergents et les pesticides) sont les vecteurs importants de la pollution des eaux naturelles. Ces contaminants auront une action sur la qualité de l'eau, des sédiments et de la vie aquatique en général (figure 3).

L'accumulation, en milieu lentique, et, en particulier, la bio-accumulation de ces contaminants sont autant de facteurs qui s'avèrent des plus importants lors de l'évaluation des répercussions par des déversements intermittents. En effet, un des points importants à retenir est que l'assimilation, la transformation ou l'accumulation d'un contaminant peut amener le dépassement d'un seuil toxique, même si à l'origine le contaminant a été déversé en quantité sub-critique (Heaney et al., 1977).

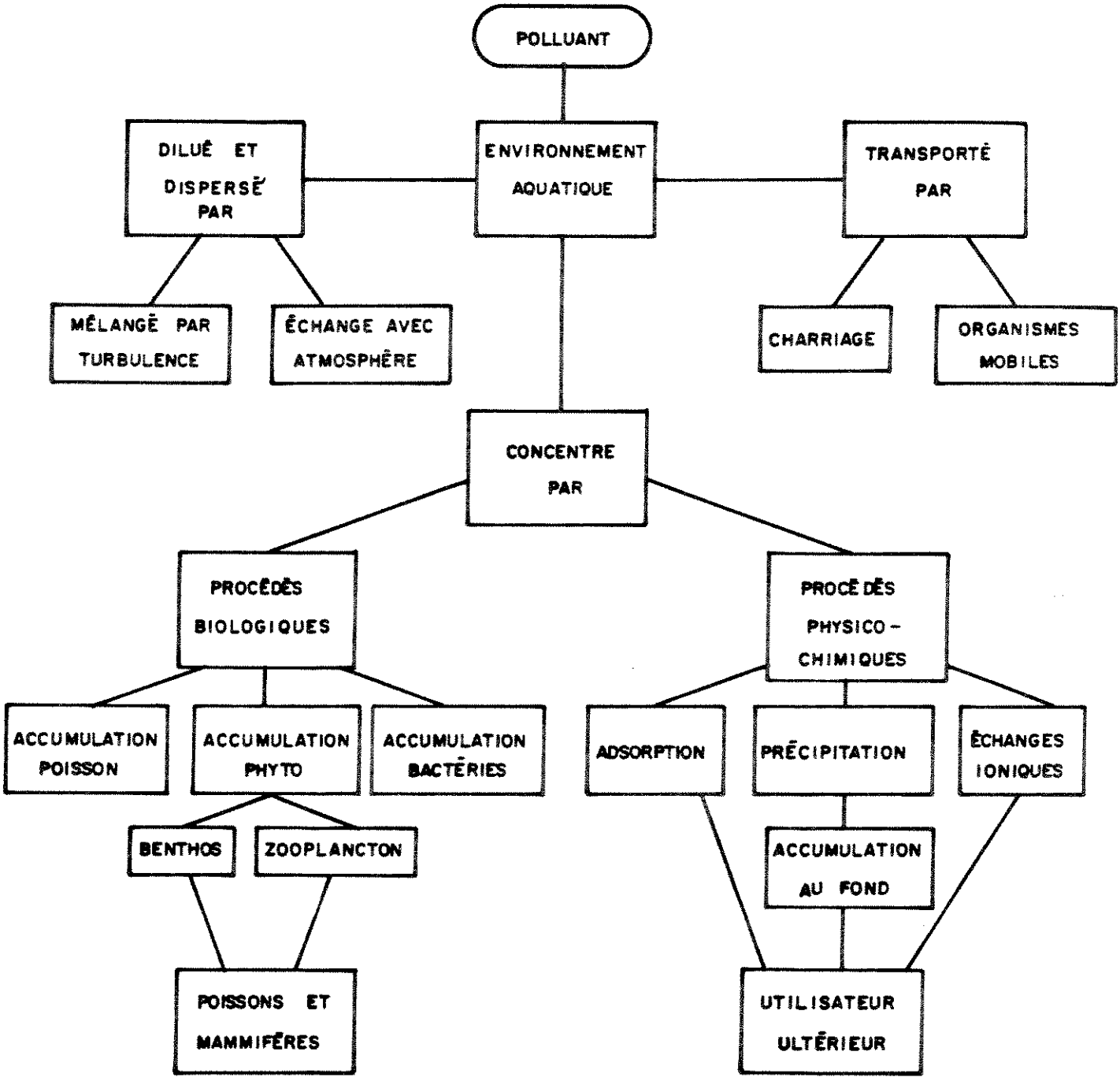
Une évaluation de l'enrichissement en métaux en aval d'une municipalité (Wilber et Hunter, 1979) (tableau) montre des augmentations de 120 à 670% pour divers métaux dans les sédiments de la rivière.

TABLEAU 1 : Comparaison des caractéristiques (charges annuelles) pour les débordements de réseau unitaire et les eaux de ruissellement urbain avec les effluents d'usine d'épuration* (kg/ha/an)

PARAMETRE	EAUX BRUTES RESEAU UNITAIRE	EAUX BRUTES PLUVIAL	DEBORDEMENT RESEAU UNITAIRE	EFFLUENT PRIMAIRE DECANTATION	EFFLUENT SECONDAIRE BIOLOGIQUE	EFFLUENT PRIMAIRE DECANTATION + FLOCULATION CHIMIQUE
S.S.	1431	17808	1344	859	143	572
DBO_5	1322	94	605	1123	198	859
N_{Total}	224	-	54	-	22	-
P_{Total}	58	3.8	74	47	44	11.6
Plomb	3.2**	3.2	-	1.9	1.9	1.9
Référence:	Eckoff *et al.* (1969)	Bryan (1972)	Burm *et al.* (1968)			

* La qualité des effluents d'usine d'épuration est évaluée à partir des mêmes eaux brutes de réseau unitaire et de l'efficacité théorique de chaque traitement mis en cause (Couillard, 1972b).

** Nous prenons la valeur obtenue de Bryan (1972) pour ce contaminant.



modifié de : Heaney et al. (1977)

Figure 3 Diagramme d'utilisation d'un polluant

REACTIONS DU MILIEU FACE A L'EFFET CHOC DES DEVERSEMENTS

Les déversements de la pollution diffuse urbaine sont intermittents, relativement très chargés en début d'orage, rejetant ainsi dans le milieu une "onde" de contaminants. Les organismes colonisant un milieu non-contaminé auront donc à résister à cette "onde de choc". Les bactéries sont probablement les organismes ayant la plus grande capacité d'adaptation de tous les organismes vivants du milieu aquatique (Kuznetsov, 1970); les espèces dominantes seront modifiées et les capacités de biodégradation diminuées par rapport aux conditions existant avant les déversements, mais il restera une certaine colonisation. Ce n'est pas le cas pour les organismes supérieurs. Les organismes pouvant migrer rechercheront un milieu propice à leur survie et ne coloniseront plus le milieu contaminé (Lavallée, 1980), même si celui-ci redevient par intermittence un milieu acceptable. Quant aux espèces fixes, si leurs sensibilités aux contaminants sont fortes, elles risquent d'être décimées.

Bien sur, ces hypothèses sont fonction du cycle, de la fréquence et de la durée de l'"effet choc". Les variations de la composition de l'eau du milieu récepteur, ainsi que les effets saisonniers (hydrologie, température, etc...) auront aussi une importance sur la dispersion des contaminants, et par conséquent sur le potentiel contaminant appliqué sur le milieu récepteur.

EVALUATION DU DEPASSEMENT DES NORMES DE QUALITE DES EFFLUENTS ET DES NORMES DE QUALITE DU MILIEU POUR LES DEBORDEMENTS DU RESEAU UNITAIRE DE LA COMMUNAUTE URBAINE DE MONTREAL

Pour évaluer les impacts sur le milieu récepteur de la pollution diffuse urbaine d'une municipalité comme la ville de Montréal; nous avons estimé le dépassement des normes de qualité, pour un milieu particulier: la rivière des Prairies[1]. Ce cours d'eau reçoit les débordements d'une partie importante de la communauté urbaine de Montréal.

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[1] cf. figure 4 pour localisation

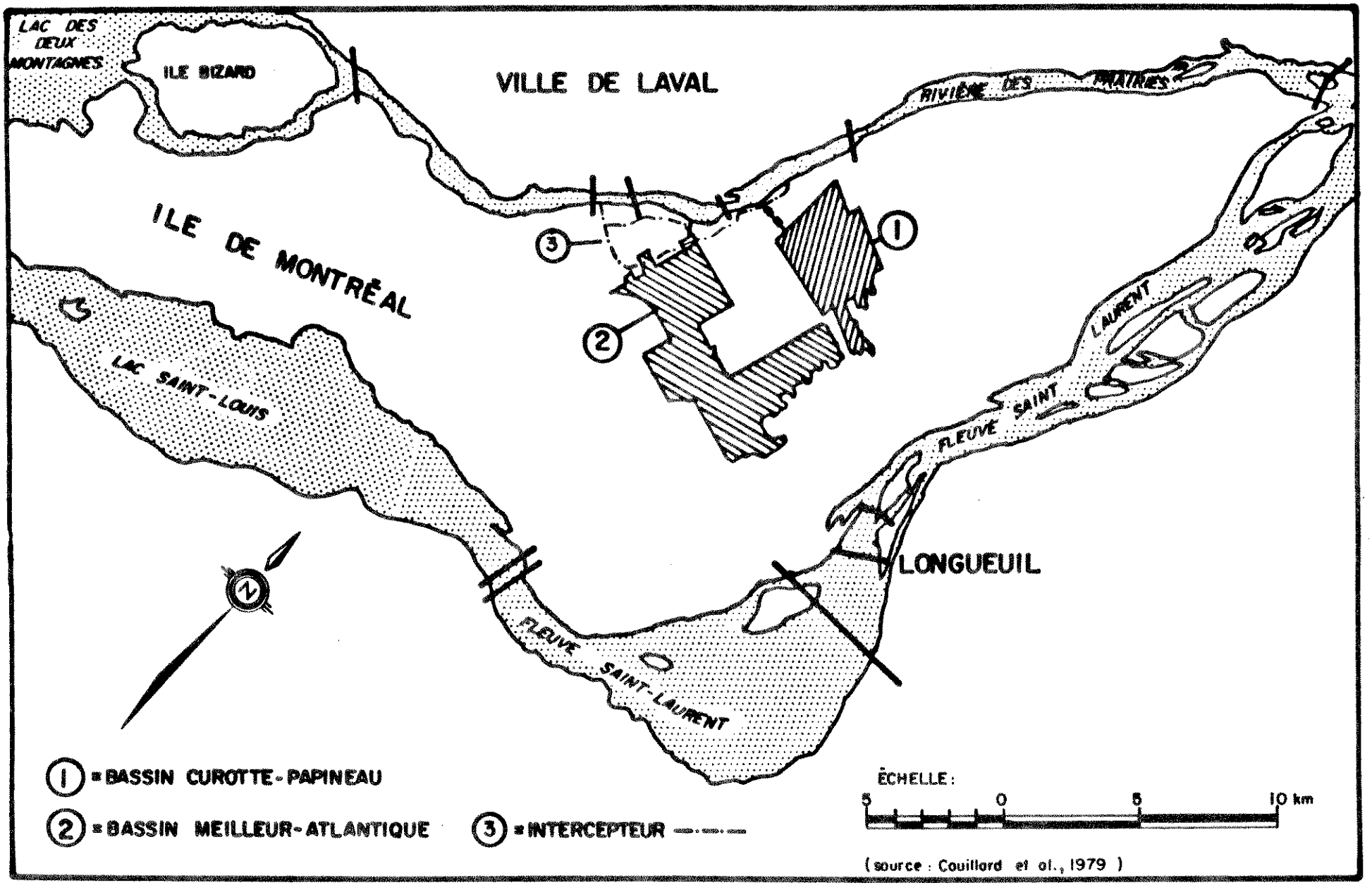

Figure 4 . Localisation géographique des bassins étudiés.

En additionnant à la qualité amont de la rivière des Prairies les seuls déversements par les trop pleins du réseau unitaire (38 débordements pour un débit total de 25 m^3/sec en temps de pluie), et en supposant que ces déversements sont dispersés sur l'ensemble de la rivière; nous remarquons que les normes de qualité du milieu permettant le plein usage d'un cours d'eau sont dépassées pour la plupart des paramètres, à l'embouchure de la Rivière des Prairies (tableau 2).

En évaluant des normes d'effluents pour respecter une qualité acceptable du cours d'eau selon le modèle de Price et Pearson (1979), nous avons déterminer que la qualité des eaux de débordements des collecteurs témoins de la ville de Montréal dépassent les normes acceptables pour les métaux et les demandes chimiques et biochimiques en oxygène (DCO et DBO_5).

EVALUATION DU POTENTIEL TOXIQUE DU RUISSELLEMENT URBAIN PAR DES TESTS BIOLOGIQUES

Dans une étude à l'INRS-Eau (Couture et al; 1980), la toxicité des eaux de ruissellement urbain a été mesurée pour une algue verte Selenastrum capricornutum. Les échantillons d'eaux usées, servant pour les tests biologiques, provenaient des eaux d'un collecteur de la C.U.M. L'occupation du sol de ce bassin est la suivante: 12% d'industriel, 33% de terrains vacants et d'équipements collectifs, 55% d'immobilisations résidentielles.

Les eaux de débordements ont été évaluées toxiques, le rapport de la biomasse calculée sur la biomasse réelle étant supérieur à 1.3 (tableau 3).

CONCLUSION

L'assainissement des eaux usées urbaines est un travail long et coûteux, pour lequel beaucoup de structures physiques, de groupes d'études, et de comités de liaison devront être mis en place. Actuellement la nécessité de traiter les eaux sanitaires et les eaux industrielles apparaît comme prioritaire et est généralement admise. Toutefois les objectifs d'un programme d'assainissement, qui sont, en général, de rejeter le moins possible de contaminants vers le milieu récepteur et de récupérer les usages d'un cours d'eau pour la population, ne peuvent être

TABLEAU 2: Comparaison de la qualité de la rivière des Prairies en aval des déversements de la pollution diffuse urbaine avec les normes de qualité du milieu et comparaison des caractéristiques des eaux de débordement de réseau unitaire avec les normes d'effluent.

PARAMETRE	COLLECTEUR UNITAIRE TEMPS PLUIE (mg/1)	NORMES D'EFFLUENT (mg/1)	DEPASSEMENT DES NORMES D'EFFLUENTS	NORMES DE QUALITE DU MILIEU (mg/1)	QUALITE AMONT DE LA RIVIERE DES PRAIRIES (mg/1)	QUALITE RIVIERE DES PRAIRIES AVAL (1) (mg/1)	DEPASSEMENT DES NORMES DE QUALITE DU MILIEU	QUALITE DEBORDEMENT / QUALITE AMONT
SS	536	865		25	4	17.4		3.4
DBO_5	157	3	x	3	-	-	-	-
DCO	644	1.1	x	1.1	15	31.1	x	1.1
NO_2+NO_3	0.75			0.3	.19	0.208		0.1
$P_{inorg.}$	0.32			0.02	.07	0.078	x	0.1
Pb	0.85	0.865	x (2)	0.025	.004	0.0253	x	5.3
Cr	0.07	1.73		0.05	.008	0.010		0.3
Zn	1.36	0.75	x	0.03	.012	0.046	x	2.8
Référence	Couillard et al. (1979)	Price et Pearson (1979)		Gouin et Malo (1978)	Gouin et Malo (1978)			

(1) Explications dans le texte

(2) Les valeurs obtenues pour le collecteur ne sont pas significativement différentes des normes d'effluents

TABLEAU 3: Mesure de la toxicité des eaux usées d'un réseau unitaire en temps de pluie (collecteur Meilleur-Atlantique, C.U.M.) par un test biologique

Série* D'ECHANTILLONS	Biomasse MESUREE (B) (mg/l)	Biomasse CALCULEE (Bc) (mg/l)	Bc/B
1	6.4	21.5	3.4
2	1.4	8.6	6.2
3	6.6	12.9	1.9
4	5.9	45.0	7.5
5	20.4	64.6	3.2
6	55.0	57.4	1.0

DE: Couture et al., 1980.

* Pour un même épisode pluvieux, le premier échantillon à t_1 est le numéro 1.

atteints que si les quantités de contaminants dans le milieu sont inférieures à un seuil critique; seuil critique qui peut être dépassé par les seuls rejets de la pollution diffuse urbaine.

Présentation visuelle

1.	Diapositive	Rivière St-Charles (Québec) après la pluie
2.	"	" " "
3.	"	" " "
4.	Acétate Figure 1	Les sources diffuses de pollution urbaine
5.	Acétate Figure 2	Facteurs influençant la qualité du ruissellement urbain
6.	Acétate Tableau 1	Comparaison des caractéristiques (charges annuelles) pour les débordements de réseau unitaire et les eaux de ruissellement urbain avec les effluents d'usine d'épuration
7.	Acétate Figure 3	Diagramme d'utilisation d'un polluant
8.	Acétate Figure 4	Localisation géographique des bassins étudiés
9.	Acétate Tableau 2	Comparaison de la qualité de la rivière des Prairies en aval des déversements de la pollution diffuse urbaine
10.	Acétate Tableau 3	Mesure de la toxicité des eaux usées d'un réseau unitaire en temps de pluie, par un test biologique

LES TESTS ECOTOXICOLOGIQUES SUR PROTOZOAIRES: ÉTAT ACTUEL ET PERSPECTIVES

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RÉSUMÉ - Les tests toxicologiques sur Protozoaires ont été basés sur de nombreux critères: éthologie (mobilité, nutrition), tests de mortalité, modification de la croissance. Parmi ces méthodes, trois seront examinées avec quelques détails: la première est basée sur la croissance totale de Colpidium campylum cultivée monoxéniquement sur bactéries. Elle a permis de tester de nombreuses substances pures (toxiques minéraux, pesticides, PCB, mycotoxines) ou des mélanges (par analyse factorielle), mais se prête mal au travail sur les effluents. La seconde méthode est un test de viabilité techniquement très simple, mais qui présente les désavantages d'une durée trop longue et d'une grande difficulté pour tester les effluents turbides. Une troisième méthode, utilisant des cultures synchrones s'avère sensible, très rapide et, selon la technique de lecture, peut s'appliquer à de nombreux types de produits à tester. Elle peut en outre être utilisée pour une étude toxicologique approfondie.

L'étude d'une mycotoxine a permis de comparer la sensibilité de diverses méthodes de test sur protozoaires avec des tests conventionnels (Artémia, Daphnia).

ECOTOXICOLOGY TESTS ON PROTOZOA: CURRENT STATUS AND PROSPECTS

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SUMMARY - The toxicology tests on Protozoa were based on a great many criteria:ethology (mobility, nutrition), mortality tests, impaired growth. Three of these methods will be examined in some detail: the first is based on the total growth of Colpidium campylum cultured axenically in the presence of a specific type of bacterial species. It can be used to test numerous substances either in the pure state (mineral toxins, pesticides, PCBs, mycotoxins) or in combination (by factor analysis) but does not lend itself well to effluents. The second method is a viability test which is technically very simple but has the disadvantages of taking too long and being extremely difficult when testing turbide effluents. The third method, using synchronous cultures, is sensitive, very fast and, depending on the reading method used, can be applied to a great many products. It can also be used for a detailed toxicology study.

Examination of a mycotoxin made it possible to compare various Protozoa test methods with conventional tests (Artemia, Daphnia) for sensitivity.

COMPARAISON INTERLABORATOIRE DES TESTS - I: TOXICITÉ DE L'AMMONIAC

G.R. Craig et K.E. Holtze
MOE, Ontario

RÉSUMÉ - Dans le cadre d'un programme conjoint réunissant sept laboratoires d'organismes de réglementation, l'influence sur les tests de toxicité en milieu aquatique de la vitesse d'intoxication des poissons, de la taille de ces derniers et de la qualité de l'eau a été évaluée. Des bio-essais de 96 heures en conditions statiques sur la truite arc-en-ciel (Salmo gairdneri) ont été menés suivant un mode opératoire uniformisé de mesure de la létalité aiguë pour les poissons. La substance toxique choisie était le chlorure d'ammonium (NH_4Cl), dont les interactions avec les paramètres de qualité de l'eau sont bien connues.

Les laboratoires ont tous déterminé la même CL_{50}-96 heures ($p = 0.05$), à deux vitesses d'intoxication des poissons, 0.5 et 1.0 litre de solution par jour et par gramme (poids du sujet). Les écarts de CL_{50} observés étaient prononcés, sauf quand la qualité de l'eau était la même. Les analyses de régression à plusieurs variables ont montré que la taille de poissons et la qualité de l'eau étaient les principales causes des variations observées.

Nous avons mis au point des modèles de prévision de la toxicité de l'ammoniac à partir de la concentration mesurée, de la taille des poissons et de la qualité de l'eau.

L'étude montre la nécessité de poursuivre l'évaluation des modes opératoires des bio-essais et montre aussi à quel point il est important d'établir un profil complet de la qualité de l'eau en vue des tests de toxicité en milieu aquatique.

INTERLABORATORY TEST COMPARISON - I AMMONIA TOXICITY

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MOE, Ontario

SUMMARY - The influence of fish loading rate, size of fish and water quality on aquatic toxicity test results were evaluated in a co-operative programme by seven regulatory laboratories. 96-hr static, bioassays, using rainbow trout (Salmo gairdneri) as the test organism were conducted according to standardized procedures for testing acute lethality to fish. Ammonium chloride (NH_4Cl) was selected for use as the test toxicant in view of its well recognized interactions with water quality parameters.

The 96-hr LC_{50}'s compared within laboratories were similar (p 0.05), when tested at two fish loading rates, 0.5 and 1.0 litres of solution per gram of fish per day of test. Differences in LC_{50}'s among laboratories were marked except where water qualities were similar. Multivariant regression analysis indicated that size of fish and water quality accounted for much of the observed variation.

Models for predicting ammonia toxicity based on measured concentration, size of fish and water quality were developed.

The study identifies the need for further evaluation of bioassay test protocols and demonstrates the importance of reporting a comprehensive water quality profile with aquatic toxicity test results.

THEME V

Courts exposés divers sur la toxicité aquatique

Diverse aquatic toxicity short exposés

ADSORPTION ET DÉSORPTION DE LA PERMÉTHRINE ET D'AUTRES PESTICIDES SUR LES OBJETS EN VERRE ET EN PLASTIQUE EMPLOYÉS POUR LES BIO-ESSAIS

M.S. Sharom et K.R. Solomon
Université de Guelph

RÉSUMÉ - Nous avons étudié en laboratoire l'adsorption et la désorption de la perméthrine par plusieurs articles en verre et en plastique qui servent à la construction de limnocoraux. Une quantité importante de l'insecticide était adsorbée par le polyéthylène et le chlorure de polyvinyle, mais non par le verre et le teflon. La perméthrine adsorbée était difficilement enlevée du polyéthylène et du chlorure de polyvinyle, même après plusieurs rinçages à l'eau. Plus la température était élevée plus l'insecticide était adsorbé. Une étude de l'adsorption de plusieurs herbicides à plusieurs solubilités dans l'eau a montré que la trifluraline était adsorbée par le verre et qu'il n'y avait aucun lien précis entre l'adsorption de l'herbicide par le chlorure de polyvinyle et la solubilité dans l'eau. Il est important de déterminer le rôle de l'adsorption des pesticides (par les articles employés pour les bio-essais) sur l'élimination globale de cet agent chimique en milieu aquatique ou sa toxicité pour les organismes aquatiques.

ADSORPTION AND DESORPTION OF PERMETHRIN AND OTHER PESTICIDES ON GLASS AND PLASTIC MATERIALS USED IN BIOASSAY PROCEDURES

M.S. Sharom and K.R. Solomon
University of Guelph

SUMMARY - Laboratory studies were conducted to determine the adsorption and desorption of permethrin on glass and several plastic materials that may be used for the construction of limnocorrals. The insecticide was substantially adsorbed on polyethylene and polyvinylchloride but not on glass and teflon. Adsorbed permethrin was not easily desorbed from polyethylene and polyvinylchloride by several water rinses. Increased adsorption of the insecticide was observed with increasing temperatures. A study on the adsorption of several herbicides of various water solubilities indicated that trifluralin was adsorbed to glass and that there was no general relationship between adsorption of the herbicide on polyvinylchloride and water solubility. It is important to determine the role of pesticide adsorption (on bioassay containers) on the overall disappearance of the chemical in aquatic systems or its toxicity to aquatic organisms.

Adsorption and Desorption of Permethrin and Other Pesticides on Glass and Plastic Materials Used for Bioassay Procedures

M.S. Sharom and K.R. Solomon

On Wednesday, Dr. Solomon spoke on the evaluation of impact of permethrin on a lake ecosystem using limnocorrals. The inactivation or disappearance of a pesticide applied to limnocorrals may be attributed to chemical and biological degradation, or adsorption on sediments, various aquatic organisms and the walls of the corrals. One of the objectives of this investigation is to determine the adsorption and desorption of permethrin on the walls of these corrals; which is made of PVC ie. polyvinylchloride. However, before doing that, one would like to know whether permethrin is adsorbed to glass, for if it is, one will have to develop a method that can differentiate between adsorption to glass and adsorption to PVC.

Several vials were filled with 3 mL of radiolabelled permethrin and left on the lab bench. At various time intervals, triplicate sample vials were removed. 1 mL was gently pipetted out and dpm (disintegration per min.) determined. The result of the study is given in this figure (SLIDE 1) and it showed that a substantial amount of the chemical was adsorbed to glass. However, adsorption was slow and equilibrium established after 48 hr. (SLIDE 2). A similar study was conducted using 5, 10 and 20 ml of water and adsorption determined after 120 hr. The results showed that about 70% of the insecticide was adsorbed on the 5 mL vial, 63% on the 10 ml vial and 42% on the 20 mL vial. The reduction in percent adsorption with increase in volume may be attributed to the increase in volume to area ratio. However, much of the adsorbed permethrin was easily desorbed from the glass surface. After 1 min. shaking by hand, as much as 93% of the adsorbed permethrin was desorbed from the 5 mL vials, 86% from the 10 mL vials, and 67% from the 20 mL vials. Studies are in progress to determine whether the weak adsorption of permethrin on glass will have any influence on

bioassay studies involving glass containers, such as the determination of EC_{50} to aquatic organism.

The next step was to determine adsorption of permethrin on PVC and for comparison, polyethylene and teflon were included in the study. The materials were placed in caps of scintillation vials. 3 mL aliquots of radiolabelled permethrin were pipetted into the vials and covered with caps lined with the appropriate materials. The vials were inverted and placed on the bench or agitated. At various time intervals, triplicate sample vials were removed and centrifuged in the upright position to remove all water droplets adhering to the materials. The caps were removed, scintillation cocktail added, and the vials closed with foil-lined caps. The vials were shaken and dpm determined (SLIDE 3). This figure showed the adsorption of permethrin on the various materials when the vials were not agitated. Adsorption of permethrin on PVC and polyethylene was slow and equilibrium not established after 120 hr. Adsorption on teflon was also slow but equilibrium seemed to be established after 48 hr. PVC and polyethylene adsorbed more permethrin than teflon (SLIDE 4). A different trend was observed when adsorption was determined with agitation. Adsorption of permethrin on PVC and polyethylene was relatively rapid with more than 80% adsorption occurring after 6 hr. and almost all the permethrin adsorbed after 96 hr. Adsorption of permethrin on teflon reached equilibrium at approximately 6 hr. and the maximum amount adsorbed was 20% of the original amount added.

I will not describe the method used to determine the desorption of permethrin from these materials. The result of the study (SLIDE 5) showed that permethrin was strongly adsorbed to PVC and polyethylene with approximately 85-95% of the applied permethrin remaining adsorbed after 4-12 mL rinses. In the case of teflon, most of the adsorbed permethrin was desorbed after the first rinse followed by proportionally less desorption with subsequent rinses.

(SLIDE 6) This slide shows the influence of temperature on permethrin adsorption. Adsorption was lowest below freezing point. Temperatures does not seem to influence permethrin adsorption on glass and teflon. For PVC and polyethylene there were significant increases in adsorption with increasesin temperature.

(SLIDE 7) This slide shows the adsorption of some herbicides on glass and PVC. With the exception of trifluralin where water solubility is less than 1 ppm, less than 1% of the applied herbicide was adsorbed to glass. However, in the case of PVC, with the exception of amitrole, whose water solubility is 280,000 ppm, substantial amounts of the herbicides were adsorbed. Thus this investigation showed that adsorption of a pesticide on walls of limnocorrals could be a significant factor in determining the disappearance and the lethal and sub-lethal effects of the chemical to organisms in model aquatic systems.

ADSORPTION, DÉSORPTION, RÉPARTITION ET DÉCOMPOSITION DE LA PERMÉTHRINE DANS DES SYSTÈMES SÉDIMENTS-EAU.

M.S. Sharom et K.R. Solomon
Université de Guelph

RÉSUMÉ - Des études en laboratoire ont montré que la perméthrine était rapidement adsorbée par les sédiments lacustres et qu'une fois adsorbée, il n'était pas facile de la déloger, même après plusieurs rinçages à l'eau. La majeure partie de l'insecticide appliqué en solution sur des sédiments n'avait pas pénétré à plus de 2 cm après trois semaines. La décomposition de la perméthrine dans l'eau ou dans les systèmes sédiments-eau a été attribuée à des processus chimiques et biologiques. Elle était accélérée en milieu non stérile, ce qui est significatif du rôle des micro-organismes dans la décomposition de la perméthrine. L'insecticide était plus persistent dans les systèmes sédiments-eau que dans l'eau. L'adsorption des molécules de perméthrine par des sédiments mettait peut-être ces dernières à l'abri de l'attaque microbienne. L'isomère cis était plus stable que la forme trans tant en ce qui concerne la décomposition chimique que la décomposition biologique. Après 8 semaines d'incubation, 98% et 76% de la trans-perméthrine introduite dans l'eau de lac non stérile et stérile, respectivement s'était décomposée. Pendant ce temps, seulement 64% et 18% de la cis-perméthrine mélangée à l'eau de lac non stérile et stérile, respectivement, s'était décomposée.

ADSORPTION, DESORPTION, DISTRIBUTION AND DEGRADATION OF PERMETHRIN IN SEDIMENT-WATER SYSTEM.

M.S. Sharom and K.R. Solomon
University of Guelph

SUMMARY - Laboratory studies indicated that permethrin was rapidly adsorbed to lake sediments and once adsorbed the insecticide was not easily desorbed by several water rinses. Most of the insecticide that was applied in solution to sediment columns remained at the top 2 cm of the columns after 3 weeks. The degradation of permethrin in water or sediment-water system was attributed to both chemical and biological processes. Degradation was more rapid in nonsterilized conditions indicating the important role of microorganisms in permethrin degradation. The insecticide was more persistant in the sediment-water system than in water. Adsorption of permethrin molecules to sediments may have made them inaccessible for microbial degradation. The cis isomer was more stable than the trans against both chemical and biological degradation. After 8 week incubation, about 98 and 76% of the applied trans permethrin was degraded in the nonsterilized and sterilized lake water, respectively. For the same period only about 64 and 18% of the cis permethrin was degraded in the nonsterilized and sterilized lake water, respectively.

The Adsorption-desorption, Degradation and Distribution of Permethrin in Aqueous Systems

M.S. Sharom and K.R. Solomon

As indicated earlier, inactivation of a pesticide applied to limnocorrals may be attributed to adsorption of the chemical on sediment. The sediment used for this study was collected from Lake St. George; it contained 43% organic matter. Preliminary investigation showed that adsorption of permethrin on the sediment was very rapid with more than 95% of the applied amount being adsorbed after 1 min. shaking. The adsorption isotherm was determined by equilibrating 5 mL of various concentrations of radiolabelled permethrin with 200 mg of freeze-dried sediment. The amount adsorbed per gm of sediment was plotted against the amount per mL of solution. The result showed that (SLIDE 1) the adsorption isotherm follows the linear pattern of increased adsorption with increased insecticide concentration.

(SLIDE 2). Once adsorbed, the insecticide was not easily desorbed by several water rinses. Approximately 8% of the initial adsorbed insecticide was desorbed from the sediment after four rinses with 10 mL of water.

The study on the degradation of permethrin in lake water was conducted in scintillation vials. The water was sterilized with sodium azide. 5 mL aliquots of sterilized and unsterilized radiolabelled permethrin solution were pipetted into several vials. The vials were capped and placed in the dark. At various time intervals triplicate sample vials were removed for analysis. Degradation of permethrin in flooded sediment was also conducted in a similar manner. Each vial contained 2 gm wet sediment with 8 mL of either sterilized or unsterilized radiolabelled permethrin. The vials were thoroughly mixed for 1 min. before placing in the dark. The result is given in this slide (SLIDE 3). Firstly it showed that the cis isomer of permethrin was more persistent than the trans. Secondly, the degradation of permethrin was more rapid in unsterilized than sterilized water indicating that microorganisms played an important role in

permethrin degradation. It also showed that the trans isomer was more susceptible to chemical degradation than the cis. Thirdly the degradation of permethrin was more rapid in water than in flooded sediment suggesting that the adsorbed permethrin molecules were less aviable for microbial and chemical degradation. Approximately 90% of the degradation product was in the form of Cl_2CA. The unidentified compound exhibited little movement from the origin of the TLC plates and comprised approximately 10% of the total radioactivity on the chromatogram after 12 weeks of incubation. (SLIDE 4) The study on the distribution of permethrin in sediment-water system was conducted in 15 cm by 1.7 cm ID glass columns. The bottom of each column was plugged with a rubber stopper. Seven gm of wet sediment followed by 7 mL of lake water were added to each column, thoroughly mixed and left overnight for the sediment to settle. 10 mL of radiolabelled permethrin solution was gently pipetted into each column. The columns were placed in the dark and at various time intervals duplicate samples were removed and frozen. While still frozen, the water column was divided into the top and bottom two cm. The sediment column was sliced into 1 cm lengths. The amount of permethrin and its metabolites in each of the slices was determined. (SLIDE 5) What can we deduce from the result of this study. Firstly total radioactivity in the water column decreased with time probably due to the gradual settling of suspended sediment. This resulted in the gradual increase of total radioactivity in the sediment with time. Secondly the concentration of permethrin in the water column also decreased with time and this may be attributed to adsorbed permethrin settling to the bottom, as well as degradation to Cl_2CA, for as you can see, Cl_2CA in the water column increased with time. Most of the unaltered permethrin was found in the top 1 cm of sediment towards the end of the experimental period. Approximately 20% of the total radioactivity penetrated into the 2nd cm sediment. No radioactivity was detected in the 3rd cm sediment.

From this investigation one can conclude that a large percentage of permethrin applied to aquatic system will be rapidly adsorbed by suspended sediments which gradually settled out. Adsorption of the insecticide will not only reduce the toxicity to target or non-target species but may also increase the persistence of the chemical in the aqueous system.

BIOLYSE ET DÉCOMPOSITION DU FENITROTHION DANS LES MICROCOSMES AQUATIQUES

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RÉSUMÉ - Du ^{14}C-fenitrothion (thiophosphate de O,O-diméthyle et de O-(3-méthyl-4-nitrophényle) marqué sur le noyau et sur une fonction méthoxy), sous l'étiquette commerciale Atlox et Aerotex ou Dowanol, a été fourni à la concentration prescrite sur le terrain (10:1:1 ppm et 50:1:5:1:5 ppm) à des microcosmes-modèles statiques ou en écoulement qui reproduisaient les conditions d'une rivière se déversant dans un lac ou celles d'un estuaire se déversant dans une baie. Des volumes d'eau distillée, d'eau de lac et d'eau d'estuaire à pH compris entre 5.5 et 8.5 et contenant des sédiments, des algues et des macrophytes endémiques ont servi à constituer des systèmes de complexités croissantes constituant les compartiments des microcosmes. Ces systèmes ont été tenus à la lumière ou tenus à l'obscurité. La décomposition et la biolyse du pesticide étaient plus rapides dans l'eau d'estuaire; le pH ne constituait pas un facteur déterminant. Les algues et les macrophytes aquatiques adsorbaient davantage de pesticides sous la forme Aérotex. L'adsorption et la désorption dans le cas des biotes vivants étaient différentes de celles des biotes morts. La biolyse dépend des espèces.

BIOLYSIS AND DEGREDATION OF FENITROTHION IN AQUATIC MICROCOSMS

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SUMMARY - ^{14}C-Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate (ring, and methoxy labelled) Atlox and Aerotex, or Dowanol were supplied as the registered field formulations (10:1:1 ppm and 50:1:5:1:5 ppm) in static and flowing model microcosms which simulated a river flowing into a lake, or an estuary into a bay. Distilled, natural lake and estuarine waters at pH levels from 5.5 to pH 8.5 with sediment, endemic algae and macrophytes in sequentially more complex systems providing the compartments of the microcosms. These were exposed to light or held in the dark. Degredation and biolysis of the pesticide were more rapid in estuarine waters, pH was not a major factor. The algae & aquatic macrophytes actively accumulated more of the pesticide in Aerotex formulations. Uptake and desorption in the living biota was different from rates from comparable dead biota. Biolysis is species specific.

PERSISTENCE OF FENITROTHION IN DISTILLED WATER, NATURAL LAKE, AND ESTUARINE WATERS

BY

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ABSTRACT

The fate, persistance, and degradation of formulated fenitrothion (Atlox/Aerotex and Atlox/Dowanol) in model aquatic flow-through, and static systems analogous to a river and a lake or bay were examined. Its short term persistence in flowing systems where the mobile phase was distilled, lake or estuarine waters at one of three levels of (pH 4.5, 6.8 and 8.5) was governed by dilution and elution. Subsequent degradation in a simulated lake or bay over a 7 day period was faster in estuarine waters but not significantly affected by pH. The Aerotex formulation preferentially catalysed degradation to more polar derivatives.

INTRODUCTION

Reports of the widespread distribution of xenobiotic residues in the global system are increasing in number (Butler 1966, Riseborough et al. 1968 a and b, Holden, 1970), however the ecological effects attributable to the accumulation of these substances is still unclear. This is partly due to incomplete information about the degradation, partitioning, biotransformation and accumulation of the xenobiotic residues.

In Canada, in the years 1968-1979 inclusive, fenitrothion [*O,O*-dimethyl *O*- (3-methyl-4-nitrophenyl) phosphorothioate] was the most widely used agricultural chemical (Symonds, 1977). Its mode of degradation in vitro by hydrolysis (Zitko and Cunningham 1974, Greenhalgh et al. 1980) and photolysis (Marshall et al. 1974, Miyamoto 1977) in distilled water and buffer solutions have been well documented. Degradation, partitioning and accumulation under limited field and laboratory conditions have also been followed (Weinberger et al. 1979, Moody et al. 1979).

The present study extended the former data by using natural lake and estuarine waters and followed the degradation and partitioning of fenitrothion in microcosms representative of a stream with 3 sampling sites flowing into lake, or an estuary into a bay. The chemical interactions were examined at three levels of pH representative of those found naturally within the Maritimes and Eastern Canada, namely pH 4.5, pH 6.8 and pH 8.5. Fenitrothion was added as a formulation with Atlox and either Aerotex or Dowanol, to determine whether the chemical degradation was modified by the type of water (lake or estuarine), pH of the medium, and/or the adjuvants with which fenitrothion was formulated.

MATERIALS AND METHODS

Materials

Chemicals: Technical grade fenitrothion (98% purity) and methoxy-^{14}C labelled fenitrothion were gifts from Sumitomo Chemical Co. Japan. Ring Labelled fenitrothion (SA 14.5 mCi/mM) was purchased from New England Nuclear Co. U.S.A. Aerotex and Atlox were gifts from Texaco Oil and Texas Chemical Co., respectively. Dowanol was obtained from Dow Chemical Co. p-Nitro-m-cresol was obtained from Aldrich Chemical Co. Ethyl iodide and ScintiVerse scintillation cocktail were purchased from Fisher Chemical Co. All oraganic solvents were pesticide grade.

Waters: In parallel experiments, deionized glass distilled water pH 6.5, lake or estuarine waters were used in the model river system. Lake water, pH 7.4, specific conductance 61±2μMHO/cm, was taken from Lac Bourgeois, Quebec, filtered through glass wool and stored at 4°C until required. Estuarine water, pH 7.5, specific conductance 49±2 mMHO/cm, density 1.018 gms/cc, was obtained from New Brunswick and stored frozen until used. 1N HCℓ or 1N NaOH was added to these waters to provide water samples of pH 4.5 and pH 8.5 respectively.

Formulations: Two formulations were used, each having field proportions of pesticide to adjuvants. An Aerotex formulation was prepared containing fenitrothion, Atlox and Aerotex in a ratio of 11;1.5:1.5 (w/v/v). The components were suspended in water and administered as an emulsion. Dowanol formulations containing fenitrothion, Atlox and Dowanol were similarly prepared.

Apparatus: The Flow-Through Model: The flow-through system consisted of a 50 ℓ feeding reservoir and three 1 litre round bottom flask, designed

to represent upstream, midstream, and downstream locations. These were connected by 21.4 m of coiled glass tubing (7 mm i.d.) into a 50 ℓ collection reservoir (Figure 1). All waters were collected in the collection reservoir and used for immediate mass balance determinations. A variable speed peristaltic pump controlled the flow of water into the upstream flask (A) into which the formulated insecticide mixture was pulsed. Air pressure controlled the flow of water through the coils. Stopcocks permitted sampling during a flow-through experiment. Cool white fluorescent lights provided an illumination intensity of 1500 LUX over the spectral range of 400 nm to 700 nm ( ). The persistence of fenitrothion in the three stations of the system was initially monitored by following the persistence of a permanganate dye (Figure 2).

Conductivity: Measurement were made with an Industrial Instruments model-RA-2A conductivity meter.

Gas Chromatography: G.C. quantitative analysed were performed on a Hewlett-Packard Model 5830 gas chromatograph equipped with a N.P. flame ionization detector and an electronic integrator. A 12 m x 6.2 mm i.d. glass column packed with 4%-SE30/6%-QF1 on chromosorb WHP (80-100 mesh) was operated at 215^{o}C with 25 ml/min carrier (N_2) flow. The injection port and detector were maintained at 215^{o} and 300^{o}C respectively.

Liquid Scintillation Counting L.S.C.: A Beckman 3133 liquid scintillation counter (^{3}H + ^{14}C channels) was used to determine the amount of ^{14}C in all organic and aqueous solutions, and in algal and plant material. All data were corrected for their respective quench and background.

Treatments: Fenitrothion enriched with the ^{14}C ring labelled isotope was transferred from methanolic stock solutions and the solvent removed

under a stream of N_2. Adjuvants were then added from concentrated aqueous stock solutions (100µℓ of a 50 mg/200 ml solution of the desired adjuvant) and the volume made up to 10 mls with the appropriate water. Resuspension of the insecticide from the walls of the vessel after vigorous shaking varied from 80-90% and was determined by scintillation counting for each preparation. The final concentration of fenitrothion and adjuvants in the solutions was 2.5 ppm, unless otherwise stated.

Sampling: At least seven samples from each station of the flow through model were analysed within the first 40 min and at 12h intervals thereafter. Each sample was extracted with 3 x 2.5 ml of ethyl acetate. The amount of label in both phases was determined by L.S.C. The amount of label in the ethyl acetate fraction recovered as fenitrothion was determined by gas chromatography. 500 ml of water in the collection reservoir were extracted with 3 x 100 ml of ethyl acetate and both fractions were analysed as described above.

Thin Layer Chromatography: Samples were spotted on U.V. sensitized silica gel plates and developed either with cyclohexane: ethyl acetate (3:1) or toluene: ethyl acetate: acetic acid (5:7:1). Spots were visualized under U.V. light and the plates were autoradiographed with Kodak no-screen X-ray films.

Photolysis and Hydrolysis in Simulated Lake or Bay: Twelve Westinghouse 20 watt, warm white fluorescent lights omitting light in the range of 500-700 nm ( ) at an intensity of 5000 lux were vertically affixed to the walls of an open-ended box (60 cm x 60 cm x 75 cm).

All samples for photolysis were prepared by the addition of 100µℓ of a concentration stock solution of either the Aerotex of Dowanol formulation to 5 ml of distilled, lake or estuarine waters adjusted to a pH of 4.5, 6.8

or 8.5. The resultant samples containing 10 ppm fenitrothion were placed on a circular rack and rotated on a turntable mounted in the base of the light box ensuring equal light exposure of all samples. The temperature was maintained at 31°C. These provided the static lake or bay microcosms. The pesticide was traced separately in all systems with ring- and methoxy-^{14}C labelled fenitrothion. All samples were prepared and analysed in triplicate. Samples wrapped in tin foil were run concurrently as thermal/hydrolytic controls.

Samples were counted directly, before and after 7 days of photolysis, to determine the persistence of the ^{14}C label in each model. Each sample was extracted with 3 x 1.5 ml of ethyl acetate and the amount of label in each fraction was determined by L.S.C. The ethyl acetate fraction was analysed by G.C. and T.L.C.

The extracted aqueous phase was then acidified to pH 1.0 with conc. HCl, saturated with NaCl, and re-extracted with 3 x 1.5 ml of ethyl acetate to remove the polar derivatives. The partition of the label between the two phases was determined by L.S.C. The ethyl acetate was removed under a gently stream of N_2 and the residue was brought up in 0.5 ml acetone. The sample was ethylated with the addition of a 10 molar excess ethyl iodide over 50 mg of potassium carbonate.

LEGENDS

Figure 1. Model Flow-Through System. Flask A, B and C represent up- upstream midstream and downstream locations. Stopcocks permitted sampling during a flowthrough run.

Figure 2. Calibration curve of the Model Flow-Through System. The 3 curves depict the expected persistence in the upstream (●) midstream (▲) and downstream (■) locii of any solvated substance pulsed into the upstream flask where disapperance is effected solely by dilution and elution.

Figure 3. Partition of the ring- and methoxy- ^{14}C labels between the aqueous (P) and the ethyl acetate (N) fractions upon extraction, the amount of fenitrothion (F) recovered in the pH 4.5 system was also shown.

Figure 4. Partition of the ring- and methoxy- ^{14}C labels between the aqueous (P) and the ethyl acetate (N) fractions upon extraction, the of fenitrothion (F) recovered in the pH 6.8 systems was also shown.

Figure 5. Partition of the ring- and methoxy- ^{14}C labels between the aqueous (P) and the ethyl acetate (N) fractions upon extraction, the amount of fenitrothion (F) recovered in the pH 8.5 systems is also shown.

RESULTS AND DISCUSSION

Flow-Through System: A typical $KMnO_4$ persistence curve is shown in Figure 2. Maximum concentrations in the midstream and downstream locations were 34% and 30% respectively of the starting concentration in the upstream station. These maxima were observed at 8 and 15 minutes in the midstream and dowstream locations respectively. The $KMnO_4$ was eluted from the system within one hour. These permanganate persistence curves served as templates on which all ^{14}C- ring fenitrothion experiments were superimposed as a measure of the extent to which the actual persistence of the pesticide followed expected values.

The Aerotex and Dowanol formulations in all 3 waters at each of the 3 levels of pH during the 60 minute flow-through run under 1500 LUX light intensity followed the template persistance curves, as shown by analyses and ^{14}C counts. No fenitrothion degradation products were observed. All ^{14}C remained extractable by ethyl acetate. Mass balances, as determined by gas chromatography and liquid scintillation counting, were quantitative (% 96±4) and indicated that no breakdown of the fenitrothion had occurred. Simple dilution and elution were the sole factors governing the persistence of fenitrothion in the dynamic model ecosystems. Essentially all of the pesticide washed downstream and accumulated in a dowstream bay or lake.

Photolysis and Hydrolysis in the Simulated Lake and Bay: Generally for each formulation at 31±2°C there was no significant difference in the mode of degradation between pH 4.5, 6.8 or 8.5 in any of the waters. Products found in the light exposed waters in either formulation set, 7 days post spray, were not significantly different from those observed in the dark controls, Figures 3,4 and 5. ^{14}C-ring- or methoxy-labelled

fenitrothion were used as markers in all cases and the partioning characteristics of the label in each of the 2 formulations, in each of the 3 waters at each level of pH was determined and compared. The products which partitioned into the aqueous phase following ethyl acetate extraction were designated as polar (P) and those which partitioned into the ethyl acetate, non-polar (N). Fenitrothion constitued the major portion of the non-polar fraction in all cases (3, 4 and 5). The labelled material in this fraction which was not fenitrothion was mainly (10-15%) p-nitro-m cresol. The unextracted label (15-30%) was probably a demethylated derivative, identification of which is presently in progress. Differences between formulations were observed. The Aerotex formulation preferentially catalysed degradation to polar derivatives.

No pH effect was clearly discerned. Degradation in estuarine water was greater than in the other waters, implicating the solvent ions in estuarine waters as playing an important role in the chemical degradation (Zapp, 1980). Acting as catalytic surfaces, they enhanced the aqueous degradation of fenitrothion in a manner comparable to that found for other pesticides (Mead, 1972; Manheim et al., 1972; Gibbs, 1977). This draws support from earlier studies (Weinberger et al., 1978) which indicated the rapid complexing of fenitrothion with a range of divalent cations.

The study highlights two facts namely, sites downstream of spray sites are highly vulnerable to pesticide perturbation and laboratory studies on the chemical degradation of pesticides in distilled water may have only marginal relevance to chemical events taking place in natural waters. In our present study, the compartment of the natural waters, broadly caterized as "lake" or "estuarine" waters, rather than the pH, were important in mediating the mode of breakdown of fenitrothion and thereby provided

a range of polar derivatives with a potential to perturb the natural ecosystems. Persistence and accumulation of these derivatives in algal and aquatic macrophyte communities is presently being followed.

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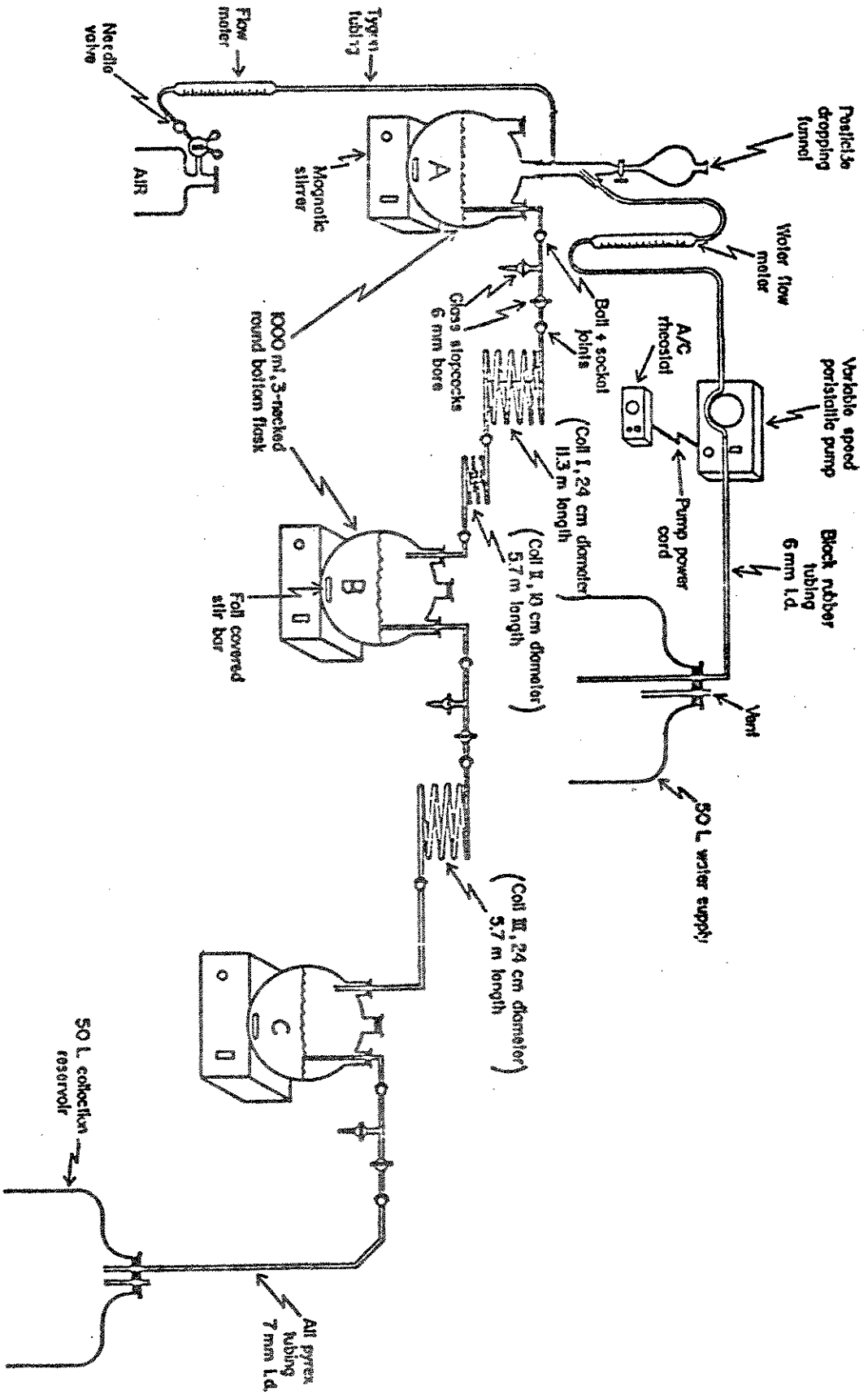
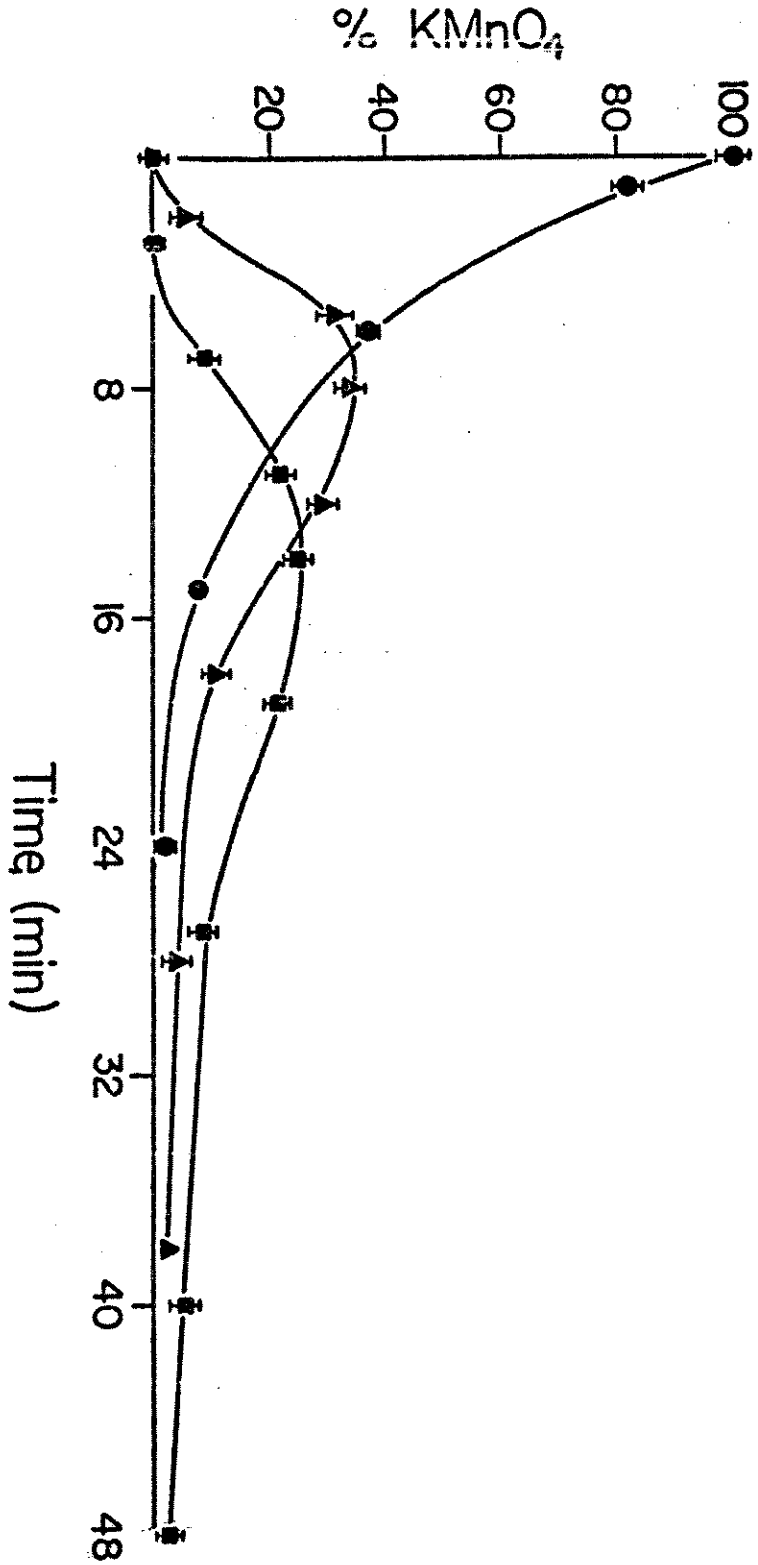

Pesticide dropping funnel
Water flow meter
Variable speed peristaltic pump
Black rubber tubing 6 mm I.d.
A/C rheostat
Pump power cord
Vent
50 L water supply
Ball + socket joints
Glass stopcocks 6 mm bore
(Coil I, 24 cm diameter 11.3 m length
1000 ml, 3-necked round bottom flask
(Coil II, 10 cm diameter 5.7 m length
Foil covered stir bar
(Coil III, 24 cm diameter 5.7 m length
Magnetic stirrer
Tygon tubing
Flow meter
Needle valve
AIR
50 L collection reservoir
All pyrex tubing 7 mm I.d.
A
B
C

FIG. 1



Persistance of $KMnO_4$ in flowthrough system at 85 ml/min (5.01 cm/sec)

FIG. 2

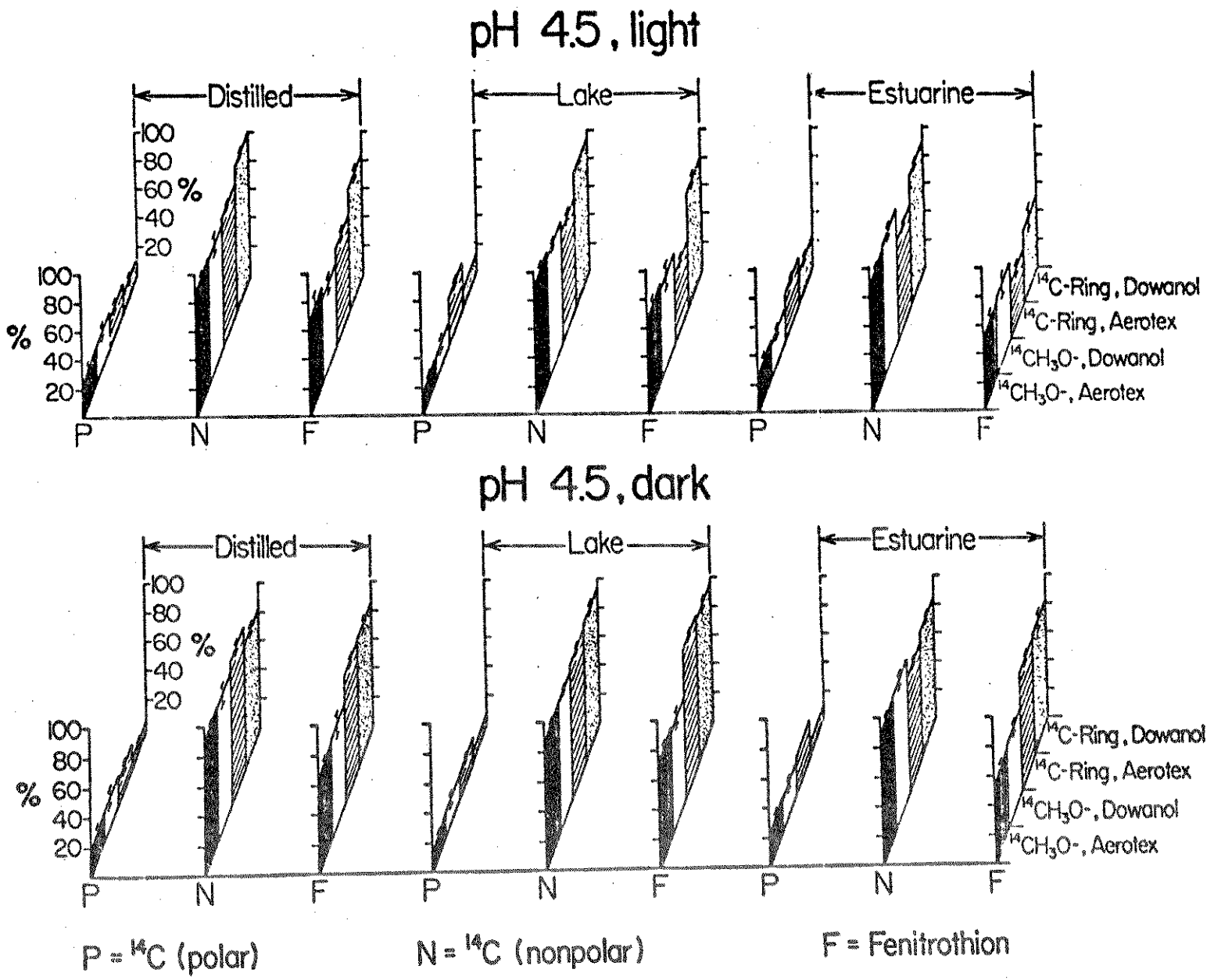

pH 4.5, light
Distilled
Lake
Estuarine
100
80
60 %
40
20
% 100 80 60 40 20
P N F P N F P N F
^{14}C-Ring, Dowanol
^{14}C-Ring, Aerotex
$^{14}CH_3O$-, Dowanol
$^{14}CH_3O$-, Aerotex
pH 4.5, dark
Distilled
Lake
Estuarine
100
80
60 %
40
20
% 100 80 60 40 20
P N F P N F P N F
^{14}C-Ring, Dowanol
^{14}C-Ring, Aerotex
$^{14}CH_3O$-, Dowanol
$^{14}CH_3O$-, Aerotex
P = ^{14}C (polar)
N = ^{14}C (nonpolar)
F = Fenitrothion

FIG. 3

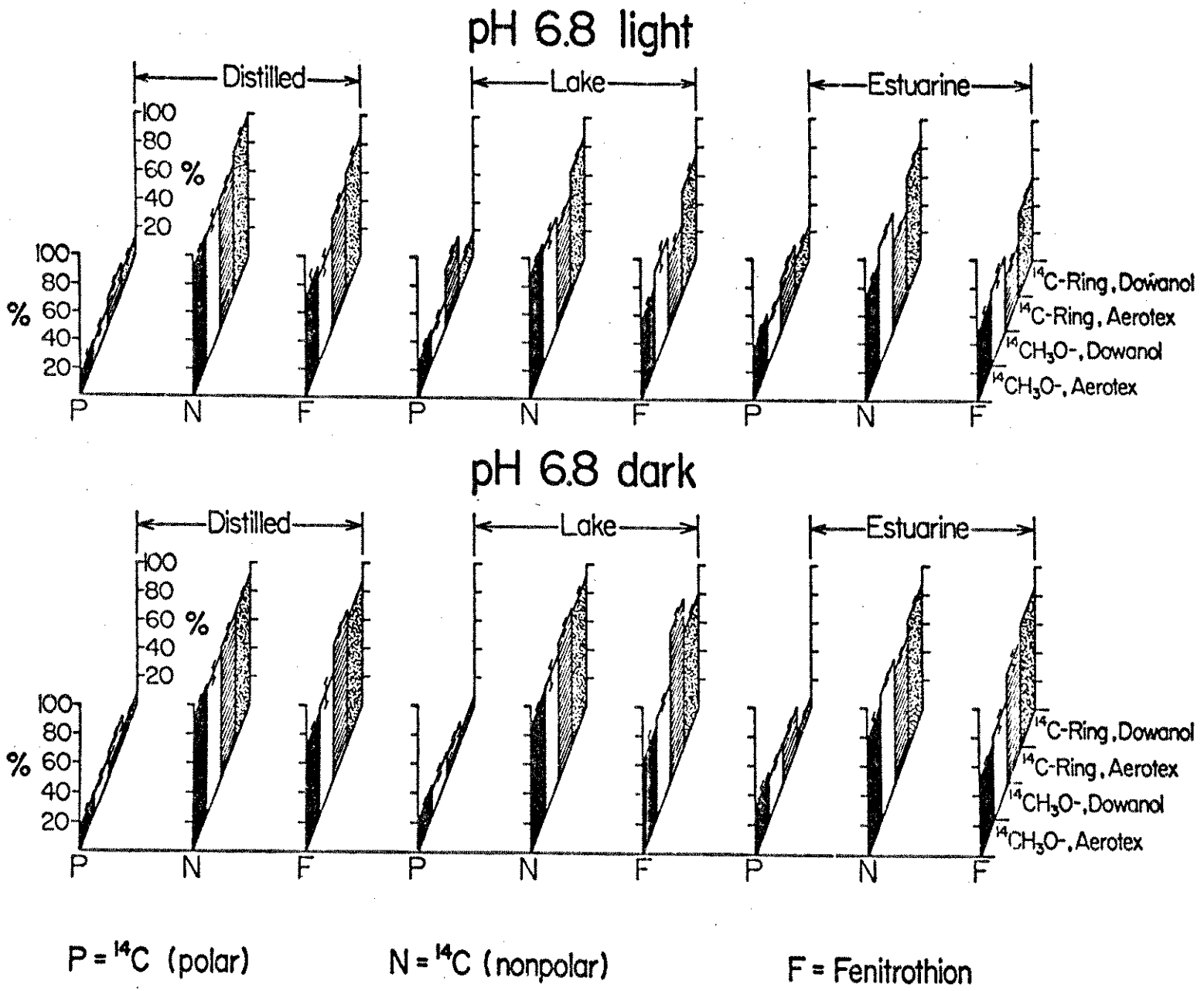

pH 6.8 light
Distilled
Lake
Estuarine
100
80
60
40
20
%
P
N
F
^{14}C-Ring, Dowanol
^{14}C-Ring, Aerotex
$^{14}CH_3O$-, Dowanol
$^{14}CH_3O$-, Aerotex
pH 6.8 dark
P = ^{14}C (polar)
N = ^{14}C (nonpolar)
F = Fenitrothion

FIG. 4

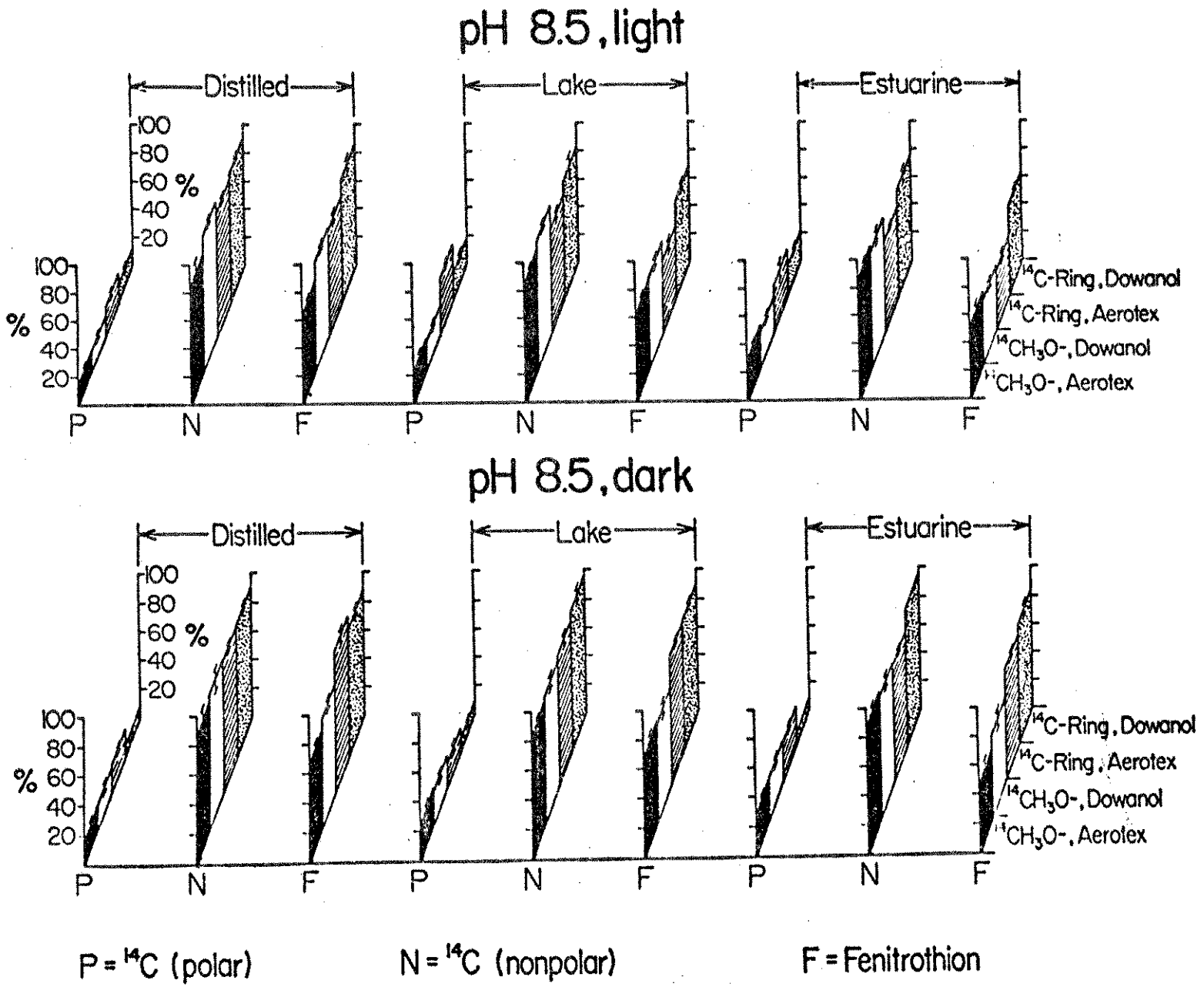

pH 8.5, light
Distilled
Lake
Estuarine
100
80
60 %
40
20
% 60
P
N
F
^{14}C-Ring, Dowanol
^{14}C-Ring, Aerotex
$^{14}CH_3O$-, Dowanol
$^{14}CH_3O$-, Aerotex
pH 8.5, dark
P = ^{14}C (polar)
N = ^{14}C (nonpolar)
F = Fenitrothion

FIG. 5

LE NONYLPHÉNOL: UN ADDITIF À EFFETS PERTURBATEURS

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RÉSUMÉ - Le nonylphénol est le solvant recommandé pour le pesticide de type carbamate Matacil. Cet additif est plus toxique que le pesticide lui-même pour toute une série de biotes constitués notamment d'algues, de limaces et de poissons arc-en-ciel. À la concentration recommandée sur le terrain de 0.5-0.7 ppm de nonylphénol, les flagelles de Chlamydomonas reinhardii se tordent et la structure fine de la cellule est perturbée. Les principaux dommages sont causés aux membranes. La dynamique démographique des algues est sérieusement perturbée, la phase de dormance de la courbe de croissance de la population étant prolongée de plusieurs semaines chez Chlorella pyrenoidosa.

NONYPHENOL: A PERTURBANT ADDITIVE

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SUMMARY - Nonylphenol is the solvent of choice in the formulation of the pesticide carbamate Matacil. This additive is more toxic than the pesticide towards a range of biota including algae, snails and guppies. In algae, at field relevant concentrations of 0.5-0.7 ppm nonyphenol the flagellae of Chlamydomonas reinhardii distort and the ultrastructural architecture of the cell is disrupted. The main sites of damage are membranes. The population dynamics of algae are seriously perturbed, the lag phase of population growth was extended by several weeks in Chlorella pyrenoidosa.

Nonylphenol: A Perturbant Additive to an Aquatic Ecosystem

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Abstract

Nonylphenol has a wide range of industrial use, from germicides, to oil and grease additives, detergents, dyestuffs and surface active and wetting agent. By their use, many millions of pounds are pulsed into the environment. As a solvent in xenobiotic formulations it is itself a toxicant towards many aquatic biota.

The population dynamics of the alga Chlorella pyrenoidosa was seriously perturbed, the lag phase of population growth was progressively extended with increasing concentrations of nonylphenol due to selective mortality. At field relevant concentrations (0.5-0.7 ppm) the flagellae of Chlamydomonas reinhardii were distorted and the ultrastructural architecture of the cells disrupted. The main sites of damage were the membranes. Changes were also observed in the behavioral and feeding characteristics of snails and guppies and some mortality observed.

Introduction

Nonylphenol is a mixture of monalkyl phenols, predominantly para-substituted. The side chains are (random) branched alkyl radicals. It is a pale yellow liquid with a relatively high viscosity and a slight, characteristic phenolic odor. It is practically insoluble in water but is soluble in a range of solvents including alcohols and ethylene glycol. It

can undergo a range of chemical reactions. If halogenated, the products may be used as germicides and the alkyl phenols and alkyl phenyl phthalates derivatives and phthalic anhydride are used as additives in lubricants. The alkyl phenols can be mercurated to form derivatives valuable as bacteriacides. Reacted with aldehyde-ammonia compounds the products inhibit oxidation when added to motor oil. Condensed with formaldehyde and morpholine it yields a compound which, on quaternization with methyl iodine, yield products suitable for use in soaps, wetting and emulsifying agents. Reacted with formaldehyde alone, a range of products are formed which are used in the painting and printing industry and as plasticizers for cellulose esters. Known applications of alkyl phenols of this molecular weight (~215), range from the uses mentioned above, to nonionic or anionic surface active agents. In all, several million pounds of nonylphenol are used for industrial products in the chemical industry and several more hundreds of thousands of pounds are used as surface active agents, in xenobiotic formulations.

The ecotoxicity of nonylphenol is presently in dispute, Wildish (1972, 1974) recorded acute toxicity to fish (goldfish, rainbow trout, Atlantic salmon) this toxicity was in the range of 1000 mg/kg fresh fish weight, and a 96 hour LC_{50} in the range of 1-10 mg/l. Holmes and Kingbury (1980), on the other hand, state that the results of their field trials and laboratory bioassays gave no cause for concern for aquatic or benthic invertebrates, or fish, at dosage rates of 0.47 l/hectare. The rates of dosage in these cases are difficult to correlate, as climatic conditions and rates of water flow were not stated. It is known, that under natural conditions 90% of the alkyl groups of nonylphenol are rapidly(3-7 days) degraded by sediment biota, and for polyethoxylates the polyethylene glycol moiety has a $t_{\frac{1}{2}}$ of ~12 days (Safe *et al*). In mammals, surfactants

such as nonylphenol have been shown to interfere with the protective mucous barrier of the stomach and synergistic carcinogenic effects have been reported in the presence of substituted guanidines (Zukushimo, 1973). Shelton (1976) documented some of the ultrastructural damage to nuclear envelope proteins caused by these nonionic detergents.

Our own studies have amplified these investigations both for terrestrial and aquatic organisms. In the present paper we report on some growth cycle and ultrastructural effects of nonylphenol on two unicellular algae; a motile flagellate Chlamydomonas reinhardii and a nonmotile alga, Chlorella pyrenoidosa. Also some behavioral effects on guppies and snails are reported.

Materials and Methods

Growth Studies: The algae were grown in axenic culture in liquid nutrient media (Bolds, 1942), and incubated at 22 ± 1°C with a 16:8 light (.5 Klux)dark cycle. Population densities were followed daily using optical density reading at 540 nm (Stein 1973), checked with haemocytometer cell counts. Nonylphenol was added to logarithmic algal cultures to give final concentrations of 0, 0.025, 0.25, 2.5, 7.5 and 25 µg/ml corresponding to minimal to high ecologically relevant levels of nonyphenol.

Microcosms: Guppies (6 per 400 ml microcosm) and six snails were housed with Chlamydomonas and Chlorella ($5X10^5$ cells/ml) in natural lake water to which nonylphenol was added to a final concentration of 0.5 ppm, and the behavior of the fish and snails were visually monitored.

Transmission Electron Microscopy: Chlamydomonas was exposed for 1 hour to 1.0 ppm nonyphenol added to the nutrient medium. Following this algae were pelleted by gentle centrifugation, fixed for 1 hour at 0°C with glutaraldehyde-osmium tetroxide (Franke et al 1969) and subsequently

infiltrated with Spurr's plastic (Spurr, 1969). Sections were cut with a DuPont diamond knife on a Sorvall Porter-Blum MT-2B ultramicrotome, mounted on acetone treated copper grids and stained 4 min. in uranyl acetate (5% in 50% ethanol) and 2 min. in lead citrate (Soto et al 1975), and then examined with a Phillips 201 microscope.

Results

Growth Studies: In Chlorella the average exponential growth rate, as determined by linear regression analysis, of the four lower concentrations of nonylphenol (0.025 to 7.5 µg/ml) was depressed and the lag phase was progressively increased with increasing concentrations to 7.5 µg/ml. At 25 µg/ml nonyphenol was a potent algicide affecting a 100% kill of the algae, Figure 1. Algal mortality was evidenced to a varying degree dependent upon concentration and this accounted for the prolongation of the lag phase. A 24h LC_{50} was obtained following exposure to 1.5 ppm nonylphenol.

Microcosms: The guppies reacted immediately to 0.5 ppm nonyphenol, initially exhibiting a startling reaction and later showing some slight disorientation when swimming. These behavioral effects were accompanied by less vigorous feeding. Two of the six guppies died within 24 hours, others recuperated and swam and fed normally after 36 hours. The snails dropped from the inner surfaces of the microcosms and did not emerge for 8 hours. Following this, 5 of the snails resumed their normal feeding behavior.

Transmission Electron Microscopy: The micrographs of treated Chlamydomonas (Plate II) showed evidence of ultrastructural damage compared to controls (Plate I). Membrane disorganization was evidenced by crennulation, vesiculation and pinocytotic invaginations of the nuclear membrane. Friability of

cytoplasm was observed in most cells. Flagellae were evidently sensitive to treatment, gross distortion was a feature of treated cells (Plate III) occuring only rarely in controls as an artifact of the embedding treatments.

Discussion

The unicellular algae used in this study are the base of many food chains leading directly to zooplankton and indirectly to a wide interweaving web of organisms at higher trophic levels. Indirectly, by its algicidal properties, and directly by affecting behavioral and feeding habits, nonylphenol has the potential to be an added stressor factor to aquatic environments. Sprayed in pesticide formulations in the late Spring, it may well perturb an aquatic environment at a time when its biota are barely recovering from the thermal stresses of winter. Although algal populations recover after several days,this haitus in their population density may result in a feeding stress for some aquatic biota. Disorientation, and lessened feeding and lack of vigor by the fish may make them easy prey to natural predators and further oscillate the stability status of the stressed ecosystem.

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Legends

Figure 1 Chlorella pyrenoidosa: Growth studies in liquid nutrient medium. Nonylphenol added to a final concentration of 0, 0.025, 0.25, 2.5, 7.5, or 25 μg/ml.

Plate I. Chlamydomonas reinhardii: T.E.M. of an untreated (control) algal cell (25000X).

Plate II. Chlamydomonas reinhardii: T.E.M. of an algal cell exposed 1 hr to 1.0 ppm nonylphenol (25000X).

Plate III. Chlamydomonas reinhardii: T.E.M. of an algal cell exposed 1 hr to 1.0 ppm nonylphenol (60000X).

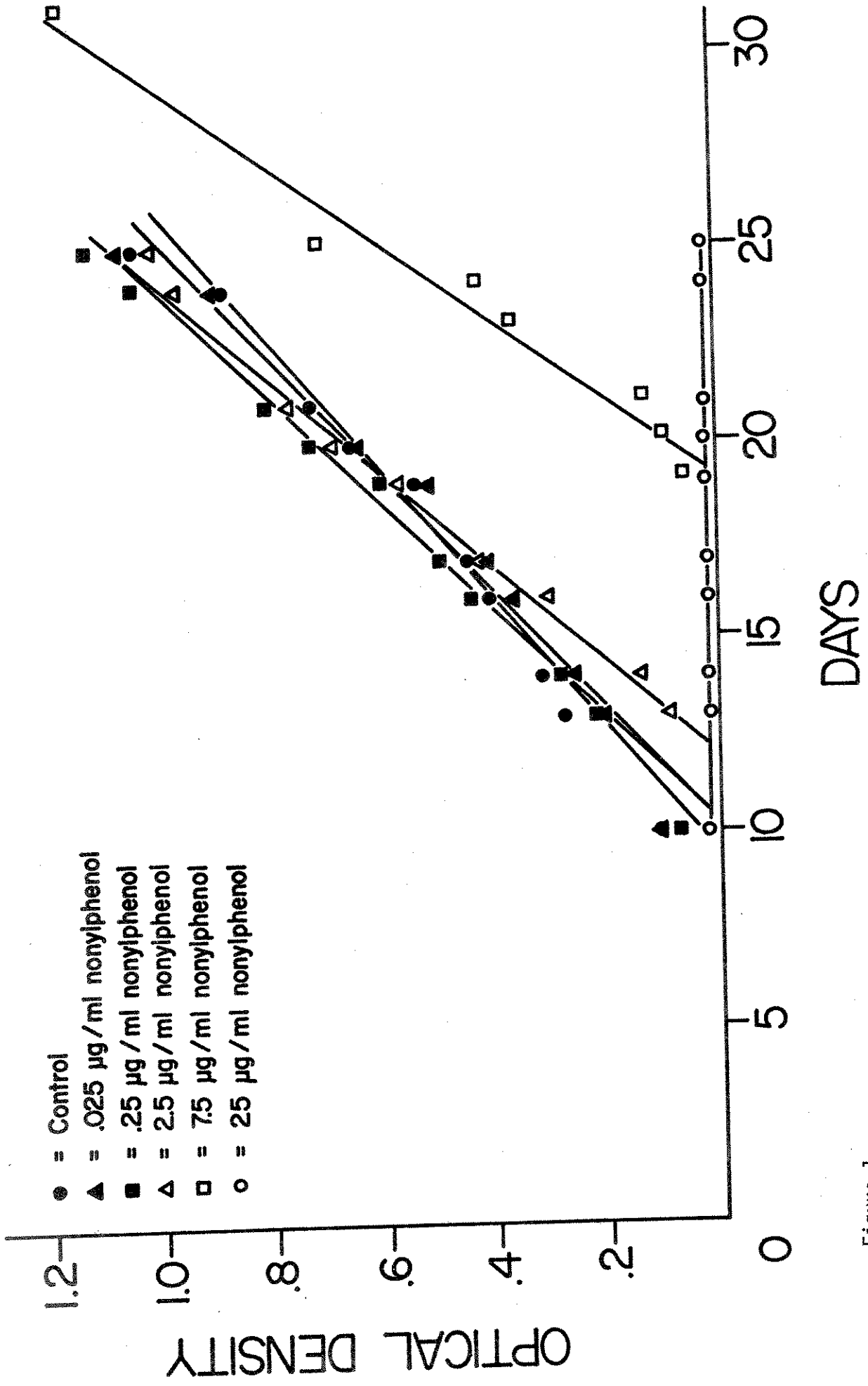

OPTICAL DENSITY
1.2
1.0
.8
.6
.4
.2
0
5
10
15
20
25
30
DAYS
● = Control
▲ = .025 µg/ml nonylphenol
■ = .25 µg/ml nonylphenol
△ = 2.5 µg/ml nonylphenol
□ = 7.5 µg/ml nonylphenol
○ = 25 µg/ml nonylphenol

Figure 1.

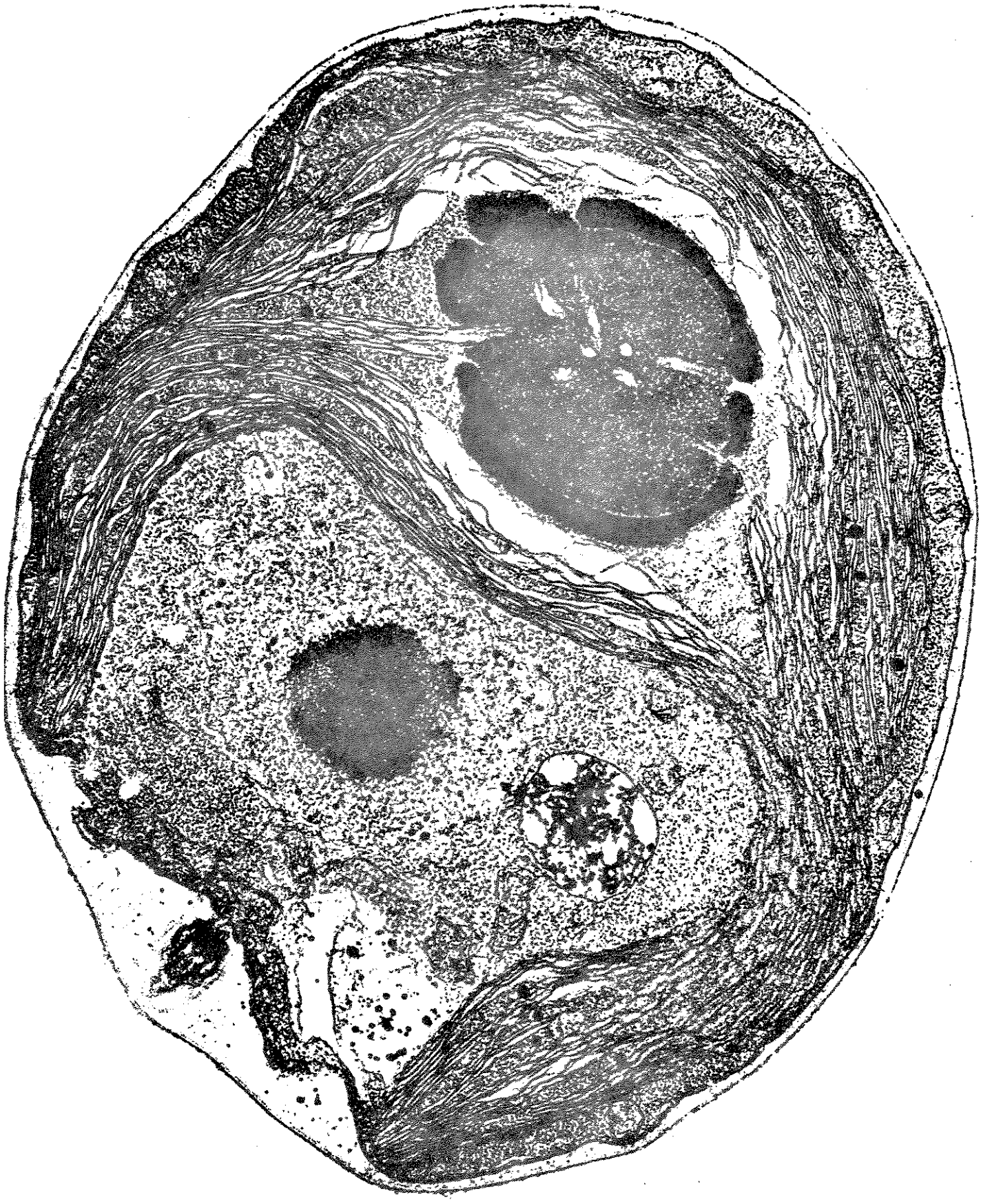
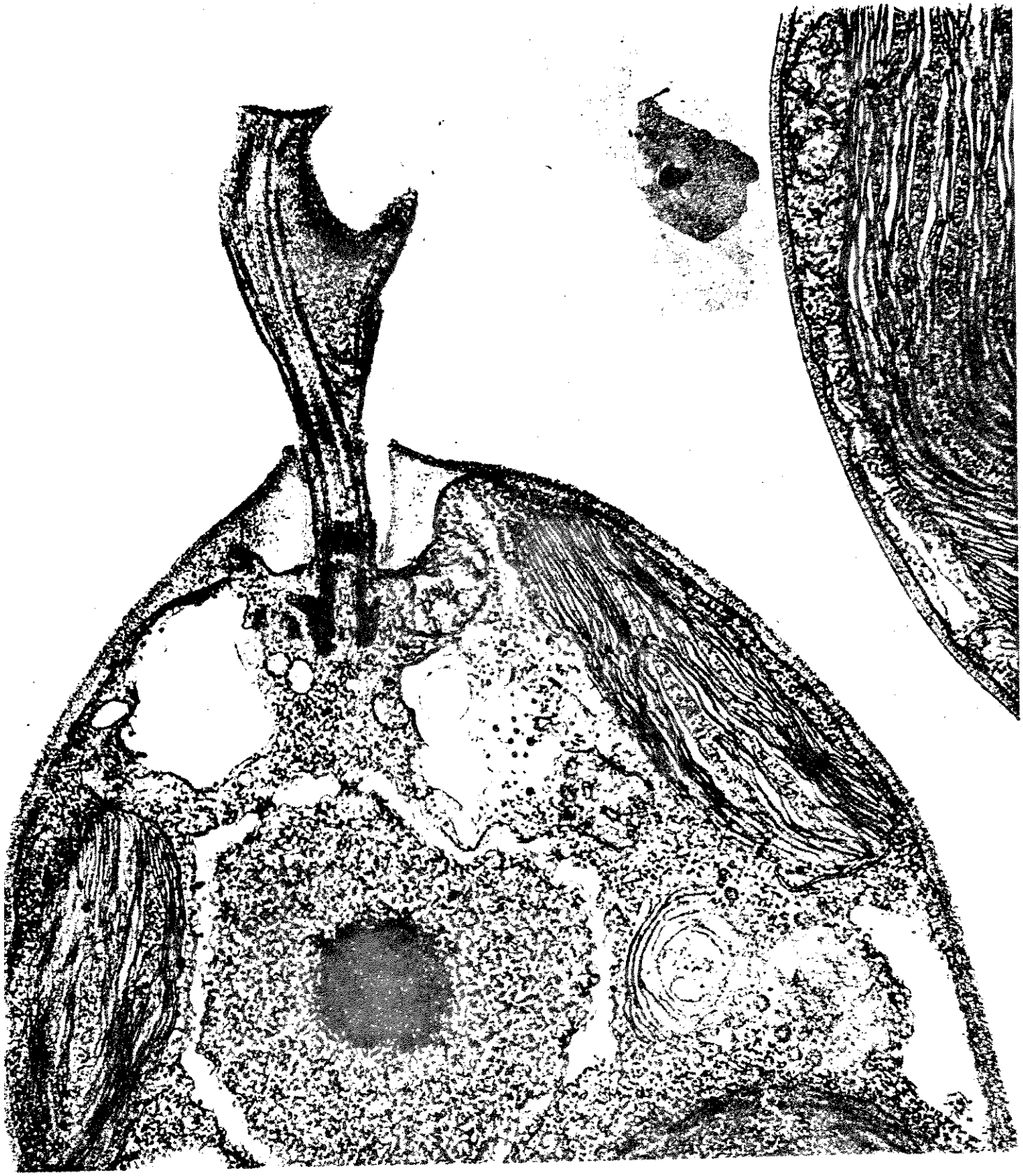


Plate I



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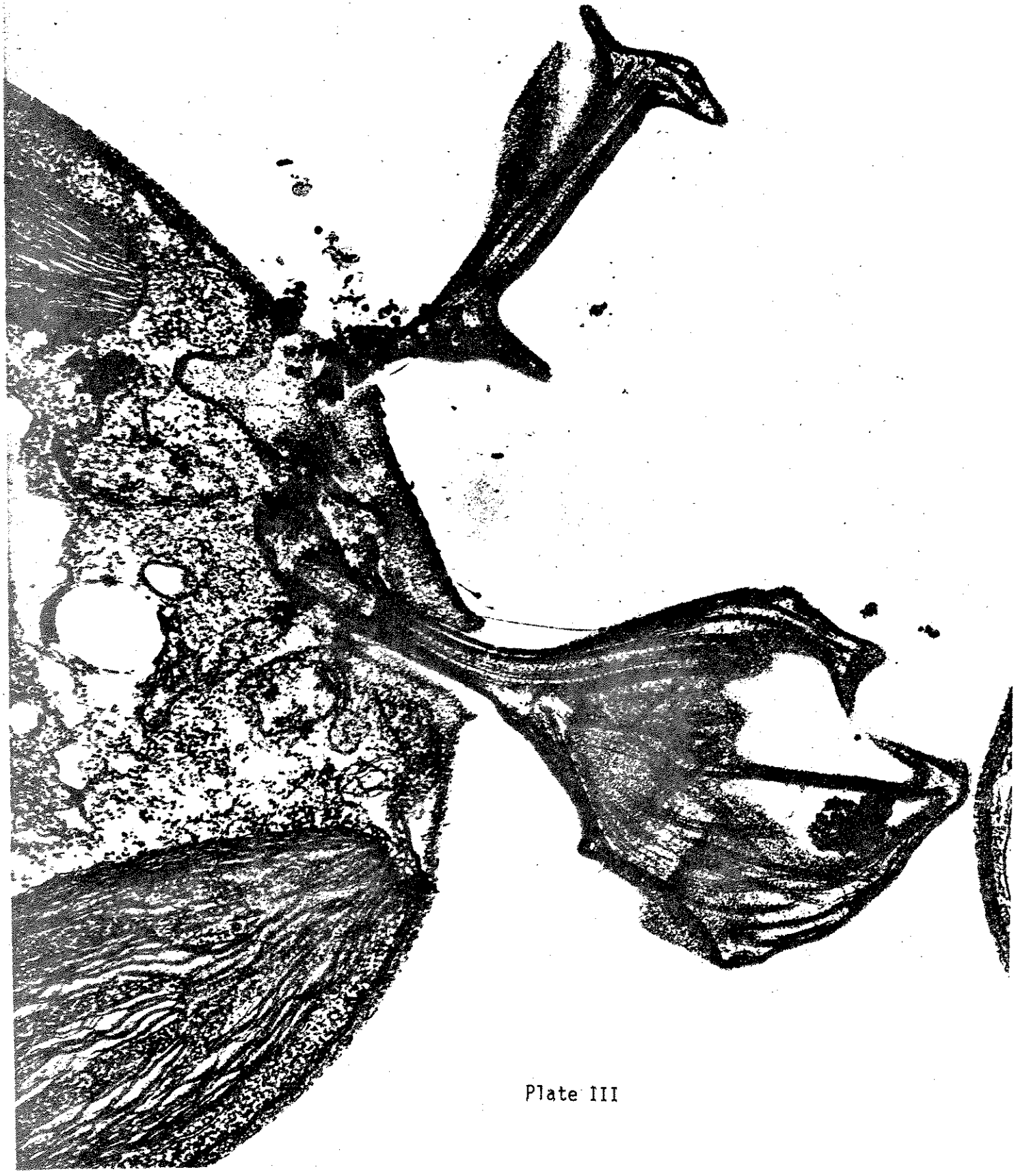

Plate III

PERSPECTIVES SUR LES FACTEURS FAVORISANT LA MÉTHYLATION BACTERIENNE DU MERCURE DANS LE CAS DU RÉSERVOIR DE LA RIVIÈRE TONGUE ET D'AUTRES ENVIRONNEMENTS A EAUX DOUCES

G.R. Phillips et P.A. Medvick
Montana State University

RÉSUMÉ - Les brochets (Essox lucius) du réservoir de la rivière Tongue, dans le sud-est du Montana, accumulent de fortes concentrations de mercure dans les muscles axiaux (jusqu'à 2.47 µg Hg/g en poids humide), bien qu'il y ait assez peu de mercure dans les sédiments (0.003-0.975 µg Hg/g en poids sec) et dans la colonne d'eau (0.05 µg Hg/l). Nous supposons donc que les conditions régnant dans le réservoir sont très favorables à la conversion du mercure inorganique en méthylmercure, qui est accumulé dans les tissus des poissons. Cette conclusion est appuyée par le fait que la marigane blanche (Pomoxis annularis) de la rivière Tongue, en aval du réservoir, accumule beaucoup plus lentement le mercure que sa congénère du réservoir.

Les facteurs accélérant la méthylation du mercure dans le réservoir comprennent: (1) le taux élevé de productivité et par conséquent, des conditions favorables à la prolifération bactérienne; (2) des périodes de débit accéléré qui libèrent le méthylmercure des sédiments de la rivière et mettent en suspension les particules organiques dans l'eau, créant ainsi des substrats pour la profilération bactérienne; (3) un mélange des eaux permettant de garder des conditions aérobies au fond de l'eau et empêcheant la stratification thermique. Ce troisième point se trouverait à supprimer la réduction du soufre, réaction tendant à précipiter le mercure, et à promouvoir l'activité bactérienne à l'interface sédiments - eau. Nous avançons l'hypothèse que les poissons de certains bassins peuvent accumuler de fortes concentrations de mercure dans leurs tissus, même en l'absence de sources anthropogènes de mercure.

PERSPECTIVES ON THE FAVORABILITY OF THE TONGUE RIVER RESERVOIR AND OTHER FRESHWATER ENVIRONMENTS FOR BACTERIAL METHYLATION OF MERCURY

G.R. Phillips and P.A. Medvick
Cooperative Fishery Research Unit Department of Biology
Montana State University

SUMMARY - Northern pike (Essox lucius) from the Tongue River Reservoir in Southeastern Montana attain high concentrations of mercury in axial muscle tissue (up to 2.47 µg Hg/g; wet basis) although relatively low mercury concentrations are present in the sediments (0.003-0.075 µg Hg/g; dry basis) and in the water colum (<0.05 µg Hg/1). Intuitively, we conclude that conditions in the reservoir are highly favorable for the conversion of inorganic mercury to methylmercury and subsequent methylmercury accumulation by fish. This conclusion is substantiated by the fact that white crappie (Pomoxis annularis) from the Tongue River downstream from the reservoir accumulate mercury at a significantly alower rate than the same species in the reservoir.

Features of this reservoir that probably enhance methylation of mercury include: (1) a high level of productivity and thus nutrient status conduscent to microbial growth; (2) high flow events that mobilize methylmercury produced in river sediments and suspend organic particulates in the water column thus creating substrates for bacterial growth; (3) sufficient mixing to maintain aerobic bottom waters and prevent thermal stratification. The third feature would suppress sulfur reduction which tends to precipitate mercury and promote bacterial activity at the sediment-water interface. We submit that fish in some waters can attain high concentrations of mercury even in the absence of an anthropogenic source of mercury.

Perspectives on the Favorability of the Tongue River Reservoir and other Freshwater Environments for Bacterial Methylation of Mercury

by

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[1]The Unit is jointly supported by the Montana Department of Fish Wildlife and Parks, the Biology Department at Montana State University, and the U.S. Fish and Wildlife Service.

INTRODUCTION

Methylmercury is the predominant mercurial present in fish tissue (Westöö 1973) and is the form responsible for neurological disorders in humans (Clarkson 1973). Bacteria present in natural waters can convert inorganic mercury to methylmercury (Jensen and Jernelöv 1969); thus, given suitable conditions, any mercurial can become hazardous.

Measurements of methylmercury in natural waters have been unattainable until very recently because of the very low concentrations present. Currently, we are aware of only one laboratory that is measuring methylmercury in natural waters and this in the highly contaminated Clay Lake-Wabigoon River System of southwestern Ontario (Park et al. 1980). Nonetheless, fish can concentrate methylmercury to measurable quantities and thus can be used as an index of how much methylmercury is present in water.

In this paper we report our observations on mercury uptake by fishes from the Tongue River and Tongue River Reservoir[2] (Fig. 1) in southeastern Montana relative to total mercury concentrations in the reservoir's sediments and water. Limnological characteristics of the reservoir are compared to conditions known to favor methylation of mercury and the potential for mercury methylation in this and other freshwater environments is discussed.

Fig. 1 near here

[2]Although surface coal mining is occurring near the Tongue River Reservoir, mining is not affecting mercury dissolution to the reservoir and therefore further discussion of mining is not relevant to this report.

MATERIALS AND METHODS

Sampling and analytical

Fishes, including northern pike (Esox lucius) and white crappie (Pomoxis annularis), were collected using trap nets or gill nets. Shortly thereafter fish were weighed and measured; a portion of axial muscle tissue was removed, placed in a whirl-pak bag, and frozen. Sediment samples were collected with an Ekman dredge, frozen in whirl-pak bags, and stored. Sediments were prepared for analyses by thawing, drying at 45-60°C, and pulverizing with a mortor and pestle. Water was collected with a VanDorn sampler, transferred to 500 ml glass bottles, preserved with nitric acid and potassium dichromate, refrigerated, and analyzed for mercury within 21 days. Water samples were oxidized with potassium permanganate and potassium persulfate prior to analysis.

Total mercury concentrations in fish tissue, sediments, and water were determined with a Varian model AA-6 atomic absorption spectrophotometer equipped with a carbon rod atomizer. Fish tissue and sediment samples were burned in a combustion chamber and aqueous mercury was vaporized. The evolved mercury was collected on a porous gold-plated tube, the tube was heated in the atomizer, and the resulting absorption signal was measured (Siemer and Woodriff 1974). Accuracy of analyses were verified by known duplicates (2-5% of samples), blind duplicates (5-10% of samples), spike and recovery (5-10% of samples), U.S. Environmental Protection Agency certified water samples, and National Bureau of Standards albacore tuna. Using our methodology, over the range of concentrations encountered, precision was estimated at ±0.01 µg Hg/l for water and ±0.05 µg Hg/g for tissue and sediment.

Limnological parameters

Limnological parameters measured included dissolved oxygen, temperature, and pH. Parameters were measured at three reservoir stations (Fig. 1); however the deepest pool located near the downstream end of the reservoir will be the only station discussed in detail. All parameters were measured twice monthly and at 2 m intervals of depth. Measurements were made using a Hydrolab Model 8000 water quality analyzer (Hydrolab Corp., Austin, Texas) that was calibrated before and after each days use. Calibration was with a standard mercury thermometer for temperature, against standard buffer solutions for pH, and relative to oxygen in water-saturated air (corrected for altitude and temperature) for dissolved oxygen.

Statistics

Regressions of fish length vs. mercury concentration in tissue were derived after a log transformation of mercury content. Degree of difference between regression lines was determined by F-test (Neter and Wasserman 1974). Student's t-test was used to compare sample means (Steel and Torrie 1960).

RESULTS

Mercury in water and sediments

During the last year (April-October 1980) we collected water samples at two week intervals from several locations in the Tongue River Reservoir and determined total mercury concentrations. Locations sampled included the inflow, the outflow, and surface, midwater, and bottom depths at three locations on the reservoir including upstream, mid-reservoir, and downstream (Fig. 1). Mercury concentrations in water from all locations were consistently low (Table 1). The average concentration for all locations combined was 0.02 µg Hg/l; no significant differences were seen between sampling locations and mercury concentrations (Student's t-test).

Table 1. Total mercury concentrations in water from various locations in the Tongue River Reservoir.

Location	n	Total mercury (µg Hg/l) mean	±SD
Reservoir inflow	12	0.01	0.01
Station 1			
surface	12	0.02	0.01
midwater	8	0.02	0.01
bottom	9	0.03	0.03
Station 2			
surface	12	0.02	0.01
midwater	12	0.02	0.01
bottom	12	0.02	0.02
Station 3			
surface	12	0.02	0.01
midwater	12	0.02	0.01
bottom	12	0.02	0.01
Reservoir outflow	12	0.02	0.01

Similarly, 176 surficial sediment samples collected from throughout the reservoir during 1979 averaged only 0.04 μg Hg/g (dry basis). The highest concentrations were associated with finer sediment particles located near the downstream end of the reservoir and the lowest concentrations were found in coarser sediments collected along the shores (Fig. 2). A similar relationship was noted for Lake Erie sediments (Thomas and Jaquet 1976) and was attributed to the higher affinity of mercury for fine clay and silt-like particles over coarser sands and gravels.

Fig. 2 near here

Mercury in fish

Notwithstanding, concentrations of mercury in some fish species from the reservoir are relatively high. Northern pike, in particular, contained up to 2.47 μg Hg/g (wet basis) in axial muscle. Data for males (Fig. 3) and females (Fig. 4) are shown separately because sex related growth differences resulted in different mercury uptake patterns. Mercury concentration in tissue increased with fish length. Data for northern pike, as well as other fish species in the reservoir, are best described by regressions of the form $\log_{10}$ Hg = a(length)-b. The logarithimic relationship probably results because older age classes cover increasingly shorter intervals of fish length. Plotting age vs. mercury concentration resulted in a linear fit (Phillips et al. 1980).

Figs. 3 and 4 near here

White crappie collected from the Tongue River immediately below the dam and from a location 52.3 river km downstream (near the town of Birney) accumulated mercury at significantly slower rates, ($p = 0.002$ and 0.001, respectively) relative to fish length, than white crappie from the reservoir (Fig. 5). Although age and growth information were not available for the river crappie, it is probably safe to assume that they grew slower than crappie from the reservoir and, thus, were not younger at a given size.

Fig. 5 near here

Reservoir limnology

Figs. 6 thru 8 near here

Seasonal isopleths (April thru October 1980) for several limnological parameters known to influence mercury methylation rates are shown in Figs. 6-8. In general, the Tongue River Reservoir is a well-mixed, eutrophic, warmwater impoundment. Primary productivity averages 1280 mg $C/m^{2} \cdot day^{-1}$ (Leathe 1980). Deep water withdrawal, a relatively fast turnover rate (mean = 67 days), and wind generated mixing maintained a relatively isothermal temperature profile (Fig. 6). However, even in the absence of a thermocline, the oxygen profile (Fig. 7) was clinograde during the summer months due to heterotrophic activity in the bottom waters combined with high oxygen production by phytoplankton in the euphotic zone. Considering the trophic status of the reservoir, this is a relatively mild oxygen deficit and is attributable to the mixing that was discussed previously. Although oxygen concentrations approached zero, bottom waters were not anaerobic.

The pH profile (Fig. 8) was similar to that for dissolved oxygen and reflected respiratory and photosynthetic processes that govern the equilibrium between free carbon dioxide, carbonate, bicarbonate, and carbonic acid. The pH of Tongue River Reservoir water is generally high (8-9) due to the high buffering capacity (high alkalinity) of the water. However, the heterotrophic consumption of oxygen and accompanying release of carbon dioxide resulted in bottom waters having lower pH values (7.7) during the warm summer months.

DISCUSSION

In comparison with other lakes and reservoirs for which information is available on mercury concentrations in both fishes and sediments (Table 2), the relatively high mercury concentrations in fishes from the Tongue River Reservoir are an anomaly. Although the fish species and the extent of information on mercury in sediments varied between studies, clearly the ratio between the maximum mercury concentration in fish and the mercury content of sediments is greater for the Tongue River Reservoir than for most of the other lakes and reservoirs.

Langley (1973) has shown that the mercury-methylating capacities of mercury-contaminated river sediments were more dependent on the ability of the sediments to promote microbial activity than on mercury concentration. Our observations lead us to believe that the physical and chemical characteristics of the Tongue River Reservoir are highly favorable for methylation of mercury. This conclusion is supported by the fact that white crappie from the Tongue River downstream from the reservoir accumulate mercury at a slower rate than the same species in the reservoir (Fig. 5). A similar relationship exists for walleye from Cookson Reservoir, Saskatchewan and from the Poplar River downstream from that reservoir (unpublished data, 1980).

Many of the limnological characteristics of the Tongue River Reservoir coincide with conditions shown to promote methylation of mercury in the laboratory. Methylation of mercury is inhibited by anaerobic conditions because of the concomitant sulfer-reducing activity that results in mercury precipitation as a sulfide (Park et al. 1980). However, low dissolved oxygen concentrations and increased water temperatures favor methylation of mercury (Bisogni and Lawrence 1975), apparently owing to

Table 2. Reports from the literature of maximum mercury concentrations in fish muscle tissue relative to mercury concentrations found in sediments from the same environment.

Location	Mercury in sediment (μg Hg/g)	Max. Hg in fish (μg Hg/g)[e]	Fish species	Reference(s)
Antelope Reservoir (Oregon)	17.1[a]	1.79	rainbow trout	Phillips and Buhler (1979); Hill et al. (1975)
Unspecified river (eastern Canada)	0.01-109.0[b]	7.0	not specified	Langley (1973)
Lake Mývatn (Iceland)	0.01-0.04[b]	0.016	arctic char	Ólafsson (1979)
Hemlock Lake (Michigan)	0.02-1.25[b]	0.42	rainbow trout	D'Itrie et al. (1971)
American Falls Reservoir (Idaho)	0.21-0.95[b,c]	1.20	rainbow trout	Kent and Johnson (1979)
Lake Powell Reservoir (Arizona)	0.30[d]	0.76	walleye	Potter et al (1975)
Lohontan Reservoir (Nevada)	0.12-1.35[b]	2.72	white bass	Richins and Risser (1975)
Clay Lake (Ontario)	0.14-7.83[b]	16.0	northern pike	Armstrong et al. (1972); Bligh (1970)
Section Four Lake (Michigan)	0.03-0.12[b]	0.45	rainbow trout	D'Itrie et al. (1971)
Lake Sangchris Reservoir (Illinois)	0.05[d]	0.30	green sunfish	Anderson and Smith (1977)
Southern Indian Lake Reservoir (Manitoba)	0.01[d]	0.51	walleye	Bodaly and Hecky (1979)
Tongue River Reservoir (Montana)	0.04[d]	2.5	northern pike	This Study
Lake Jocasse Reservoir (South Carolina)	0.04[a]	4.49[f]	largemouth bass	Abernathy and Cumbie (1977)

[a] Only one sample taken.

[b] Range.

[c] Wet basis.

[d] Mean.

[e] Reported for axial muscle on wet weight basis.

[f] Mean of largest size group.

increased microbial activity. The high temperatures and low oxygen concentrations that occur at the sediment-water interface in the Tongue River Reservoir during the summer, combined with the high level of biological productivity of overlying waters (thus settling of organics) appear to provide ideal conditions for bacterial methylation of mercury. The sediment-water interface was the major site of methylmercury production in Clay Lake, Ontario (Park et al. 1980). Further, the mildly oxidizing conditions near the bottom and concomitant lowering of pH further favor monomethylmercury formation over volatile dimethylmercury (Fagerström and Jernelöv 1972).

The river influence may also contribute to the accumulation of mercury by fishes in the Tongue River Reservoir. The Tongue River rises in the spring and early summer due to snowmelt in the high country. Methylmercury produced in river sediments may be transported to the reservoir at this time. Park et al. (1980) have shown that scowering of Wabigoon River sediments during high flow events mobilized methylmercury and increased the loading of that compound into Clay Lake. High flow also imparts considerable turbidity on the upstream end of the reservoir. Organic particulates suspended in the water column may create additional substrates for bacterial growth, thereby enhancing methylation of mercury.

We submit that some bodies of water may develop considerable mercury problems, owing to their physical and chemical characteristics, even in the absence of an anthropogenic source of mercury. Although little information is available on methylmercury concentrations in natural waters, insight into the relative capacities of different environments for methylation of mercury can be gained by comparing mercury concentrations in fishes (an index of methylmercury concentrations in water) to mercury

concentrations in sediments (an index of the total amount of mercury present).

ACKNOWLEDGMENTS

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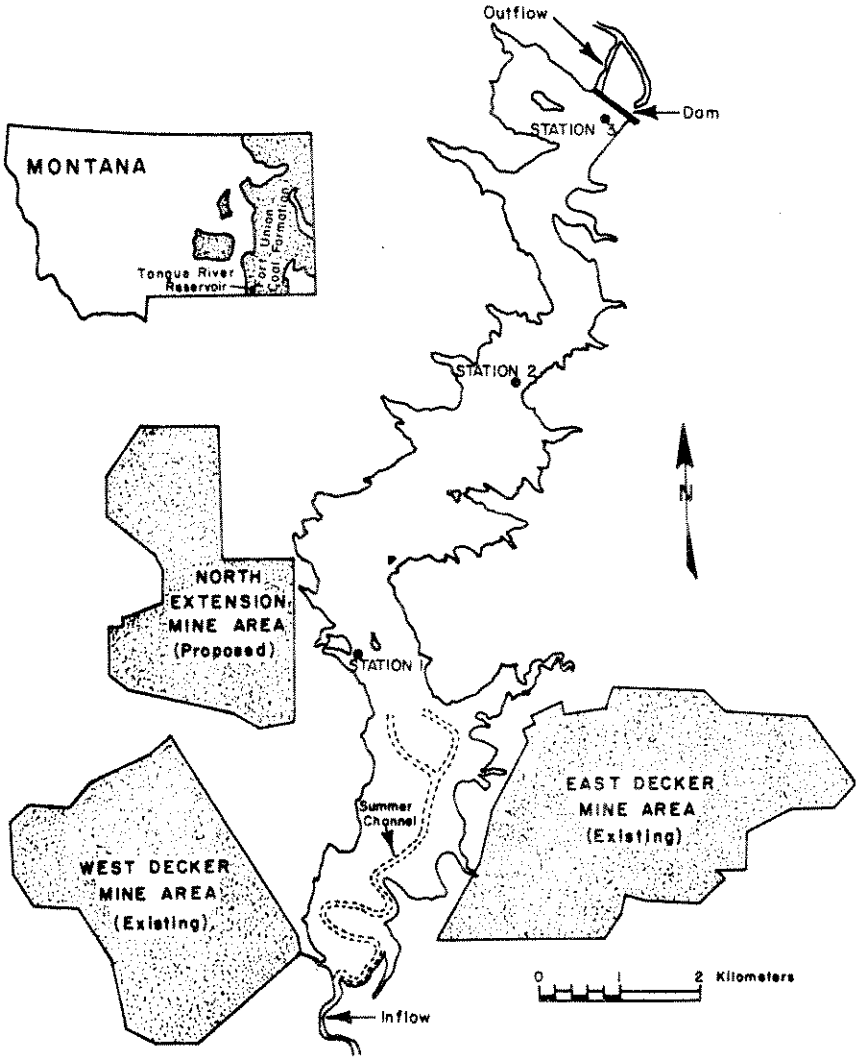
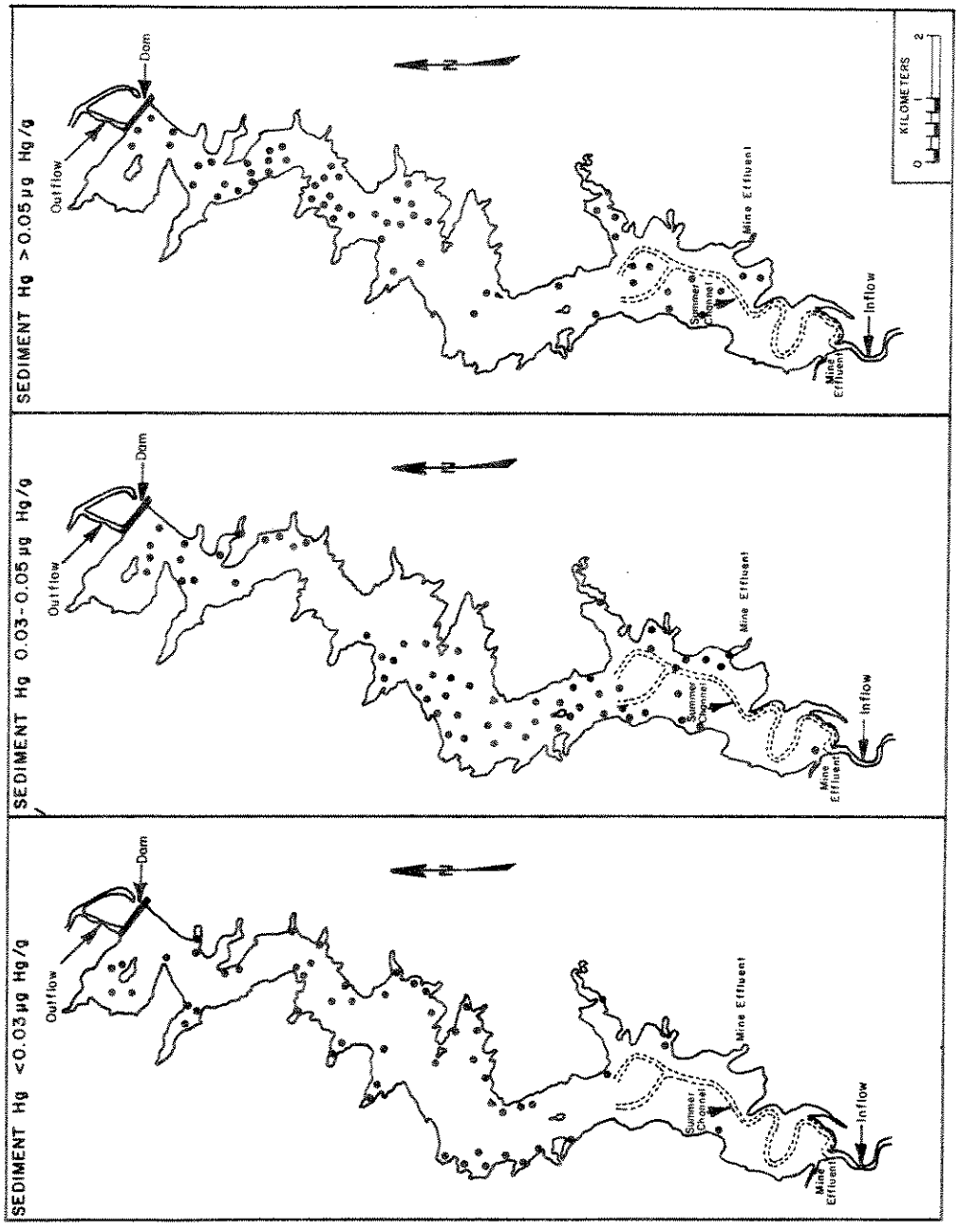
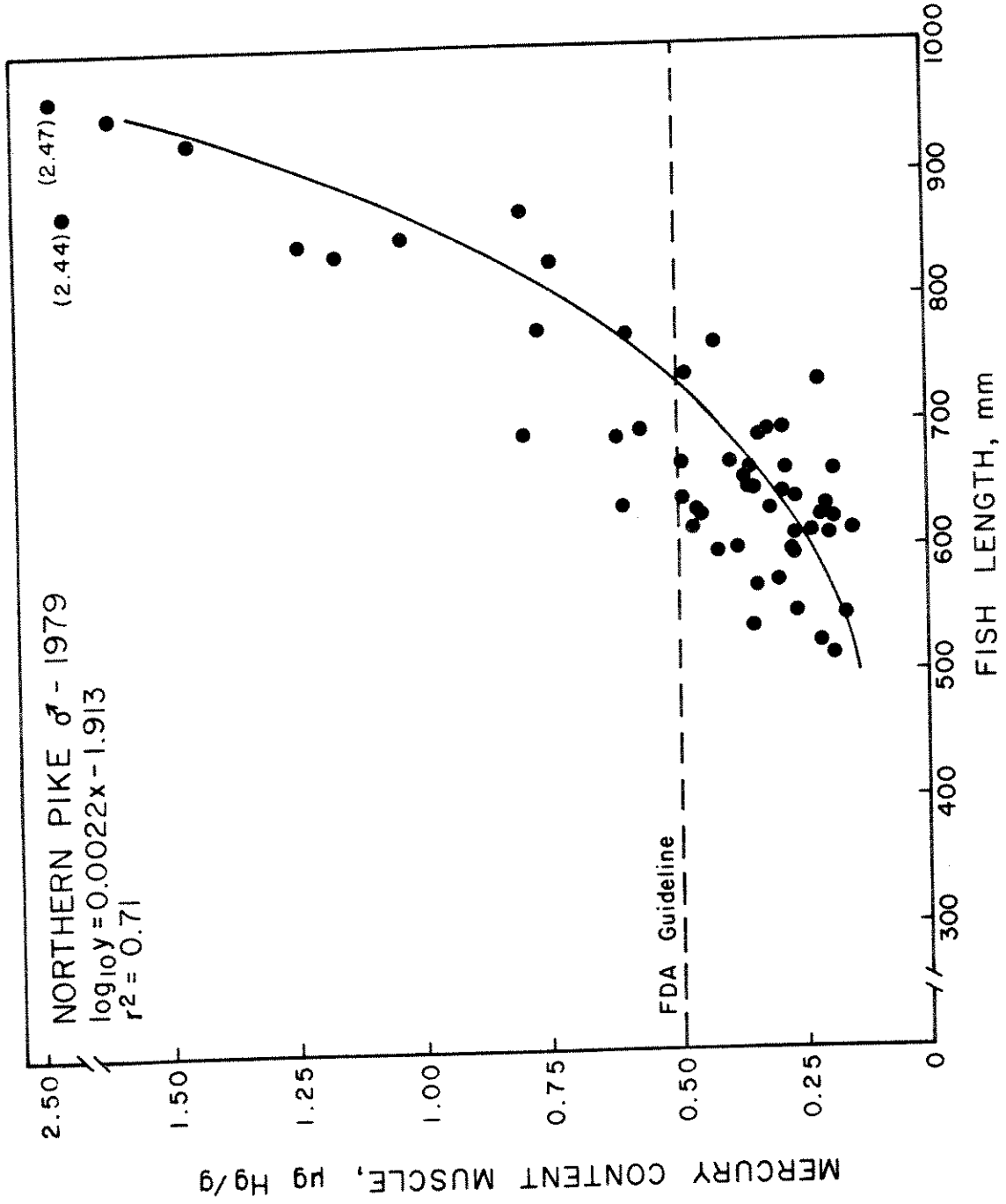
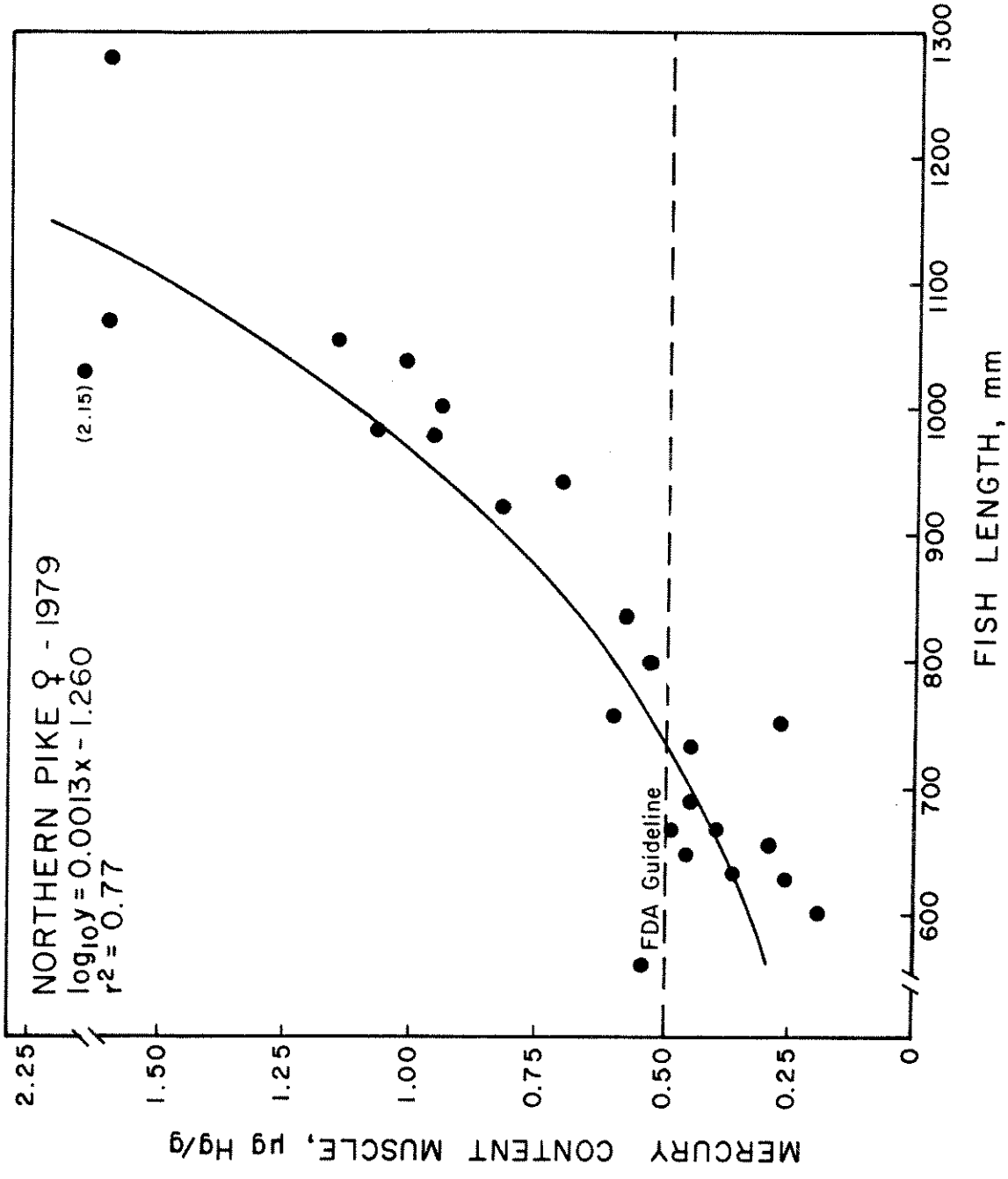
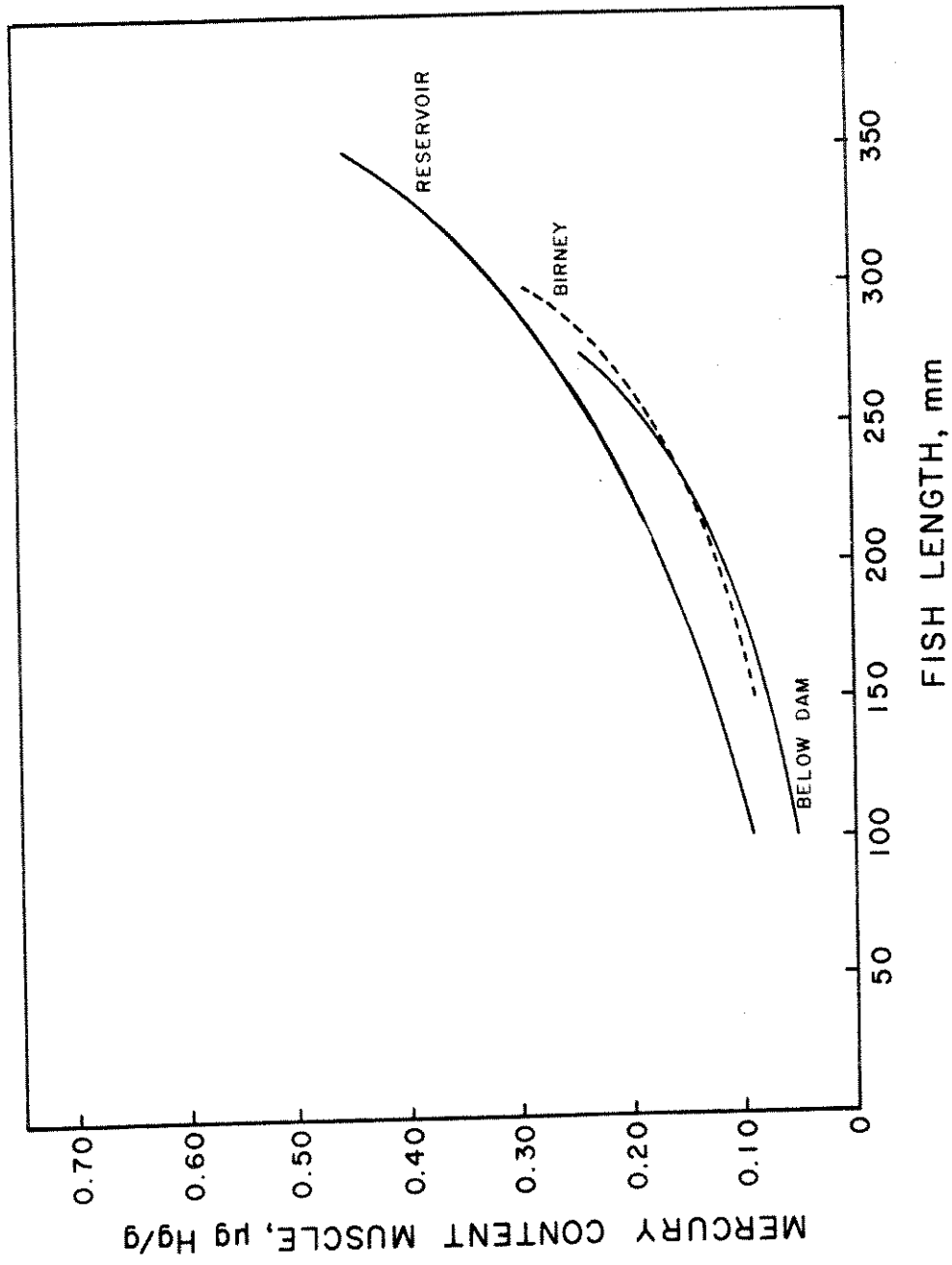
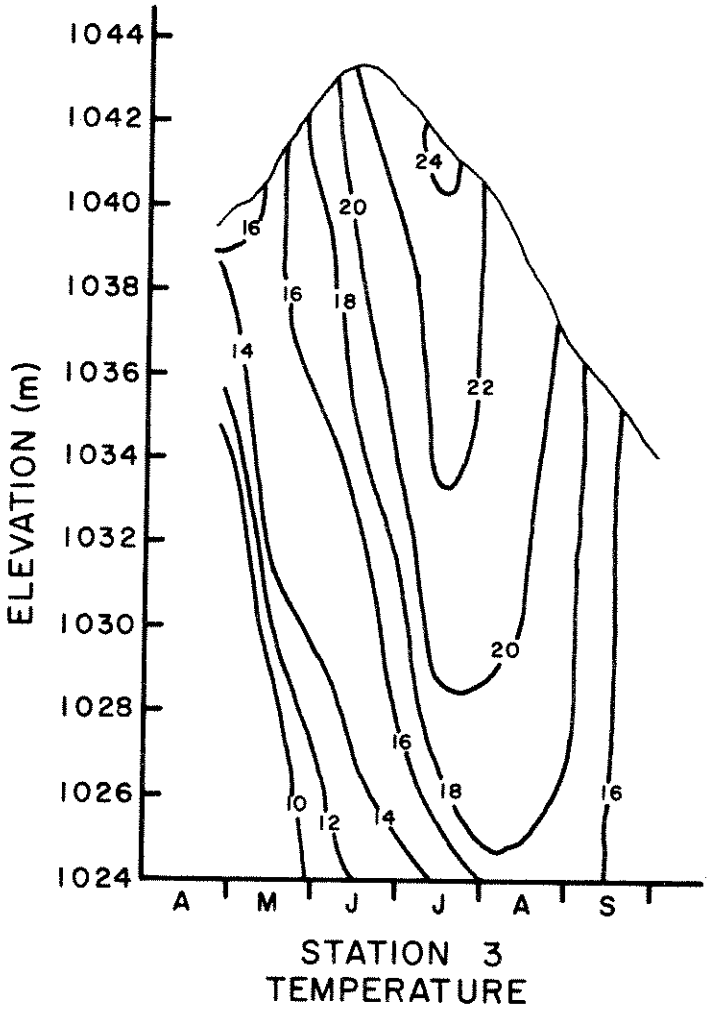

Outflow
Dam
STATION 3
MONTANA
Fort Union Coal Formation
Tongue River Reservoir
STATION 2
N
NORTH EXTENSION MINE AREA (Proposed)
STATION 1
EAST DECKER MINE AREA (Existing)
Summer Channel
WEST DECKER MINE AREA (Existing)
0 1 2 Kilometers
Inflow


SEDIMENT Hg <0.03 µg Hg/g
SEDIMENT Hg 0.03-0.05 µg Hg/g
SEDIMENT Hg >0.05 µg Hg/g
Outflow
Dam
Summer Channel
Mine Effluent
Inflow
KILOMETERS
0
1
2

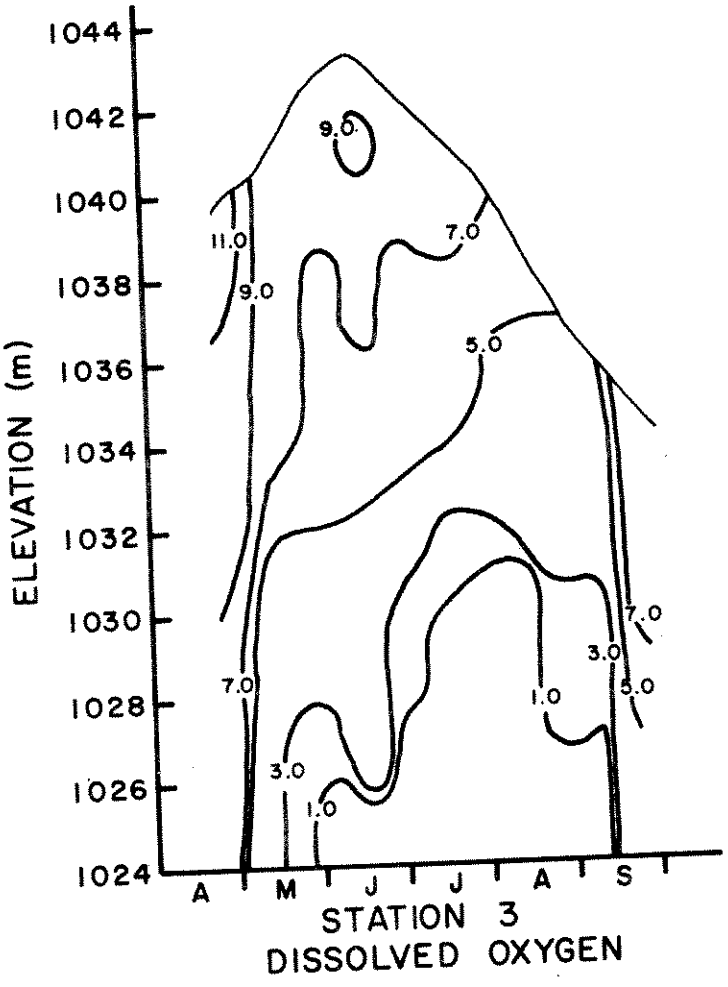

NORTHERN PIKE ♂ - 1979
log10y = 0.0022x - 1.913
r² = 0.71
(2.44)
(2.47)
FDA Guideline
MERCURY CONTENT MUSCLE, µg Hg/g
FISH LENGTH, mm
2.50
1.50
1.25
1.00
0.75
0.50
0.25
0
300
400
500
600
700
800
900
1000


NORTHERN PIKE ♀ - 1979
$\log_{10} y = 0.0013x - 1.260$
$r^2 = 0.77$
(2.15)
FDA Guideline
MERCURY CONTENT MUSCLE, µg Hg/g
FISH LENGTH, mm
2.25
1.50
1.25
1.00
0.75
0.50
0.25
0
600
700
800
900
1000
1100
1200
1300


RESERVOIR
BIRNEY
BELOW DAM
MERCURY CONTENT MUSCLE, µg Hg/g
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
50
100
150
200
250
300
350
FISH LENGTH, mm



STATION 3
TEMPERATURE



STATION 3
DISSOLVED OXYGEN

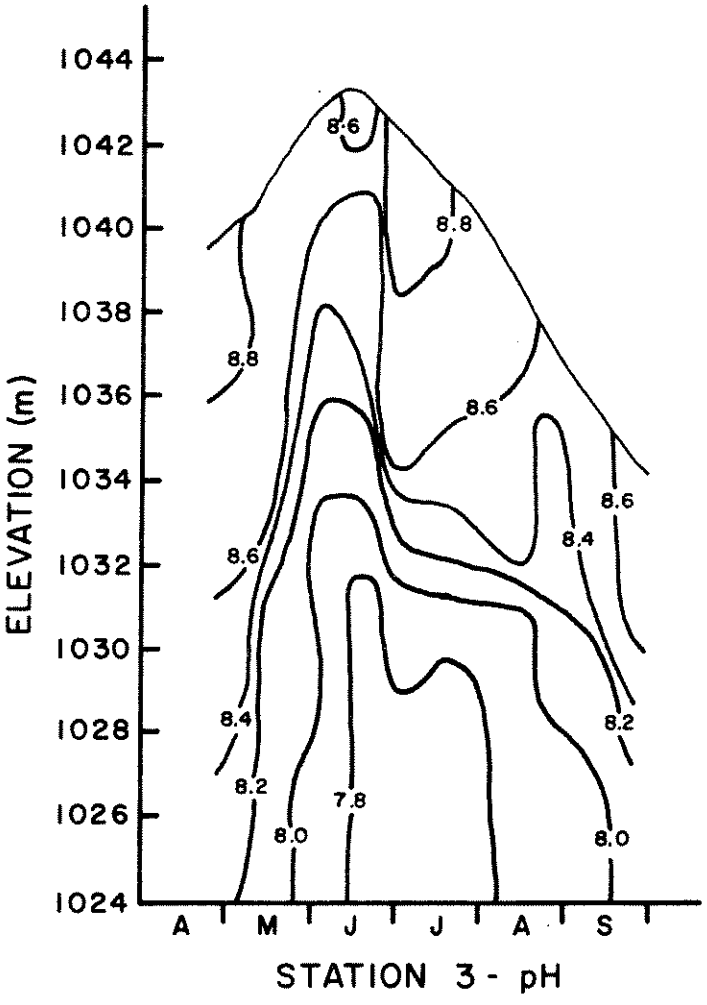

ELEVATION (m)
1044
1042
1040
1038
1036
1034
1032
1030
1028
1026
1024
A
M
J
J
A
S
8.6
8.8
8.8
8.6
8.6
8.4
8.6
8.4
8.2
8.2
7.8
8.0
8.0
STATION 3 - pH

FIGURE CAPTIONS

Figure 1. Map of the Tongue River Reservoir showing sampling locations for limnological measurements and mercury monitoring.

Figure 2. Map showing the distribution of mercury in the surficial sediments of the Tongue River Reservoir.

Figure 3. Relation between total fish length and mercury concentration in axial muscle tissue for male northern pike taken from the Tongue River Reservoir during spring and summer 1979.

Figure 4. Relation between total fish length and mercury in axial muscle tissue for female northern pike taken from the Tongue River Reservoir during spring and summer 1979.

Figure 5. Comparison of the relationships between total fish length and mercury concentration in axial muscle tissue of white crappie collected from the Tongue River Reservoir ($\log_{10}$ Hg = 0.0034 length -1.47; r^2 = 0.67; n = 35), from the Tongue River below the dam ($\log_{10}$ Hg = 0.0038 length -1.66; r^2 = 0.50; n = 30), and from the Tongue River near Birney ($\log_{10}$ Hg = 0.0040 length -1.73; r^2 = 0.64; n = 14) during 1978.

Figure 6. Temperature isopleths for the Tongue River Reservoir between April and October 1980.

Figure 7. Dissolved oxygen isopleths for of the Tongue River Reservoir between April and October 1980.

Figure 8. Isopleths for pH at the Tongue River Reservoir between April and October 1980.

#36/C

Le 5 novembre 1980 à 15:35 hres

ATELIER: A C T I V I T E I

L'UTILITE DES ESSAIS BIOLOGIQUES POUR PREDIRE LES RISQUES SUR LA SANTE

Selon le Conseil Consultatif Canadien sur l'Environnement, l'utilisation, la perte, et le rejet ultime de substances chimiques variées occasionnent l'infiltration dans l'environnement de celles-ci de façon massive et insidieuse. Il est impossible d'en localiser les effets à cause de leur dispersion rapide et de leur pouvoir d'envahissement. Par conséquent, l'environnement entier, en passant par l'homme, est en train de s'embourber dans un océan chimique. Le terme décrivant ce genre de souillure environnementale est l'écotoxicité.

Par ailleurs, le Conseil énonce qu'en élevant la toxicologie à un statut notable lors de la mise en place de politiques environnementales, la législation gouvernementale est inconsciemment devenue l'esclave des trois limitations principales de la toxicologie classique, soit, les substances chimiques individuelles, les effets aigus et les niveaux à concentration élevées. En toute justice à ceux qui élaborent les politiques, existe-t-il une alternative? Y a-t-il une science d'écotoxicologie?

Une des conclusions du Conseil stipule que le ministère de l'Environnement devrait prendre l'initiative afin d'assurer rapidement le développement de méthodologies faciles, activées et peu coûteuses, dont le but serait de déterminer les effets toxiques de contaminants environnementaux, particulièrement ceux soupçonnés de propriétés cancérigènes, mutagènes et tératogènes.

Cette recommandation est-elle concevable et pratique? Advenant le cas, comment ceci peut-il le mieux s'accomplir?

Cette vue est-elle trop restreinte? Si oui, vers quelle avenue devrait-elle s'étendre?

RAPPORT D'ACTIVITÉ DE L'ATELIER I

UTILITÉ DES ESSAIS BIOLOGIQUES POUR PRÉDIRE LES RISQUES SUR LA SANTÉ

K. Solomon
Officier rapporteur désigné

RÉSUMÉ - Après de longues discussions sur le sujet, le consensus ci-dessous a été obtenu.

En réponse à la question n^{o} 1, "L'écotoxicologie est-elle une science?", la réponse est oui, mais avec la restriction qu'il s'agit d'une science à ses débuts et, bien que personne ne semble qualifié dans cette science actuellement, on pouvait voir à cet atelier l'esprit qui anime cette science.

Pour ce qui est du développement d'essais nouveaux, simples et rapides pour vérifier les effets toxiques des contaminants, on était d'avis que:

1. On insistait trop sur la muta-, carcino- et tératogenèse, même si ces domaines devraient être étudiés en plus des autres.

2. Il n'y avait pas de solution pour remplacer les essais de toxicité chimique particuliers, car ceux-ci étaient considérés comme la première étape d'un système à plusieurs étapes d'essais de toxicité.

3. L'un des principaux points de la discussion était l'importance des essais d'écotoxicité pour les humains et selon les principaux arguments, ces essais étaient pertinents parce que la qualité de la vie et de l'écosystème est souvent plus sensible que le système humain et sert ainsi à établir les seuils d'exposition qui protégeront automatiquement les humains.. Par ailleurs, on a soutenu que les toxicologistes du système humain n'étaient pas

bien représentés à cette réunion et que nous ne disposions pas des connaissances nécessaires pour faire de telles extrapolations.

4. En bref, pour ne pas trop entamer la matière de la question 2, les autres discussions sur l'écotoxicologie étaient les suivantes:

   a) Les priorités relatives aux organismes devraient être décidées selon la biologie de la région de l'impact.

   b) La méthodologie et la technique détaillées des bio-essais devraient être évaluées en tenant compte des bio-essais passés, présents et futurs, pour faire en sorte que les résultats soient valides.

   c) Il faut utiliser des conditions et des concentrations d'exposition aussi réelles que possible dans les modes opératoires des essais à long terme.

   d) Il faut essayer de prévoir la toxicité chronique à partir des données des essais toxicologiques à court terme.

WORKSHOP ACTIVITY I

November 5, 1980 at 15:35 hr

USEFULNESS OF BIOASSAYS IN PREDICTING HEALTH HAZARDS

According to the Canadian Environmental Advisory Council, the "Use, waste and ultimate discard of chemicals in all forms pervade the environment in massive and insidious ways. Their rapid dispersion and invasive power make it impossible to localize their effects. Consequently, the whole environment, including humans, is being contaminated in a sea of chemicals. The term for this environmental defilement is ecotoxicity".

Furthermore the council states "Government legislation in elevating toxicology to a dominant place in environmental policy making, unwittingly absorbed the three dominant limitations of classic toxicology - single chemicals, acute effects and high dosage levels. In fairness to policy-makers, is there an alternative? Is there a science of ecotoxicology?".

A conclusion from the council is that "The Department of Environment should take initiative in ensuring the rapid development of simple, quick and inexpensive testing procedures for determining the toxic effects of environmental contaminants, especially those suspected of carcinogenicity, mutagenicity and teratoginicity".

Is this conceivable, practical and if so, how best can it be accomplished? Is this too restrictive? If so, how should it be expanded?

REPORT OF WORKSHOP ACTIVITY I

USEFULNESS IN PREDICTING HEALTH HAZARDS

K. Solomon
Mandated Reporting Officer

There was considerable and varied discussion on the subject but the following consensus was obtained;

In answer to question #1, "Is there a science of ecotoxicology?", the answer was yes, but, with the proviso that this science was very much in the formative stages at present and, while no particular person was seen to be qualified in this science at present, the spirit of this science was seen at this workshop.

With regard to the development of new, rapid and simple tests for toxic effects of contaminants, it was felt that:

1. Undue emphasis was placed on muta-carcino- and teratogenesis although these areas should be studied in addition to others.
2. There is not an alternative to single chemical acute tests as these were seen to be the very necessary first steps in a tiered system of toxicity testing.
3. A major area of discussion was the relevance of ecotoxicity testing to humans and main points of this discussion were that it is relevant because of the quality of life and the ecosystem is in many cases more sensitive than the human system and thus serves to set thresholds of exposure which will automatically protect humans. Another point made in this regard was that the human toxicologists are not well represented at this meeting and we lack the knowledge to make these extrapolations.
4. Without overlapping too much into the subject of question 2, other discussions on ecotoxicology were:

   (a) Organism priorities should be decided according to the biology of the

impact area.

(b) Methodological and technical details of bioassays should be critically evaluated in past, present and future bioassay to ensure that the results are valid.

(c) That realistic conditions and exposure concentrations should be used in all long-term testing procedures.

(d) An attempt should be made to predict chronic toxicity from short term toxicity test data.

Le 5 novembre 1980 à 16:35 hres

ATELIER: A C T I V I T E I I

CHEMINEMENT DES TOXIQUES DANS L'ENVIRONNEMENT

L'O.C.D.E a récemment discuté des conséquences de mesures législatives appliquées dans divers pays afin de réduire les effets nocifs des produits chimiques envers l'homme et son environnement. De telles mesures peuvent causer des problèmes commerciaux et des pertes économiques, puisque des règlements spécifiques à chaque pays sont ainsi élaborés. De plus, les procédures de tests sont si différentes que la comparaison des résultats serait difficile; tout ceci oblige donc les manufacturiers à présenter différents résultats de test qui ont essentiellement le même objectif.

L'O.C.D.E. se propose donc d'établir une série de tests qui pourraient être utilisés dans le but d'évaluer les effets toxiques potentiels de produits chimiques dans l'environnement; celle-ci proposerait une méthodologie qui correspondrait à un système d'étude "par étape" où des points de décision, permettant de passer d'une étape à l'autre, seraient définis.

Attendu que la séléction de tests dits appropries peut être définie à l'intérieur d'un concept de tests en séquence étapiste, ce qui permettrait d'éviter une duplication inutile du travail, d'orienter le fabricant et même le chercheur.

Attendu qu'il n'est pas possible d'établir des méthodes d'analyse

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standard appropriées à tous les produits chimiques, à cause de la spécificité des effets de chaque produit chimique dans un environnement particulier.

Quel serait le concept d'étude écotoxicologique qui devrait être élaboré lors de la mise en marché d'un nouveau produit chimique?

Mots Clefs:

Nature du produit chimique.

Espèces biologiques.

Effets anticipés

Reproductibilité

Aspects économiques et politiques etc.,

RAPPORT D'ACTIVITE DE L'ATELIER II

CHEMINEMENT DES TOXIQUES DANS L'ENVIRONNEMENT

Kern Marshall
Officier rapporteur désigné

RESUME - L'atelier II a été marqué par une confusion remarquable à propos du sujet à discuter. Le titre de l'atelier avait suggéré à un certain nombre de personnes un certain nombre d'idées qui ne correspondaient pas au thème présenté dans le texte.

Le texte se terminait par la question: "Quels devraient être les principes de base des essais écotoxicologiques d'un nouveau produit chimique?"
Je vais tenter de résumer et d'interpréter les discussions dans les petits groupes pour voir si une réponse peut être trouvée à cette question.

Il semble y avoir dans les divers groupes un consensus général appuyant le texte, selon lequel des essais et des modes opératoires normalisés sont requis. Toutefois, il a été souligné qu'à titre de toxicologistes de l'environnement, nous sommes confrontés à deux problèmes. D'abord, il y a le problème des nouveaux produits chimiques placés sur le marché international chaque année. Deuxièmement, nous sommes confrontés au problème de la découverte des produits chimiques déjà dans l'environnement qui peuvent constituer un danger (ou sont déjà reconnus comme tels). Ces deux problèmes sont rendus plus difficiles par le grand nombre de nouveaux produits chimiques créés en laboratoire et découverts dans l'environnement chaque année.

Si nous commençons à appliquer des séries d'essais normalisés pour résoudre ces problèmes, nous devons d'abord répondre à certaines questions fondamentales au sujet de ces essais:

Est-ce que la série d'essais sera acceptable pour les scientifiques qui effectuent les essais, pour les législateurs qui demandent ces essais, pour les fabricants des produits à l'étude qui doivent effectuer les essais et pour le public qui devra s'accommoder des produits chimiques qui sont jugés "sans danger" à la suite des essais?

Qui va interpréter les résultats des séries d'essais normalisés? Les scientifiques, les législateurs, les fabricants et le public sans connaissances techniques du pays qui exporte ces composés ou leur vis-à-vis dans le pays qui importe ces produits? Il faut noter que cette question ne s'applique pas seulement aux nouveaux produits chimiques, encore dans leurs contenants, mais aussi aux produits chimiques qui ont été libérés dans l'environnement et qui ne respectent pas les frontières politiques ou économiques.

Est-il possible de développer un ensemble d'essais qui représente la diversité des climats et des écosystèmes qu'on trouve sur la terre? Ou, autrement dit:

Est-ce que chaque essai choisi, et la série dans son ensemble, est crédible du point de vue environnemental et acceptable du point de vue écologique?

Finalement, est-ce que les essais normalisés le seront par rapport au plus haut ou au plus bas commun dénominateur? C'est-à-dire: Est-ce que les critères pour l'acceptabilité des résultats d'un essai seront fixés à la valeur la plus sévère, ou la plus faible, actuellement en vigueur?

Si nous avions plus de temps, nous aurions peut-être pu nous entendre sur les réponses à ces questions, ou du moins rechercher les bonnes réponses à celles-ci. Toutefois, le rapport de ces discussions ne fournit pas de réponses, non seulement à cause du manque de temps, mais aussi à cause de la confusion que nous avons notée à ce propos.

Bien que certaines personnes se soient posées les questions que j'ai mentionnées ci-dessus et les aient étudiées, d'autres ont proposé une approche pour les essais, et la plus grande partie du temps de l'Atelier I a été consacrée à cette approche.

Ces derniers semblent favoriser une série d'essais basés sur un système à plusieurs étapes. Tous les composés seraient soumis à des essais rapides à la première étape, qui filtreraient les composés présentant les plus grands dangers.

La première étape nécessiterait l'évaluation du risque présenté par un produit chimique lors de sa fabrication, de son stockage, de son transport, de son utilisation et enfin, de son rejet final. Une partie de cette évaluation devrait comprendre des informations précises sur les propriétés physiques et chimiques du composé, et sur ses métabolites ou produits de dégradation.

Les étapes suivantes étudieraient les effets physiologiques et écologiques du composé à l'essai.

Il a été affirmé à plusieurs reprises que le simple essai de mortalité aiguë (succès/échec) et l'essai de CL_{50} étaient insuffisants pour évaluer un composé. Les effets sublétaux et chroniques doivent être étudiés avant qu'un composé soit déclaré "sans danger". Il a aussi été dit qu'il n'est pas nécessaire de mettre au point de nouveaux essais pour évaluer ces effets, car nous disposons probablement déjà des essais appropriés, ou des travailleurs dans d'autres domaines peuvent déjà les avoir mis au point. Il faut seulement appliquer l'essai pertinent au bon moment.

Plusieurs groupes ont noté que le mode opératoire d'essais ne doit pas être compliqué au point qu'il soit peu pratique économiquement. Les gens qui mettent au point les séries d'essais normalisés et qui appliquent ces essais seront indubitablement sujets à des pressions économiques et politiques à cause du temps, du coût, et

de la sélectivité des essais. Il a été suggéré par plusieurs personnes qu'un ensemble international d'essais obligatoires pourraient être élaborés, avec certains essais additionnels qui seraient requis avant l'acceptation finale par le pays dans lequel seront écoulés les produits chimiques. Par exemple, un composé peut être soumis à des essais de toxicité aiguë pour être conforme à la norme internationale, mais il doit être soumis à des essais sublétaux en eau douce froide pour être commercialisé au Canada et en eau salée tiède pour être commercialisé à la Jamaïque.

En outre, il a été mentionné que les scientifiques devront être impliqués dans des questions de responsabilité juridique si les essais ou l'interprétation de leurs résultats s'avèrent inadéquats et si le danger d'un composé a été sous-estimé.

Pour conclure, bien qu'un grand nombre d'idées importantes aient été exprimées dans différents groupes, nous sommes incapables de fournir une réponse complète à la question: "Quels devraient être les principes de base des essais écotoxicologiques d'un nouveau produit chimique?"

Toutefois, nous avons identifié certains des aspects complexes de cette question, et nous nous sommes posé d'autres questions qui devraient nous aider à explorer le problème auquel nous sommes confrontés, et nous avons discuté d'une méthode pratique pouvant être utilisée pour évaluer les produits chimiques.

Il se peut que cette question revienne à la surface lors de l'Atelier sur la toxicité aquatique de l'année prochaine.

November 5, 1980 at 16:35 hr

WORKSHOP ACTIVITY II

PATHWAYS OF TOXIC SUBSTANCES IN THE ENVIRONMENT

Recently, the O.E.C.D. discussed the consequences of regulatory measures taken in some countries to reduce the adverse effects of chemicals on man and the ecosystem. It was considered to be important that such measures should not lead to trade barriers and economic losses, because of differences in testing requirements in different countries. Divergent test procedures in different countries could make the comparison of test results difficult and also require manufacturers to perform several tests for essentially the same objective.

The task set to the Group was to establish which tests should be used for assessing potential effects of chemicals in the environment and to advise on testing that should be carried out at the different levels of a tierred system and on the decision points to be used for going from one level to another.

Because selection of appropriate tests may be defined in a concept of step sequence testing; to avoid unnecessary duplication of work and to give guidance to those developing and assessing chemicals.

Because it is not possible to devise standard test methods appropriate to all chemicals because of specificity of effects of each chemical in a particular environment.

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What should be the concept of ecotoxicological testing of a new chemical product?

Key Words:

Nature of Chemicals

Biological Species

Anticipated effects

Reproductibility

Economical & Political aspects etc.,

REPORT OF WORKSHOP ACTIVITY II

PATHWAYS OF TOXIC SUBSTANCES IN THE ENVIRONMENT

Kern Marshall
Mandated Reporting Officer

Workshop Activity II was marked by considerable confusion over the topic to be discussed. The title suggested a number of ideas to a number of people which did not correspond to the theme presented in the text.

The text ended with the question "What should be the concept of ecotoxicological testing of a new chemical product"? I shall try to summarize and interpret the discussion in the small groups to see if an answer can be found to this question.

There appeared to be a general concensus in the various groups agreeing with the text that standard tests and standard testing are required. However, it was pointed out that 2 problems face us as environmental toxicologists. First, we face the problem of new chemical products placed on the international market each year. Secondly, we face the problem of discovery of chemicals, already in the environment, which may, (or already do) pose a hazard. Both of these problems are made more difficult by the numbers of new chemicals created in the laboratory and discovered in the environment each year.

If we start to apply some series of standard tests to these problems, there are some very basic questions we must ask about the tests:

Will the series of tests be acceptable to the scientists who conduct the tests, to the legislators who require the tests, to the manufacturers of the compounds under study who must conduct to the tests and to the public who will have to cope with the chemicals which are deemed "safe" by the tests?

Who will interpret the results of the standard test series" Will it be the scientists, legislators, manufacturers and lay public of the country exporting these compounds or their counterparts in the country importing these compounds? It must be noted that this question applies not only to new chemicals, still in their containers, but also to chemicals which have been released to the environment and do not respect political or economic borders.

Can a group of tests be found which will represent the diversity of climates and ecosystems found on earth? To restate that:

Does each selected test, and the series as a whole, have environmental credibility and ecological acceptability?

Finally, will the standardized tests be standardized to the highest or lowest common denominator? By this it was meant; will the criteria for acceptability of the results of a test be set at the most stringent level currently existing, or at the lowest level currently existing?

Perhaps, if we had more time, we could agree upon answers to those questions, or at least look for answers to those questions. However, no answers appeared in the discussion as reported. This is due not only to the lack of time, but it is also due to the confusion we experienced on this topic.

Though some people asked the questions I've just stated, and puzzled over them some other participants forged on to suggested a test strategy. Indeed, the greatest amount of time seems to have been devoted to this, as in Workshop I.

This other number of participants appeared to favour a series of tests based on a tier system. All compounds would be tested quickly at the first tier which would reject those compounds of greatest hazard.

The first tier would involve assessing the risk or hazard a chemical could potentially pose in its manufacture, storage, transport, use, and ultimate disposal. Part of this assessment would have to include accurate information about the physical and chemical properties of the compound, and its metabolites or degradation products.

Higher tiers would consider the physiologic and ecologic effects of the test compound.

It was repeatedly stated that simple pass/fail acute mortality testing and LC_{50} testing is not enough to evaluate a compound. Sublethal effects and chronic effects must be considered before a compound is declared "safe". It was also stated that we may not need to develop new tests to assess these effects, we may already have the appropriate tests, or workers in other fields may already have the appropriate tests. All we need to do is apply the right test at the right time.

Several groups noted that the testing procedure must not become so cumbersome that it becomes economically unfeasible. The people that develop the standard test series and apply the tests will undoubtedly be subject to economic and political pressures because of the time, cost and, selective processes

of the tests. It was suggested by several people that an international set of mandatory tests could be developed, with some additional tests to be required prior to final acceptance by the country which will be the market for the chemicals. For example, a compound may be tested for acute toxicity to meet the international standard, but must be tested for sub-lethal effects in cold, fresh water to be marketed in Canada and in warm salt water to be marketed in Jamaica.

Also, it was mentioned that scientists will have to be involved in questions of liability if the tests, or the interpretation of the results proves inadequate and the hazard of a compound is underestimated.

To conclude then, though a great number of important ideas were expressed in the various groups, we were unable to completely answer the question posed: "What should be the concept of ecotoxicological testing of a new chemical product"?

However, we did identify some of the complexity of the question, we did ask some further questions whidh will help us explore the question presented to us, and we did discuss a possible practical process for assess chemicals.

Perhaps we may see this question presented to us again at next year's Aquatic Toxicity Workshop.

Le 6 novembre 1980 à 15:25 hres

ATELIER: A C T I V I T E I I I

PRISE DE DECISION SUITE À UN DESASTRE PETROLIER MARITIME

A) Introduction

Le "Deep Threat", navire de forage de la Panglobal Drill Ship qui forait depuis seize (16) jours au site A (annexe 1) a subi, le 1er août, une perte de circulation et a dû quitter le puits en raison des fuites de gaz.

Incapable de fermer la vanne d'éruption et ayant perdu tout espoir de contrôler le puits en surface, ils ont entrepris de forer un puits d'intervention. En étant optimiste, ce puits incliné devrait atteindre la profondeur nécessaire au colmatage en un mois.

B) Information sur l'éruption

Le puits produit 1000 M^3 (600 barils) d'huile brute légère par jour et à peu près 100 fois ce volume de gaz (mesuré à 1 atmosphère). L'huile fait surface quelques kilomètres SSW du puits et produit une traînée d'huile de 5 Km x 1 Km. Cette huile fraîche et non émulsifiée se retrouve à 80% dans une région de 1 Km^2 avec une épaisseur entre 0.1 et 0.3 Mm. De petits îlots d'huile de 1 Km^2 et de 0.1 Mm d'épaisseur parsèment les 10 à 20 kilomètres suivants en direction SSW.

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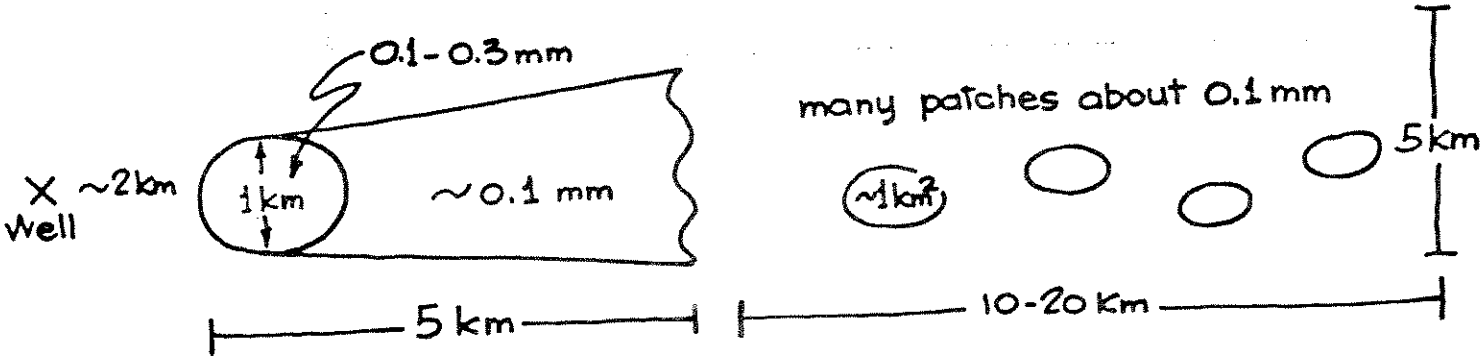
L'OSC vous fournit l'esquisse suivante de la situation (figure 1).



De plus, un vol de reconnaissance rapporte la présence de plusieurs îlots d'huile de quelques Km^2 situés à 100-120 Km SSW du puits.

C) Conditions météorologiques et océanographiques

Le navire de forage a effectué des mesures océanographiques sur le site mais, seul le profil de température est disponible et présenté ici en annexe 2.

Une tempête vient de traverser ce secteur avec des vents du NE de 60-70 Km/h. et une condition maritime de 6. Il y a maintenant des vents d'Est de 20 Km/h. et une condition maritime de 3. Aucun changement dans les conditions météorologiques n'est prévu pour les cinq (5) prochains jours.

D) Impact sur l'environnement

La compagnie a également fourni une carte localisant les différentes populations présentes dans ces régions à ce temps-ci de l'année (annexe 1).

E) Situation présente

Nous sommes le 8 août a.m. et vous êtes le comité consultatif sur l'environnement de l'OSC. Présentement, tout l'équipement anti-pollution est sur le lieu de résurgence, et celui-ci ne permet la récupération que de 20% de l'huile.

La compagnie ne peut fournir le matériel nécessaire au nettoyage des côtes; celui-ci ne leur ayant pas été livré. Dans l'immédiat, il y a sur place assez de dispersant pour alimenter un épandage massif durant une semaine. Si cette opération est déclenché dans l'heure qui suit, on prévoit une émulsification efficace à 80%. Dans le cas contraire, le tout est reporté de 24 heures et les premiers îlots d'huile risquent d'atteindre les côtes.

Les impacts sur les différentes populations de cette communauté écologique, évalués à la hâte par vos biologistes, sont résumés dans une page fournie à l'annexe 3.

Quelles seraient vos recommandations visant à préserver l'environnement et à quelle ressource accorderiez-vous une protection prioritaire?

EXPECTED IMPACT DATA SHEET

Ressources Resources	Avec dispersants With dispersants			Sans dispersants Without dispersants		
	A court terme Short term	A moyen terme Medium term (>1,<5 yrs) (>1,<5 ans)	A long terme Long term (>5 yrs) (>5 ans)	A court terme Short term	A moyen terme Medium term (>1 ,<5 yrs) (>1 ,<5 ans)	A long terme Long term (>1, < 5 yrs) (>1, < 5 ans)
Mammifères marins Marine Mammals	-	-	--	--	--	---
Oiseaux Birds	--	-	0	---	--	0
Benthos Benthos	0	0	0	0	0	0
Phytoplancton Phytoplankton	--	0	0	-	0	0
Zooplancton Zooplankton	--	0	0	-	0	0
Communauté des zones de marée Intertidal Community	-	0	0	---	--	-
Poisson (adulte) Fish (adult)	--	-	0	0	-	0
Icthyoplancton Icthyoplankton	--	-	0	-	0	0
Berges Shoreline	-	0	0	---	--	-

dommageable	--- -- -	detrimental
aucun impact	0	no impact
rehaussement	+	enhancement

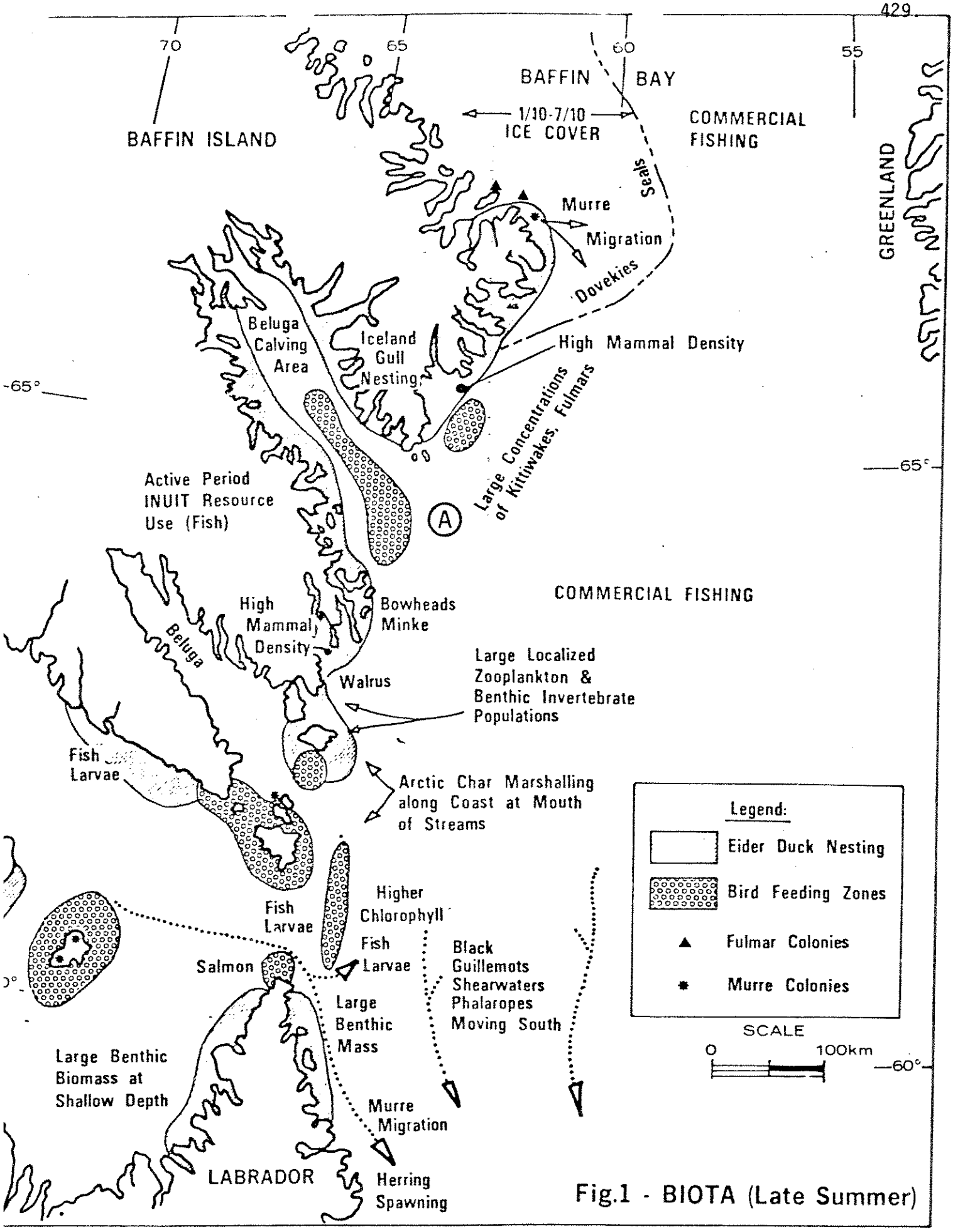
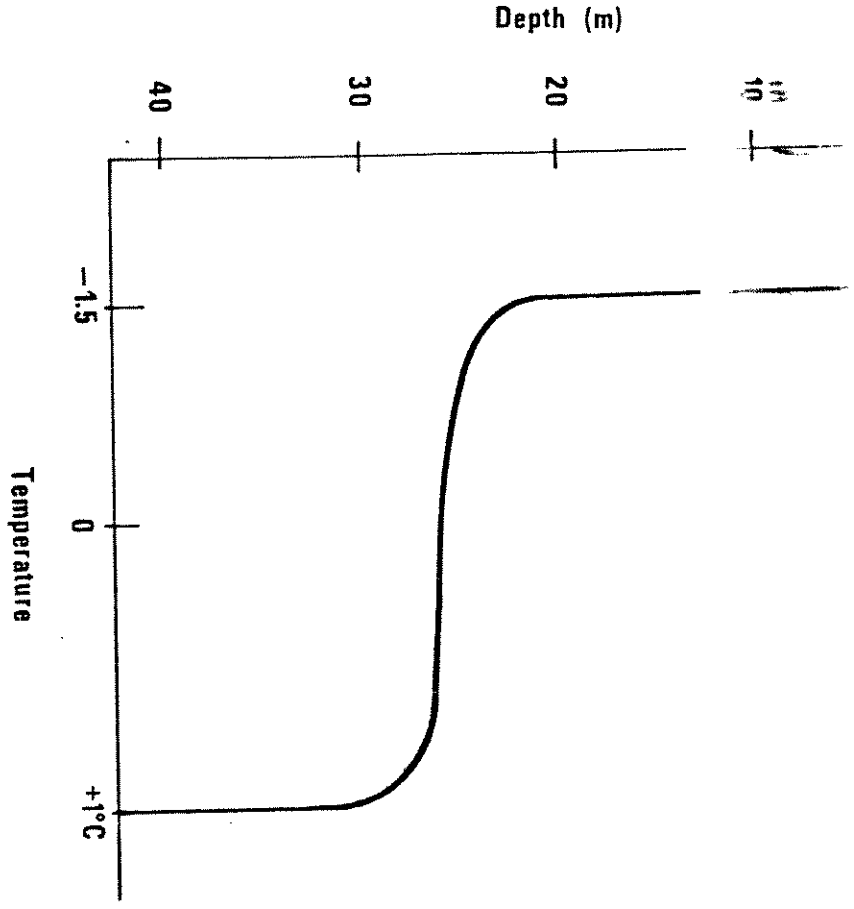


Fig.1 - BIOTA (Late Summer)



Temperature Profile at Blow-out site

RAPPORT D'ACTIVITÉ DE L'ATELIER III
PRISE DE DÉCISIONS SUITE A UN DÉSASTRE EN MILIEU MARIN

C. Rivet
Officier Rapporteur désigné

RÉSUMÉ - L'objectif de cet atelier était de mettre les participants en situation telle qu'ils devaient, en tant que membres d'un groupe d'experts, prendre rapidement une décision qui se doit d'être la plus logique considérant les données disponibles.

Tous les groupes ont d'abord fait une évaluation de la situation à l'aide de la carte fournit.

La deuxième étape fut, à l'aide du tableau (Expected impact data sheet) et de l'expérience personnelle, d'évaluer l'impact relatif sur les diverses ressources.

De là, la question proprement dite fut peut-être abordée non plus en terme de "Quelles ressources doit-on protéger?" mais plutôt "Quelles ressources doit-on sacrifier?".

Le consensus fut surtout de protéger la niche ou l'habitat plutôt que l'individu, d'éviter les effets à long terme plutôt que les impacts à court terme.

L'utilisation de dispersants fut donc favorisée à la lumière de l'efficacité décrite dans la plupart des ateliers mais avec des restrictions visant à protéger le littoral.

De plus, des considérations autres que strictement toxicologique ont été abordées tels que, l'impact sur le public, le coût de l'opération suggéré et les recherches à entreprendre pour mieux comprendre le devenir et les effets du pétrole en mer.

Note du rapporteur

Dans de tels cas, le groupe d'experts doit ne donner qu'une solution ainsi que les raisons motivant ce choix, les alternatives possibles et les raisons s'opposant à ces alternatives.

A toute fin pratique, le groupe d'experts doit être en mesure de dire que toutes les conséquences ont été pesées et que sa solution est celle du moindre mal.

W O R K S H O P A C T I V I T Y III

November 6, 1980 at 15:25 hr

DECISION MAKING SUBSEQUENT TO A MARINE ENVIRONMENTAL DISASTER.....

A. Introduction

The Panglobal Drill Ship "Deep Threat" has been drilling at Site A on the map provided as Fig. 1 for 16 days and, on August 1, lost circulation and was forced to move off the hole due to escaping gas. They were not able to close the BOP, have now totally abandoned that hole and are drilling a relief well. An optimistic estimate of the time to reach a suitable depth to shut down the flow is 1 month.

B. Blowout Characteristics

The well is producing 1000 m^3 (600 bbl) of light crude oil/day and approx. 100 times that volume of gas (measured at 1 atmosphere). The oil is surfacing, over an area of 5 sq. km, a few km SSW of the blowout site. This patch is about 1 km wide. This oil is fresh, not emulsified and most of the oil (80%) is localized in an area about 1 km^2, with a thickness of about 0.1-0.3 mm. There is also a visible oil trail about 10-20 km away. It is in many patches about 1 km^2 in area and the average thickness is estimated to be as approx. 0.1 mm.

The OSC has provided a sketch of the situation.

E. Present Situation

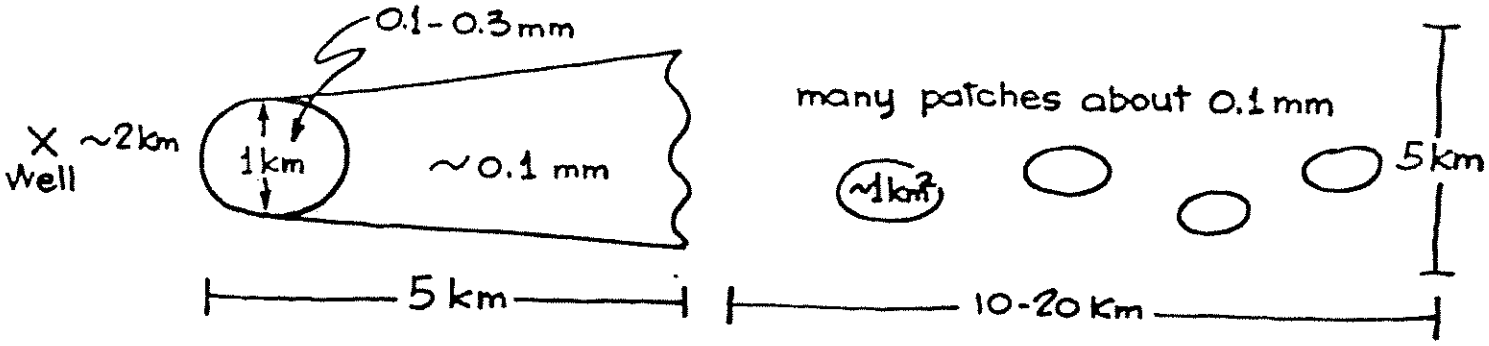
It is August 8 a.m. and you are the Environmental Advisory Team to the OSC. At this moment, all clean-up equipment at the surfacing site has been deployed recovering only 20% of the oil.

There is no shoreline clean-up equipment available from the company's stockpile. It was not delivered on time.

Sufficient dispersant is readily on hand for one week extensive spraying activity. 80% efficiency is expected in emulsifying the existing oil slicks if spraying is commenced immediately. If orders to spray are not given within one hour spraying activities would have to be retarded 24 hours and the first oil slicks could reach the shoreline.

The expected impact has been hastily evaluated by your local biologists and is summarized on the attached sheet.

What recommendations would you give to protect the environment and which ressource should be protected in priority?



In addition, there is a report of an oil sighting by reconnaissance aircraft of many patches of oil 100-120 km SSW of the well. These patches are a few km^2 in area.

C. Oceanography and Meteorology

The drill ship has been doing oceanography monitoring at the site but only the temperature profile is available and has been supplied to you.

A storm has just passed through the area with winds NE 60-70 km/hr and a seastate of 6. The winds are currently 20 km/hr from the East and a seastate of 3. The 5 day forecast is for no change.

D. Environmental Concerns

You have also been provided with the company's "Biota" map for this time of year.

Ressources Resources	Avec dispersants With dispersants A court terme Short term	A moyen terme Medium term (>1,<5 yrs) (>1,<5 ans)	A long terme Long term (>5 yrs) (>5 ans)	Sans dispersants Without dispersants A court terme Short term	A moyen terme Medium term (>1 ,<5 yrs) (>1 ,<5 ans)	A long terme Long term (>1, < 5 yrs) (>1, < 5 ans)
Mammifères marins Marine Mammals	-	-	--	--	--	---
Oiseaux Birds	--	-	0	---	--	0
Benthos Benthos	0	0	0	0	0	0
Phytoplancton Phytoplankton	--	0	0	-	0	0
Zooplancton Zooplankton	--	0	0	-	0	0
Communauté des zones de marée Intertidal Community	-	0	0	---	--	-
Poisson (adulte) Fish (adult)	--	-	0	0	-	0
Icthyoplancton Icthyoplankton	--	-	0	-	0	0
Berges Shoreline	-	0	0	---	--	-

dommageable	--- -- -	detrimental
aucun impact	0	no impact
rehaussement	+ ++ +++	enhancement

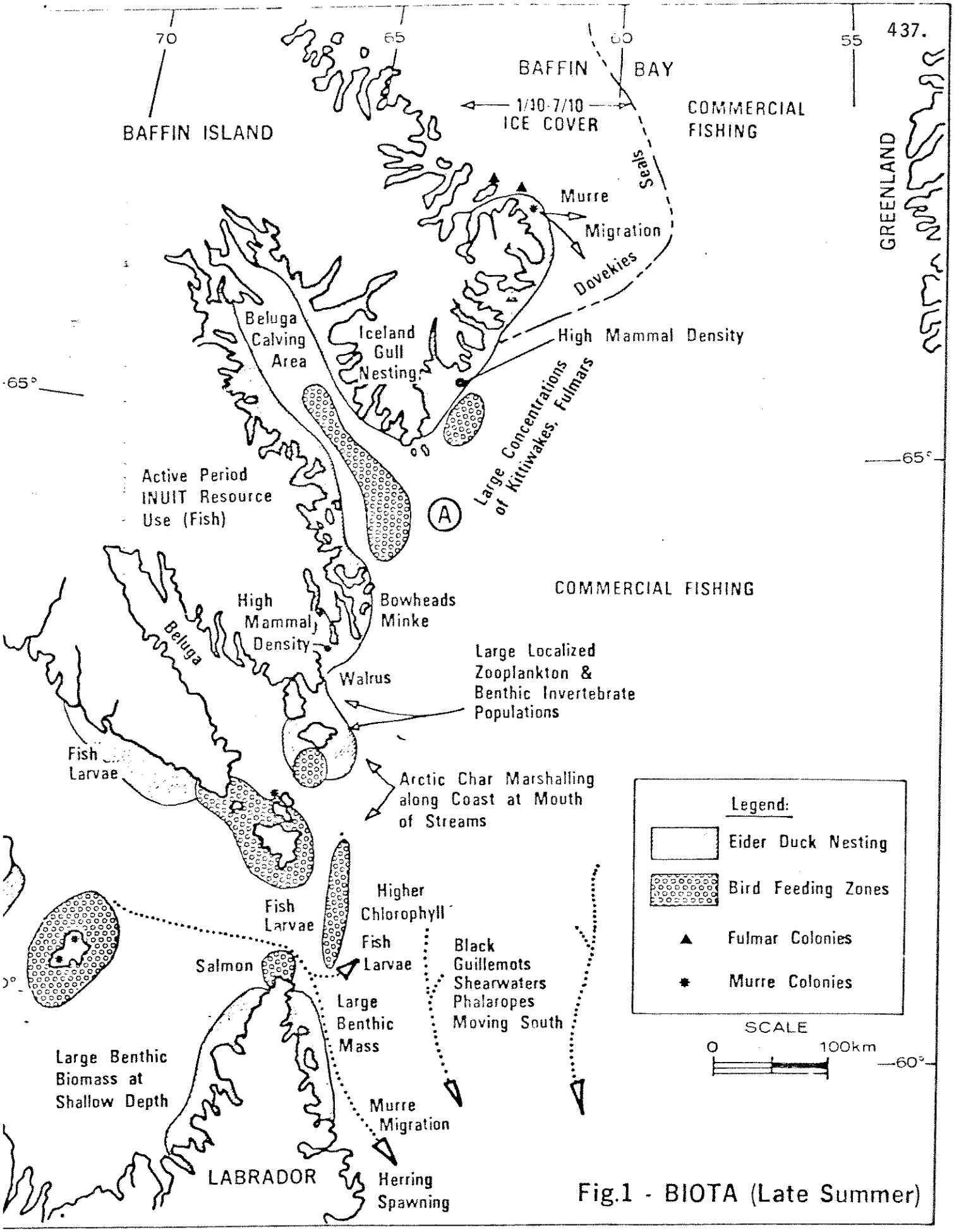
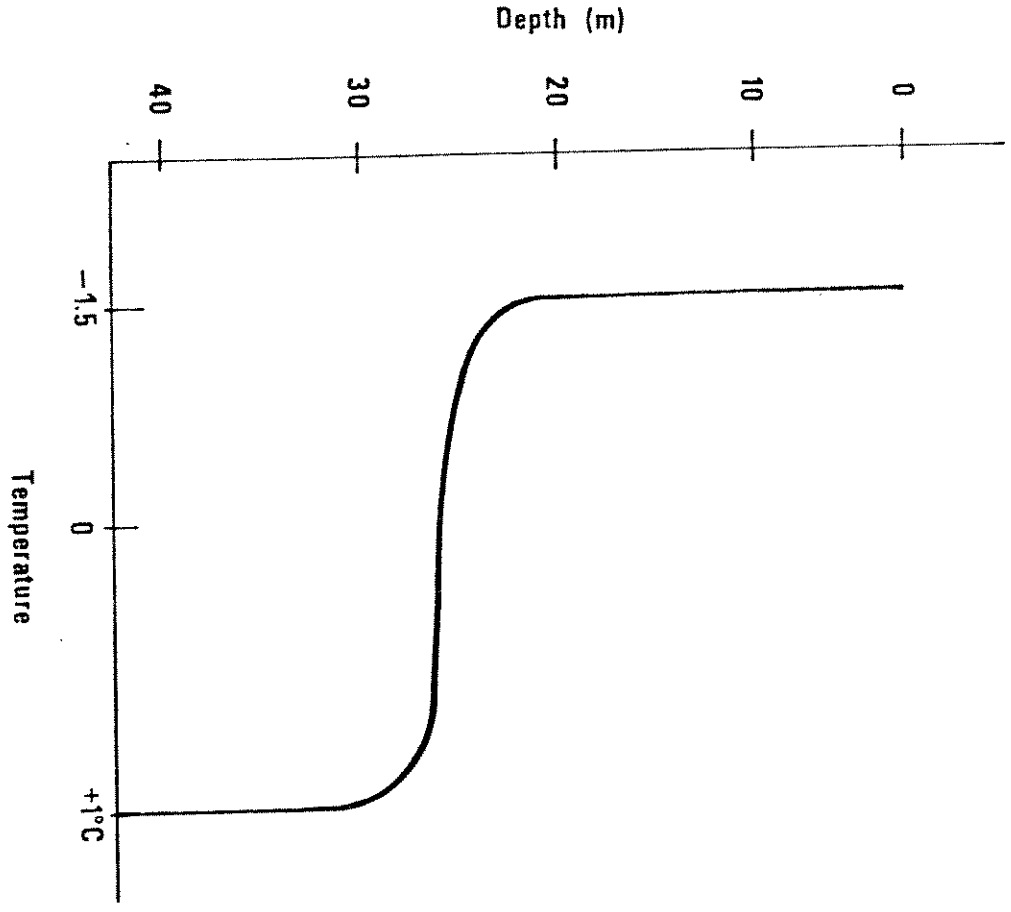


Fig.1 - BIOTA (Late Summer)



Temperature Profile at Blow-out site

REPORT OF WORKSHOP ACTIVITY III
DECISION MAKING SUBSEQUENT TO A MARINE ENVIRONMENTAL DISASTER

C. Rivet
Mandated Reporting Officer

SUMMARY - The purpose of the workshop was to present participants with a situation in which, in their capacity as members of a group of experts, they had to make a rapid decision that, given all the data available, was the most logical one.

All groups first assessed the situation with the help of the map supplied.

The next stage was to evaluate, on the basis of the Expected Impact Data sheet and personal experience, the relative impact on the various resources.

Then the problem in its true sense was tackled, not in terms of "what resources must be protected?" so much as "what resources must be sacrificed?".

The consensus was that the niche or habitat should be protected rather than the individual and that long-range effects should be avoided rather than the short-range.

Thus, there was support for the use of dispersants, since most workshops had spoken of their effectiveness, with the caveat that there should be restrictions on their use so as to protect the shoreline.

Considerations other than the purely toxicologic were dealt with too, such as the impact on the public, the cost of the proposed operation and the research necessary to gain a better understanding of what happens to oil in the sea and waht effects it has.

Rapporteur's note

In such cases, the group of experts is required to provide only one solution together with the reasons for adopting it, possible alternatives and the reasons for not adopting them.

To all practical intents, the group of experts must be able to say it has weighed up all the possible consequences and its solution will cause the least harm.

Le 6 novembre 1980 à 16:25 hres

ATELIER: A C T I V I T E I V

APPROCHE BIOANALYTIQUE DANS L'EVALUATION DES REPERCUSSIONS ENVIRONNEMENTALES D'UN REJET MINIER

Depuis le début du siècle, les activités minières dans une forêt de l'ouest ont amené la disparition complète de la pêche à la truite dans la rivière Black Creek. Une autre rivière, la Cat Creek, qui reçoit les eaux de la Black Creek, en aval du confluent avec la Black Creek, soutient une faible industrie de pêche. Avant 1950, la Cat Creek était utilisée comme frayère pour le saumon "chinook" et la truite "steelhead" et comme habitat pour les truites fardée, mouchetée et arc-en-ciel.

La guerre de Corée et le besoin d'approvisionnement en métaux de base, ont engendré une exploitation considérable de la région, sans égards à l'environnement. Une gestion des déchets inadéquate a occasionné une infiltration d'eau de drainage acide provenant des amoncellements de résidus miniers. Cette eau se charge de métaux, particulièrement de cuivre. Les nouveaux propriétaires de la mine se sont vu dans l'obligation d'améliorer les conditions environnementales; ils ont réclamé une étude impliquant des bioessais afin de déterminer les teneurs ou le niveau de cuivre qui seraient non nuisible au rétablissement d'une industrie de pêche dans la Cat Creek.

... /2

On a suggéré d'aménager un laboratoire de bioessais au confluent des deux cours d'eau pour déterminer les niveaux de toxicité aïgue et chronique de l'eau de la Black Creek.

Les problèmes envisagés sont la variation du débit d'eau, la température, les niveaux d'oxygène dessous, le pH, les concentrations de cuivre et également les effets synergiques possibles d'autres éléments comme le cobalt, l'arsénic et le fer.

L'objectif premier de l'étude serait de déterminer les concentrations de cuivre pouvant être tolérés par les salmonidés indigènes et les conditions nécessaires au recyclement de la rivière Cat Creeké

Quelle serait la façon de réaliser ces bioessais et quel échéancier devrions-nous considérer pour obtenir des résultats significatifs?

INFORMATIONS ADDITIONNELLES

Concentrations de cuivre

	Black Creek	0.1 - 3.0 mg/l
avant confluent	Cat Creek	0.05 - 0.2 mg/l
après confluent	Cat Creek	0.01 - 0.02 mg/l

RAPPORT D'ACTIVITE DE L'ATELIER IV

APPROCHE ANALYTIQUE DANS L'ÉVALUATION DES RÉPERCUSSIONS ENVIRONNEMENTALES D'UN REJET MINIER

D. Scarfe
Officier rapporteur désigné

RÉSUMÉ - La situation décrite dans le scénario en était une qui prévalait vraisemblablement dans l'un des états centralisés des Etats-Unis. Après avoir d'abord évalué les conditions environnementales à partir des données disponibles, les participants suggérèrent plusieurs plans d'attaque différents afin de comprendre le plein impact des polluants prédominants. Finalement, des mesures de nettoyage furent proposées afin d'assurer au moins la restauration des populations naturelles concernées.

L'environnement riverain en question, parsemé d'une communauté aquatique véritablement décimée, semblait sujet au ruissellement de métaux lourds (particulièrement le cuivre) dont l'origine paraissait associée aux rejets miniers présents dans la zone de drainage du Ruisseau Black. L'apport relativement important des eaux du Ruisseau Cat contribuait néanmoins à l'amélioration de cette situation, ce qui y permettait la présence de certains poissons, et, il faut le croire, d'une certaine faune et flore. En plus des métaux lourds, d'autres facteurs physico-chimiques, tels que le pH et la dureté, jouaient sûrement un rôle dans l'établissement d'un climat adverse envers les communautés biologiques endémiques. Malgré l'opinion que cet environnement pourrait en être un qui serait déjà très sélectif à l'état naturel, il fut reconnu qu'il incombait à toute société la responsabilité d'établir un milieu capable de faire vivre un nombre minimal de communautés naturelles. Cette responsabilité, cependant, ne visa pas un secteur de la société en particulier.

On jugea que le problème soulevé par la situation de ce lieu devait faire l'objet d'une préoccupation spécifique où l'importance

des polluants potentiels, ainsi que celle de leurs effets synergistiques, devait être d'abord évaluée. On requièrera ensuite d'avantage de données spécifiques à ce milieu (c.d. autres métaux lourds, niveaux organiques et inorganiques, variations saisonnières, etc.). On estima que ces données devraient être comparées aux données publiées afin d'évaluer les impacts négatifs en ce qui concerne l'espèce la plus abondante ou "importante" (c.d. les salmonidés). A défaut d'autres données physico-chimiques, il faudrait supplémenter celles-ci immédiatement.

C'est à ce moment que deux lignes de pensées émergèrent alors que les uns revendiquèrent que les données physico-chimiques et que la connaissance d'autres situations similaires permettraient d'établir les concentrations maximales allouables en fonction des variables physico-chimiques. Les autres, plus nombreux, préférèrent corroborer ces trouvailles par l'entremise de bioanalyses et suggérèrent la détermination des plus grands niveaux tolérables de toutes les variables, individuellement et cumulativement, en utilisant l'étape vitale la plus sensible (embryo-larvaire) de l'espèce prédominante et "importante" (c.d. les salmonidés). La réalisation de bioanalyses de types chronique et aigu fut également recommandée en utilisant l'eau du Ruisseau Black comme diluant et ce, sur des poissons élevés localement et en pisciculture. Ces premières évaluations pourraient être terminées à l'intérieur d'une période variant de six mois à un an.

Plusieurs pensèrent que les métaux lourds (en particulier le cuivre) étaient les plus nocifs et lancèrent l'idée que ce problème pourrait être résolu rapidement par un procédé qui en ferait précipiter la plus grande partie.

Ceci pourrait s'accomplir en alcalinisant avec de la chaux les rejets liquides miniers et/ou des sections du Ruisseau Black qui seraient dirigés vers des lagunes d'épandage aménagées à cette fin. Les eaux neutralisées pourraient alors être redirigés vers le ruisseau. Une telle action nécessiterait l'étude de techniques industrielles présentement employées à cette fin.

Pour récapituler, l'effort immédiat consista d'abord à réduire les concentrations métalliques et d'estimer, en fonction des espèces biologiques de ce milieu, les niveaux acceptables de tous les constituants susceptibles d'affecter collectivement la qualité de ce cours d'eau en se basant sur des études déjà publiées et/ou sur des bioanalyses réalisées avec les embryons et larves de salmonidés. On plaça une importance secondaire en ce qui avait trait à l'étude de tous les niveaux trophiques et des communautés actives dans ce ruisseau ainsi que sur le besoin d'estimer l'effet de polluants sur la densité et la diversité de la flore et de la faune. De telles études à long terme devraient être entreprises suite à la réalisation d'une action première. En général, il fut suggéré qu'il y ait, en plus des relevés écologiques, une continuité quant aux programmes de monitoring biologique jusqu'à l'obtention d'une salubrité environnementale pour ce milieu ou, au moins, jusqu'à la reconnaissance que celui-ci soit exempt de toute perturbation humaine.

W O R K S H O P A C T I V I T Y IV

November 6, 1980 at 16:25 hr

BIOASSAYS IN ASSESSING THE ENVIRONMENTAL EFFECTS OF A MINE EFFLUENT

Mining activity since the turn of the century in a national forest of a western state has resulted in the total elimination of a trout fishery in Black Creek. Cat Creek, below its convergence with Black Creek, supports a meager trout fishery. Prior to 1950, Cat Creek was used as a spawning area for chinook salmon and steelhead trout, as well as a habitat for cutthroat, brook and rainbow trout.

The onset of the Korean War and the need for more base metals, resulted in extensive exploitation of the area regardless of the environmental aspects. Poor waste management has resulted in acid drainage seeping from waste rock piles. This run-off water becomes loaded with heavy metals, particularly copper. The new owners of this mine were faced with the prospect of ameliorating the poor conditions and requested a study involving bioassays to determine the amount of copper removal from the Black Creek water required to restore a viable fishery to Cat Creek.

It was proposed to erect a mobile bioassay lab at the junction of the two creeks to determine the acute and chronic toxic levels of dilution of Black Creek. Problems that would have to be overcome by the researchers are fluctuations in water flow, temperature, dissolved oxygen levels, pH and copper concentrations as well as possible synergistic effects of other elements such as Co, As and Fe.

The main objective of the study would be to assess what levels of copper indigenous salmonids could tolerate and under what conditions they could successfully repopulate Cat Creek.

To conduct these bioassays what approach would you take and what time frame should be expected to get meaningful data?

Additional Information

Cu Concentrations

	Black Creek	0.1 - 3.0 mg/L
(below convergence)	Cat Creek	0.05 - 0.2 mg/L
(above convergence)	Cat Creek	0.01 - 0.02 mg/L

Water Hardness

Cat Creek	30-50 mg/L as CaCO

pH

Black Creek	4.5-6.5
Cat Creek	5.5-7.5

Flows (CFS)

Black Creek	20-200
Cat Creek	60-1000

REPORT OF WORKSHOP ACTIVITY IV

BIOASSAYS IN ASSESSING THE ENVIRONMENTAL EFFECTS OF A MINE EFFLUENT

D. Scarfe
Mandated reporting officer

SUMMARY - This situation was apparently one which existed in one of the north central, U.S. states. The workshop participants first assessed the environmental conditions from available data, then suggested several different approaches to assess the full impact of the predominant pollutants. Finally clean up protocols were generally proposed to, minimally allow natural populations to be restored.

This riverine environment contained an extremely suppressed aquatic community which appeared to be a result of runoff of heavy metals (especially copper) originating predominantly from mine tailings in the Black Creek drainage area. The relatively large inflow from the Cat Creek ameliorated the situation to some extent allowing the presence of some fish, and presumably other fauna and flora. In addition to the presence of the heavy metals, other physio-chemical factors, such as pH and water hardness, may contribute significantly to an adverse environment for natural biota. It was generally agreed that, even though the environment could be or was a naturally harsh one, it was incumbent upon society to provide an environment that would minimally support natural communities. This responsibility was not directed to any one sector of society.

The participants considered that this situation should be dealt with as a site-specific one in which the relative importance of all potential pollutants, as well as their synergistic effects should be first assessed. More site-specific data (e.g., other heavy metal, organic and inorganic levels, seasonal fluctuations, etc.) should be required. These data should be related to published data to estimate the adverse impacts on the most abundant or "important" biota (e.g., salmonids). If no additional physio-chemical data were available,

measurements should begin immediately.

At this point a dichotomy arose with some participants claiming that the physio-chemical data and knowledge of other existing situations would allow determination of the maximum permissible levels of all physio-chemical variables. Another larger group preferred to substantiate such findings with bioassays and suggested determining the greatest tolerable levels of all variables, separately and cumulatively, using the most sensitive stages (embryo-larval) of the predominant or most "important" species (e.g., salmonids). Both chronic and acute assays should be undertaken using dilutions of local Black Creek water on both indigenous and hatchery reared stock. These initial assessments could possibly be completed within a period of six months to one year.

Several participants considered that heavy metals (predominantly copper) presented the major environmental hazard and suggested that this problem could possibly be dealt with promptly, by crudely precipitating out much of the metal content. This could be accomplished by basifying rechanneled tailing drainage and/or sections of Black Creek water in bulldozed ponds, with lime. Neutralized water could then be returned to the creek. Such action necessitates examining industrial techniques presently used for such purposes.

The immediate reaction therefore was to emphasize initial reduction of the heavy metal load and to estimate biologically tolerable levels of combined riverine constituents from other published studies and/or bioassays using salmonid embryo-larvae. A secondary emphasis was placed on examining all trophic levels and community structures of the whole riverine system, and estimating the pollutant effects on, for example, densitites and diversities of all flora and fauna. These relatively long-term studies should begin after some initial action has been taken. It was generally suggested that along with ecological surveys, other biomonitoring programs should continue until the system is considered environmentally sound or, minimally, free of human disturbance.

REMERCIEMENTS

Nous tenons à remercier toutes les personnes qui ont participé à la rédaction de ce compte rendu, particulièrement: les auteurs qui, par leur diligence, nous ont permis de rencontrer notre échéancier, les officiers rapporteurs qui ont rédigé les comptes rendus des activités de travail en atelier, le Bureau de Traduction dont Mme Willems, du Secrétariat d'Etat qui, en dépit de l'échéancier très court, a pu traduire la majeure partie des résumés, les secrétaires qui ont collaboré à la dactylographie des textes: H. Caron, L. Champagne, M. Lapierrière et L. Morency.

ACKNOWLEDGMENTS

We wish to tank all those who have participated in the composition of the proceedings and mostly: the Authors whose diligence has permitted us to respect our time schedule, the Reporting Officers of the Workshop Activities who summarised the outcome of those activities, the Translator, especially Mrs. Willems' group at the Department of the Secretary of State who, in spite of the tight schedule, produced most of the translations, the Secretaries: H. Caron, L. Champagne, M. Lapierrière and L. Morency, who collaborated in setting up the texts.

APPENDICE I

RÉSULTAT D'ENQUÊTE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

APPENDIX I

RESULTS FROM THE SURVEY OF AQUATIC TOXICITY RESEARCH

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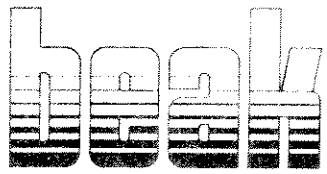

Spécialistes de l'environnement
Environmental Specialists

Reference: 7th Annual aquatic Toxicity Conference, 5, 6, 7 November 1980, Montréal, Québec, Canada.

Dear Sir:

This year's aquatic toxicity conference will be held in Montréal, Québec, and you and your confrères are cordially invited for three days of scientific dialogue and joie de vivre. Simultaneous translation will also be featured at all conference presentations.

In an attempt to provide a concise and timely overview of ongoing aquatic toxicity research, the Organizing Committee of this year's aquatic toxicity conference requests that you complete the enclosed form and forward it to this office within the next few weeks.

The data will be compiled and made available to all conference delegates. Please note also that you are encouraged to actively solicite information from your co-workers in order that this survey be as complete as possible. With this goal in mind, a number of American institutions are being canvassed this year, as well.

Your cooperation is appreciated and we look forward to seeing you in Montréal.

Blaine L. Hummel
Organizing Committee

BLH/dp

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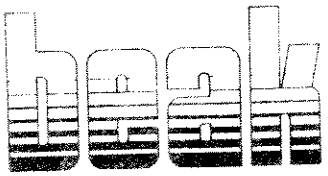

Spécialistes de l'environnement
Environmental Specialists

Objet: 7ième Atelier annuel sur la toxicité aquatique, 5, 6, 7 novembre 1980, Montréal, Québec, Canada.

Cher collègue,

Cette année, l'atelier sur la toxicité aquatique se tiendra à Montréal (Québec). Vous et vos collègues êtes cordialement invités à participer à cette rencontre où se mêleront les discussions scientifiques et les pauses propices à la détente et aux distractions de toutes sortes. Toutes les communications scientifiques bénéficieront d'un service de traduction simultanée.

Afin de pouvoir présenter un aperçu à la fois concis et à jour de la recherche en toxicité dans le milieu aquatique, le Comité organisateur de la conférence de cette année vous demande de bien vouloir remplir le formulaire ci-joint et le retourner au soussigné d'ici quelques semaines.

Les données recueillies seront compilées et mises à la disposition des délégués. Pour obtenir un maximum de représentativité des résultats, un certain nombre d'institutions américaines ont été invitées à participer à l'enquête. Pour la même raison, nous vous encourageons aussi à faire remplir ce questionnaire par vos collègues de travail.

Nous vous remercions à l'avance de votre collaboration et nous vous souhaitons une fois de plus la bienvenue à Montréal.

Blaine L. Hummel
Comité organisateur

BLH/dp

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Fisheries and Environmental Sciences, Department of Fisheries and Oceans				
Fisheries and Environmental Research (St.Andrews)	D. W. McLeese	Lobster, Crangon, Pandalus, Nereis, Mytilus	Lethality, sublethal effects, accumulation' excretion	Organochlorine compounds, Organophosphate pesticides, pyrethroids, alkylphenols
	S. Ray	Lobsters, Crangon, Pandalus, Nereis, Mytilus	Accumulation, excretion, interactions	Heavy metals
	K. Haya	Lobster, flounder	Adenylate energy charge	Heavy metals
	V. Zitko	Atlantic salmon	Lethality, accumution, excretion	Organic chemicals
Fisheries and Environmental Research (Halifax)	H. Freeman	Cod	Steroidogenesis	Phthalates
	J. F. Uthe	Cod, lobsters, bivalves	Histological indicators of effects	Heavy metals, organic chemicals

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Brock University Department of Biological Sciences St.Catharines, Ontario	Mike Dickman	Amphipods, trout	Cyanamid of Canada Ltd., Ammonia-rich wastes	Inorganic nitrogen compounds

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada Environmental Protection Service Aquatic Toxicology Laboratory 14317-128 Avenue Edmonton, Alberta	Art Beckett Wendy Neufeld Walt Golebiowski	*Salmo gairdneri* *Daphnia pulex* *Gasterosteus aculeatus* *Selenastrum capricornutum* *Brachydanio rerio*	Acute Lethality -freshwater -marine Sublethal Response -embryo/larval -breeding success -multi-generation Algal -algicidal	Industrial effluents and chemicals Hazardous materials Environmental contaminants and pollutants

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
McMaster University Hamilton, Ontario	R.A. Sonstegard	Fish, rat, bird	Carcinogenesis Endocrinology Pathology Epizoothology	Many
	C.M. Wood, D.G. McDonald D.J. Spry	Trout, suckers and crayfish	Physiology; acid-base and ion regulation;	Acid at varying water hardnesses and zinc toxicity vs. water pH.
	T.T. Chen	Trout, salmon	Endocrinology	Halogenated Hydrocarbons; polycyclic aromatic and heterocyclic hydrocarbons
	D.J. Spry	Rainbow trout	Physiological effects of zinc.	Zinc.

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
E.V.S. Consultants Ltd. North Vancouver, B.C.	G.A. Vigers	Rainbow trout, Pacific herring, Coho & Chum salmon, Stickle-back	acute toxicity avoidance behaviour inter laboratory comparisons;	Sulfite mill effluent
		Prickly and Staghorn Sculpins	bioaccumulation, muscle/liver	chlorophenols
	E.R. McGreer	Macoma balthica Mytilus edulis	bioavailability from contaminated marine sediments;	Cu, Pb, Zn, Hg, As, Cd, PCB
			mine tailings	Cu, Pb, Zn, Cd, Fe
	D. Konasewich		literature reviews on pathways, proces-ses transformations, fate and effects of environmental con-taminants	Us., EPA 129 Priority Pollutants Chlorinated organics and isomers from pulp and paper effluents
	P.M. Chapman	11 species of oligochaete worms	acute lethal and sublethal effects	Cd, Hg Pulp mill effluent
			salinity, dissolved oxygen, temperature	NaPCP Sewage sludge

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Concordia University Montréal, Québec	TEAM Dr. Anderson Dr. Leduc Dr. Ruby Dr. Maly Prof. Dick	Fish Invertebrates algae	In test populations Survival Growth Movement Behaviour Development Reproduction In simulated Trophic systems Productivity Distribution Biomass Standing crop Succession	Inorganics (particularly heavy metals industrial organics (e.g. Cyanide, phenols) agricultural organics Dieldin Fenitrothion Methoxychlor

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
National Water Research Institute Canada Centre for Inland Water Burlington, Ontario	Dr. Y.K. Chau	Freshwate algae	Algae Toxicity and Chemical structure relationship.	Organometal compounds, organotin organolead, etc.

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
University of Ottawa Biology dept. Ottawa, Ontario	Dr. S.U. Qodin and students	Crustaceans, fish	Accumulation, uptake, release and effects on growth and reproduction.	Arsenic Matacil Mirex

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Nova Scotia Research Foundation Corporation Dartmouth, N.S.	K. Hellenbrand	Rainbow trout	Toxicity	Various industrial effluents

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
University of Victoria British Columbia Environmental Toxicology Group	Dr. A. T. Matheson co-ordinator	Salmonids	Growth, uptake, distribution, secretion, metallothionein tissue protein	Copper & zinc

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
United States Environmental Protection Agency Environmental Research Laboratory Duluth, Minnesota	J.H. McCormick	Largemouth bass vs Fathead minnows (exposed-vs-unexposed).	differential predation rate Threshold of effect concentration detection, method development.	General

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Beak Consultants Ltd. 6870 Goreway Drive Toxicity Group Mississauga, Ontario	R. Scroggins	Rainbow Trout, Smallmouth bass Yellow perch, Flagfish, Gammarus, Daphnia, Chlorella.	Acute and chronic toxicity Bioaccumulation Tainting, Behaviour	Industrial effluents, oils and Dispersants, Pesticides, etc.

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
University of Guelph Guelph, Ontario	J.B. Wilson & J.C. Roff	Micro-crustaceans, plankton	Sublethal changes in light responses	Lead, cadmium
	D.E. Gaskin, R. Frank, B. Braune, & G. Smith	Harbour porpoise pelagic seabirds, and food-chain organisms	Tissue levels with respect to differences in organ, season, region	Mercury, cadmium, lead, selenium
	A.K. Kumaraguru & F.W.H. Beamish	Rainbow Trout	Sublethal physiology physiology	Permethrin
	D.W. Rodgers & F.W.H. Beamish	Rainbow Trout	Uptake mechanisms	Methyl mercury

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Plansearch Inc. (formerly MacLaren Marex Inc.)	Dr. M. Hutcheson	Arctic bivalves 3-Spine Stick-lebacks Rainbow Trout	Scope for growth Acute lethal mortality	Crude oil and chemically dispersed oil Assorted industrial effluents and chemicals

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada Fisheries and Oceans West Vancouver Laboratory	Dr. M. Waldichuk	*Mytilus edulis* and *Macoma balthica*	Bioaccumulation under different salinities and temperatures	Copper, lead, zinc cadmium and iron in mine tailings
		Anisogammarus confervicolus and *A. pugettensis*	Dissolved oxygen concentrations, salinity and temperature	Low dissolved oxygen concentrations

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Institute for Environmental Studies, University of Toronto Toronto, Ontario c/o Marine Ecology Laboratory B.I.O. P.O. Box 1006 Dartmouth, N.S.	D. Mackay P.G. Wells W.Y. Shiu S.A. Abernethy	*Artemia salina* *Acartia clavsi* *Pseudocalanus spp.* *Corophium volutator* *Homarus americanus*	Lethality, Behaviour Molting Success Rate of Development	3 crude oils 3 oilspill dispersants

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Alberta Oil Sands Environmental Research Program Edmonton, Alberta	Philip T.P. Tsui Brian R. McMahon Peter J. McCart	Caenis diminuta (Ephemeroptera) Paraleptophlebia bicornuta (Ephemeroptera) Hyalella azteca (Amphipoda)	Survivorship (Acute and chronic toxicities); Development Moulting frequency; Emergence; Allometric growth; Osmoregulation; Tissue ion levels	Mine depressurization Groundwater from Alberta's Athabasca Tar Sand Area.
	Mobil Oil Canada Ltd., Calgary Univ. of Calgary Aquatic Environments Ltd. Calgary	Rainbow Trout; Lake Chub; White Sucker; Lake whitefish (eggs only)	Chronic Lethality; Respiratory and coughing frequency; hematocrit; blood and tissue ion levels; gill histology; growth; fertilization success.	
Mobil Oil Canada Ltd Ltd St.John's, Nfld.	Environmental planning and management department Mobil Oil Canada Ltd., (attn: Philip T.P. Tsui)	Blue mussel; Green seaurchin; soft shelled clam; sand shrimp.	Acute toxicity (96hr LC_{50})	Hibernia Crude oil; and sodium lauryl sulphate (as standard toxicant)

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
University of Guelph Guelph Ontario	R.W. Bradley & J.B. Sprague	Rainbow Trout	Effects of pH, hardness, alkalinity on toxicity.	Zinc
	N.J. Hutchison & J.B. Sprague	Flagfish, etc.	Potential role of metals in reproductive failure in acid lakes.	Mixture of metals
	W.P. Banas & J.B. Sprague	Rainbow Trout	Acclimation to toxicants.	Organophosphates
	J.F. Leatherland R.A. Sonsetgard (McMaster)	Oncorhynchus	Thyroid and liver physiology and iono-regulation.	Unknown goitrogen in Great Lakes
	N.E. Down & J.F. Leatherland	Cyprinids	Gonodal dysfunction.	Unknown agent in Great Lakes
	K. Munkittrick & J.F. Leatherland	Cyprinids	Osmo- and iono-regulation processes associated wilth gonadal problems.	Unkonwn agent in Great Lakes
	M. Stephenson & G.L. Mackie	Sphaeriid clams	Growth, reproduction, other sublethal effects in lab and field experiments.	2, 4D ester and amine
	B. Rooke & G.L. Mackie	Molluscs	Growth and reproduction as affected by pH and sulphate levels in acid lakes.	Components of acid lakewater
	C. Wren & H.R. MacCrimmon	Lake Trout	Physico-chemical and trophic relationships governing residue levels lin field and lab.	Mercury

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Ministry of the Environment Water Resources Branch Limnology and Toxicity Section P.O. Box 213 Rexdale, Ontario Environmental Contaminants (Research) Laboratory	C. Inniss K. Holtze T. Heiman	Rainbow Trout Small mouth bass Yellow perch Zebrafish Flagfish Fathead Minnow *Daphnia* sp.	Chemical: Aluminum, manganese Water hardness alkalinity, pH, acidity Biological: Egg hatchability Growth and survival during the embryonic and alevin developmental periods Histopathology of eggs, larvae and adult fish	Hydrogen ion-aluminum and manganese Chlorine Sewage Treatment Plant Effluents

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ontario	U. Borgmann	Zooplankton invertebrates	Production mixed function oxidases	Metals
	D.G. Dixon	Fish	Structure: toxicity correlations	Chlorobenzenes, parasubstituted phenols.
	P.V. Hodson	Fish	Chronic toxicity Embryo-larval tests	Alkyl lead compounds, pentachlorophenol.
			Partial life cycle	Dietary and waterborne Se. Dietary As. Great Lakes contaminated fish as diet.
			Indicators of fish health-biochemical	Lead organics
	S. Millard	Plankton to fish	Transfer and accumulation	PCB
	A.J. Niimi	Fish	Physiology and kinetics	HCB, PCP
	P.T.S. Wong Y.K. Chau, O. Kramar, G. Bengert	Pure algal species and natural phytoplankton	Primary production; cell multiplication; adaptation; morphology	Individual metals (Pb, As, Se, Cd, Sn, Hg, Zn, Cu, Ni, Co), and mixtures of metals
			Enzymes	Triaryl phosphates HCB, PCP

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Great Lakes Biolimnology Laboratory, Burlington, Ontario	P.T.S. Wong Y.K. Chau O. Kramar	Sediment microorganisms	Methylation	Pb, As, Se, Sn
	G. Bengert	Fish	Uptake and depuration	Tetramethyllead
Environmental Contaminants Division, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario Organics Properties Section	K.L.E. Kaiser	Structure-activity correlations of various groups of contaminants with varius organisms and parameters, based on literature data.		

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada Freshwater Institute Winnipeg, Manitoba	S. Leonhard	*Daphnia* sp.	Uptake of calcium in post-moult females	Heavy metals, acid
	W.K. Friesen	*Hexagenia vigida* Ephemeroptera-the burrowing mayfly	Nymphs Toxicity of contaminated sediment: long & short term exposures Eggs: hatchability	Permethrin Ambush (R) ^{14}C isotope

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada Environmental Protection Service Aquatic Toxicity Lab, 1801 Welch Street North Vancouver, B.C.	R. Watts J. Baumann D. Moul	Rainbow Trout Salmon	Acute Lethal Static & Flow-through 96hr LC50/LT50	Mining pulp and paper, municipal sewage, petrol-chemicals, pure and processed chemicals, detergents, leachates.

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Noranda Research Centre Pointe-Claire, Québec	M. R. Speyer, R. Levaque-Charron C. Wood and R. Prairie	Salmo salar, Daphnia magna S. gairdneri Homarus americanus Gammarus sp.	Comparative Toxicity LC50/LT50 Acute toxicity at varying pH, hardness temperature Chronic effects on growth Acute toxicity	 Cu, Al, Mn, Th. Pb, Cd, and other metals. pH, metals

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Fisheries & Oceans Freshwater Institute Limnology Section Northern Reservoir Biology & Fisheries Winnipeg, Manitoba	D. Bodaly R. Hecky	Lake whitefish, walleye, northern pike.	Muscle levels of Hg and Se; mechanisms of elevation of Hg levels in freshwater fishes as a result of lake river impoundment.	Hg, Se
Experimental Limnology Winnipeg, Manitoba	D. W. Schindler	Whole lake systems	Limnology	Acid
	C. Kelly	Microbial systems	Methane production	Acid, heavy metals and their interactions.
	J. Shearer	Algae	Primary production	Acid
	D. Findlay	Algae	Community composition	Acid
	K. Mills S. Chalanchuk	Lake Trout	Population size and and age structure; growth	Acid
	I. Davies	Crayfish (Oronectes virilis).	Population size and age structure, fecundity.	Acid
	D. Malley P.S.S. Chang W. Findlay	Crayfish (O. virilis)	Ca^{++} balance	Acid, Aluminum

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Experimental Limnology	R. France (Grad student with D.W. Schindler)	Crayfish (*O. virilis*)	Growth and reproduction.	Acid
	R. Nero (Grad student with D.W. Schindler)	Opposum shrimp (*Mysis relicta*)	Population size and age structure	Acid
	D. Ramsey (Grad student with K. Patalas)	Zooplankton	Population responses; community composition	Acid
	P. Olesiuk (Grad student with D.W. Schindler)	Amphiphod *Pontoporeia affinis*	Growth, reproduction, mortality	Acid

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada EPS, Atlantic Region, Halifax, Nova Scotia	Roy Parker	Rainbow Trout	LC50, LT50 (Static/flow-through)	Industrial effluents and pure chemical
		3-spine stickleback	LC50, LT50 (Static/flow-through)	Industrial effluents and pure chemical
		Daphnia pulex	Static LC50	Industrial effluents and pure chemical
		Rainbow Trout Eggs	LC50	Insecticides

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Environment Canada SPE, Région du Québec Longueuil, Québec	C. Blaise. R. Legault et N. Birmingham	Salmo gairdneri S. capricornutum Microtox (Bechman)	LC50, LT50 CI50 et bio-accumulation CI50	Effluents industriels

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada EPS, Atlantic Region, Halifax, Nova Scotia	Roy Parker	Rainbow Trout	LC50, LT50 (Static/flow-through)	Industrial effluents and pure chemical
		3-spine stickleback	LC50, LT50 (Static/flow-through)	Industrial effluents and pure chemical
		Daphnia pulex	Static LC50	Industrial effluents and pure chemical
		Rainbow Trout Eggs	LC50	Insecticides

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Environment Canada SPE, Région du Québec Longueuil, Québec	C. Blaise. R. Legault et N. Birmingham	Salmo gairdneri S. capricornutum Microtox (Bechman)	LC50, LT50 CI50 et bio-accumulation CI50	Effluents industriels

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
INRS-Eau	Tessier, A. Campbell, P. Bates, S. Auclair, J.C. Bisson, M. Couture, P.	Algues: Chlamydomonas	Assimilation C-14, S-35; Taux de croissance; adsorption et assi- milation de toxique fluorescence.	Zn, Cu
	Couillars, D. Croteau, G. Couture, P.	Algue Selenastrum	Croissance	Eaux usées.
	Couture, P. Visser, S. Lambert, M.	Algues: Chlamydomonas Selenastrum Crustacé: Daphnia	Motilité Croissance Assimilation C-14 Motilité	Pesticides: Fénitrothion Aminocarb Matière organique (eau)
Université Laval Faculté d'agriculture Dept. des sols Laval, Québec	Visser, S.	Algues: Chlamydomonas Selenastrum Crustacé: Daphnia	Motilité Croissance Motilité	Matière humique (sol)

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
IRCHA (France)	Cabridenc, R. Lepailleur, H.	Algues: Scenedesmus	croissance	substances organiques et inorganiques.
		Crustacé: Daphnia	Mobilité	" "
		Poisson Brachydanio	Mortalité	" "
	Huet, M.C.	Bactéries	Assimilation C-14	Eaux de rivières

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Université de Montréal Montréal, Québec	Marc Lalande du Laboratoire de Mme B.P. Alloul	Crustacés zooplanctoniques	Toxicités aigue et chronique.	Métaux lourds et organo-chlorés et inorganiques.

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Eco-Recherches Ltée Pointe-Claire, Québec	Ecotoxicologie	Poissons	Toxicité létale aigües	Effluents industriels. Produits chimiques
			Bio-dégradation	Produits chimiques
			Bio-accumulation	Produits chimiques
			Toxicité sous-létale	Effluents
		Oeufs de poisson	Toxicité sous-létale et retardée	Produits chimiques
		Invertébrés	Toxicité létale aigües	Effluents simulés
			Bio-accumulation	Effluents industriels Produits chimiques
		Algues	Croissance Bio-accumulation	Effluents industriels Produits chimiques
			Toxicité retardée	Stimulateurs
		Ecosystème expérimental	Bio-accumulation Transfert trophique Effets additifs	Produits chimiques

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Université du Québec à Trois-Rivières	Dr. Pierre-F. Blanchet Groupe de recherche sur les insectes piqueurs		Dégradation des pesticides organo-phosphorés en milieu aqueux	Abate, Chlorpyrifosméthyl Chlorpyrifos, etc.

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Université Laval Québec, Québec	Claude Vézina*	Salmo gairdneri	Concentration d'ABS dans les tissus Structure histologique des bourgeons du goût des branchies et de la langue	ABS (alkylbenzène sulfonate)

* adresse actuelle:
Roche Associé Ltée
2535 boul. Laurier
Sainte-Foy, Québec

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Ecole Polytechnique de Montréal Montréal, Québec	C.E. Delisle et étudiants gradués	D. magna S. gairdneri Guppies	Evitement (avoidance) Accumulation	Différents composés Methylmercure radioactif Ammoniaque (Ammonia)

ENQUETE SUR LA RECHERCHE DANS LE MILIEU AQUATIQUE

ORGANISME, INSTITUTION OU ENTREPRISE	INDIVIDU OU GROUPE	ORGANISME D'ESSAI	PARAMETRES EVALUES	PRODUIT ETUDIE
Environnement Québec Division Biologie Ste-Foy, Québec	G. Joubert	Selenastrum capricornutum	CI50 (LC50)	Effluents variés et eaux de surface
		Chlamydomonas variabilis	CI50	" "
		Daphnia magna	CI50	" "
		Daphnia pulex	CI50	" "
		Microtox (R) de Beckman	CE50	" "

APPENCIDE II

LISTE DES PARTICIPANTS

Cette liste n'est que partielle, ceux qui ont omis de signer la feuille réservée à cet effet ne sont pas comptabilisés.

APPENDIX II

LIST OF PARTICIPANTS

This is only a partial list of the actual presence. Those who have omited to sign the list intended for this compilation are unfortunately not accounted for.

NOM	AFFILIATION	ADRESSE	TEL.
Richard Legault	SPE	585 Jacques Cartier	655-7690
Perie Couture	INRS - Eau	2700 Einstein Ste-Foy	657-2278
R. Van Coillie	ECO Recherches C.I.L.	121 boul. Hymus	697-3273
Claude Thellen	ECO - C.I.L.	121 boul. Hymus	697-3273
Gérald Joubert	Environ. Québec	2700 Einstein, Ste-Foy	643-8208
Serge Metikosh	Dept. Indian Aff. and Northern Dev.	P.O. Box 1500 Yellowknife, N.W.T.	(403)920-8223
Roy Parker	EPS, Halifax	5151 George St., N.S. B3J 1M5	(902)426-3287
Art Beckett	EPS, Edmonton	Aquatic Toxicology Laboratory 14317-128 Ave Edmonton, Alberta T5L 3H3	(403)420-2610
Rick McCubbin	EPS NFLD	P.O. Box 5037 St.John's, NFLD	(709)737-5488
Brian Hammond	Alberta Environ.	9820-106st, Edmonton	(403)427-6254
Margaret Friesen	DFO, Freshwater Institute	501, University Cr.	(204)269-7379
Sharon L. Leonhard	DFO, Freshwater Institute	501, University Cr.	(204)269-7379
Gérard Leduc	BIOL. Sc.	Concordia Univ.	(514)879-5989
A. David Scarfe	U.S. EPA	Envir. Res. Lab. Narragansett RI	(401)789-1071
Ian R. McCracken	R.P.C.	P.O. Box 6000 Fredericton, N.B.	(506)455-8330
Gloria Sangalang	Dept. of Fisheries and Oceans	1707	(902)426-6278

NOMS	AFFILIATION	ADRESSE	TEL.
J. Howard & McCormick	U.S. - EPA, Oceans	6201 Congdon Blvd	(218)727-6692
Don C. Miller	U.S. EPA, ERL Narragansett	50 Ferry Rd., Narra. RI	(401)789-1071
Anthony Calabrese	National Marine Fisheries Service	Milford, Connecticut	(203)878-2459
Marta Griffiths	Minis. of Envir.	1 St. Clair W. Toronto	965-6957
Keith R. Solomon	University of Guelph	Guelph	(519)824-7259
Ron G. Watts	DOE - EPS	1801 Welch St. N.Van, B.C. V7P 1B7	(604)980-6917
Gordon Craig	Ont. Min. of Env.	P.O. Box 213 Roxdale, Ont.	(416)248-3011
M. Sharom	Uni. of Guelph	89 Shadybrook Cr. Guelph	824-2712
W. Lake	Alberta Env. Center	Bag 4000 Vapreville, Albe.	632-6767
Barry Munson	Alberta Env.	9820-106thSt. Edmonton	427-3943
John Retallack	Syncrude Canada Ltd.	10030-107 St Edmonton T5J 3E5	429-9848
Margaret C. Taylor	Environment Canada Water Quality Branch	Ottawa K1A 0E7	(819)997-1920
Georges Mezzetta	Env. Canada	SPE Montréal	283-2335
Bev. Blunt	C.C.I.W.	867 Lakeshore Box 5050 Burlington	637-4507
O. Kramar	C.C.I.W	" "	637-4565
Lynne Luxon	C.C.I.W.	" "	637-4565
P. Wong	C.C.I.W.	" "	637-4559
V.B. Wilson	U. Guelph	Guelph, Ont.	824-4120
C.D. Wren	"	"	"

NOM	AFFILIATION	ADRESSE	TEL.
Scott Millard	C.C.I.W.	867 Lakeshore Blvd. Burlington	637-4507
Karen Ralph	"	"	637-4559
P.V. Hodson	"	"	637-4559
Pierre Lavallée	Min. Env. Gouv. du Qué.	2360 Chemin Ste-Foy Québec	643-2474
Betty Hillaby	Dept. Fisheries and Oceans	1090 W. Pender St. Van.	666-1309
William C. Phoel	NOAA, NMFS Sandy Hook Lab	Highlands N.J M U.S.A. 07732	(201)872-0200
Kern Marshall	Pollutech Ltd.	10905 peers Rd. Oakville	416-844-1900
Peter G. Wells	Univ. of Toronto and Bedford Inst. Dartmouth, N.S.	P.O. 1006 Dartmouth, N.S. B2Y 4A2	902-426-3276 416-978-7358
Glen H. Geen	Simon Fraser Univ.	Burnaby, B.Ci	291-3536
George Dixon	C.C.I.W. Burlington	Box 5050 Burlington L7A 4A6	416-637-4203
Keith Holtze	Min. of the Env.	Toronto, Ont.	(Work) 248-3011
Lawrence Kina	EPS Ontario	25 St. Clair Ave. Toronto, Ont.	(Work) 248-3011 (Hou.) 533-9575
Ross Norstrom	CWS, Ottawa	Ottawa	997-1410
Stephen Bates	Uni. du Québec INRS - Eau	Ste-Foy, Québec	657-2543
Jargo Kiceinuts	F & O	St. Johns, Nfld. Box 5667	
John Munro	Ontario Minis. of Environment	P.O. Box 213 Rexdale	416-248-3011
John W. Hilton	Univ. of Guelph	Guelph Ont. N1G 2W1	519-824-4120 (3608)
L.S. McCarty	MacLaren Ltd.	Willowdale, Ont.	494-7640 499-0880 ex 383

NOM	AFFILIATION	ADRESSE	TEL.
Peddrick Weis	N.J. Medical School	Newark NJ U.S.A.	201-456-4409
Bruce Reid	E.V.S. Consultants Ltd.	Vancouver B.C.	604-986-4331
M.Z. Speyer	Noranda Research Ct.	240 Hymus Blvd. Pointe-Claire, Qué.	514-697-6640
Henry S. Majewski	Freshwater Institute	501 University Cres.	269-7379
Daniel Dive	INSERM	Lille (France)	20(91-14-62)
Simon Visser	Univ. Laval	Ste-Foy, Qué.	656-3280
Blaise Christian	Env. Canada	Longueuil, P.Q.	653-4975
James M. McKim	Env. Res. Lab,	Duluth, Mn	218-727-6692
William Penrose	Argonne Nat'l Lab	Argonne IL 60439	312-972-4260
Jacques D. Duval	CIE Minière Québec Cartier	Port Cartier Qué. G5B 2H3	418-768-2279
Claudy Deschenes	"	"	418-768-4596
Pierre Mineau	Service Canadien de la Faune	C.C.E.I., B.P. 5050 Burlington, Ont.	637-4264
Marcel-Marie Lebel	Asso. industrielle Laval	12,500 boul. industrielle Pointe-aux-Trembles	645-2258
Michel Bienvenue	ECO - Recherches	121 Boul. Hymus Pointe Claire	697-3273
Jean-Claude Dol	Université Ste-Anne	Church Point Digby Nouv. Ecosse	769-2114
J. Tom Buckley	University Victoria	Victoria B.C.	
Helena M. Da Costa	Concordia University	3535 Papineau #1416 Montréal	525-5166
Glenn R. Phillips	Montana State Univ. Biol. Dept.	Lewis Hall, Bozeman Mt.	406-994-3560
Pierre Raymond	Université Concordia	3547 Université, app 16 Montréal, H3A 2B1	
Mary Gregory	Université Concordia	2250 Guy, #1901, Mtl.	933-7423

NOM	AFFILIATION	ADRESSE	TEL.
Barry Jessimar	University Ottawa	515 Bathurst Ave.	733-7017
Ken Trudel	S.L. Ross Env. Res. Ltd.	2496 Alta Vista Dr. Ottawa	731-2547
Michael Gregory	Concordia Univ.	1445 blvd. De Maisonneuve	879-4216
GB Bacon	Research & Productivity Covneil	Frederiction NB	506-454-0625
Bernadette	Université de Mtl.	Montréal	343-7691
Lucie Martin	"	"	"
Marthe Carillo	Concordia Univ.	1445 De Maisonneuve	879-4216
Daniel Green	S.V.P.	C.P. 65 Place d'Armes	844-5477
Michael Ailbertoon	EPS Ottawa	Env. Canada, Ottawa	994-0706
Charles Walbourn	Beckman Inst.	6200 El Camino Real	(714)438-9151
Tibor Kovacs	Pulp & Paper Research Inst. of Canada	570 St. Jean, Pointe Claire, Qué.	(514)697-4110
Gary Sprules	Univ. of Toronto (Erindale)	Mississauga	(416)828-5366
Craig Wood	Noranda Research Centre	Pointe Claire, Que. H9R 1G5	(514)697-6640
Danielle Chenier	Env. Canada	Montréal	283-4670
Gino Sabatini	McGill	Montréal	725-4469
Louis Hamel	Union Carbide du Canada Ltée	Montréal-Est 10555 Métropolitain est	645-5311
Madelyn Webb	York University	RM302 Petrie, 4700 Keele St. Downsview, Ont. M3J 1P3	416-667-3861
Brian Murley	"	"	"
Larry Onisto	"	"	"
Luc Valiquette	Env. Canada	1550 Maisonneuve	283-7309
Marc Lalande	Université de Mtl.	90 ave. Vincent d'Indy Mtl.	343-7691
Lucie Martin	Université Mtl.	90 ave. "	"

NOM	AFFILIATION	ADRESSE	TEL.
Denis Brunet	Beekman Inst.	6465 Hotthon Dr.	255-4039
Pat Duffy	Vanier College	5160 Décarie, Mtl.	333-4050
Ray Broules	Env. Can.	Ottawa	997-3513
Francine Perron	Env. Canada	Mtl.	283-7305

APPENDIX III

MINUTES OF THE 1st MEETING OF THE STEERING COMMITTEE FOR THE AQUATIC TOXICITY WORKSHOP

AQUATIC TOXICITY WORKSHOP

Minutes of the 1st Meeting of the Steering Committee

DATE/TIME: Thursday, 6 November, 1980/17:30h.
LOCATION: Holiday Inn, 420 Sherbrooke, Montreal, Quebec

ATTENDANCE:

Norman Bermingham	1001 Pierre Dupuy EPS Environment Canada Montreal, Quebec J4K 1A1 Tel: (514) 651-6860
Gordon Craig	Ministry of Environment Limnology and Toxicity Section P.O. Box 213 Rexdale, Ontario Tel: (416) 248-3011
Peter Hodson	Great Lakes Biolimnology Lab Canada Centre for Inland Waters Box 5050, 867 Lakeshore Rd. Burlington, Ontario Tel: (416) 637-4559
Marshall Kern	Pollutech Ltd. 1090 Speers Rd. Oakville, Ontario L6L 2X4 Tel: (416) 844-1900
Bill Lake (Brian Hammond attended for him)	Aquatic Bioassay Section Animal Sciences Wing Alberta Environmental Centre Box 399 Vegreville, Alberta T0B 4L0 Tel: (403) 632-3361
Gerard Leduc	Dept. of Biological Sciences Concordia University Maisonneuve Blvd Montreal, Quebec Tel: (514) 879-5989
Sharon Leonhard	Freshwater Institute 501 University Crescent Winnipeg, Manitoba, R3T 2N6 Tel: (204) 269-7379

Keith Solomon

Environmental Biology
University of Guelph
Guelph, Ontario N1Y 2W1
Tel: (519) 824-4120 Ext 2748
or Ext 8334

Peter G. Wells

Marine Ecology Lab
B.I.O., Dartmouth
Nova Scotia, B2Y 4A2
Tel: (902) 426-3276
(902) 826-2479 Home
and
Inst. for Environ. Studies
U. of T., Toronto, Ont. M5S 1A4

...2/

As directed by the general consent of the assembly at the 7th Annual Aquatic Toxicity Workshop Banquet Meeting on 5 November, 1980, a steering committee for the Aquatic Toxicity Workshop was established. Designates and volunteers met to discuss the composition of a permanent committee and to outline its functions. These members agreed that the organization should consider being federally incorporated as a non-profit group.

ACTION: S. LEONHARD
Investigate the terms and conditions for federal incorporation as the Aquatic Toxicity Workshop. Report findings to the members of the steering committee for evaluation.

The "officers" of this corporation shall consist of the members of the steering committee; any two of whom may have signing authority. This committee shall have a minimum of seven (7) members with power to add. These shall be determined as follows:

REPRESENTATIVE	DUTY	INCUMBENT
Chairman of the Workshop 2 years past	Chairman of the Steering Committee	P. Hodson
Chairman of the Workshop 1 year past	Recording Secretary	S. Leonhard
Present Chairman	Treasurer	N. Bermingham & G. Leduc
Chairman of the Workshop 1 year future		G. Craig & K. Solomon
Chairman of the Workshop 2 years future		B. Lake
Continuity Chairman	(duties follow)	K. Marshall
Regional representatives		

The functions of the steering committee shall be:

- to provide continuity between workshops by establishing a permanent mailing address, maintaining and updating a mailing list and maintaining a running record of workshop activities, including "proceedings" files.
- to determine the hosts of future workshops.
- to provide advice to the host committee concerning the organization of the annual meeting of the Aquatic Toxicity Workshop with respect to style format, theme, language, schedules, correspondence, workloads, publicity, publications.

This committee shall meet annually at the Aquatic Toxicity Workshop.

...4/

The steering committee views the functions of the host committee as:

- to establish a working committee
- to designate chairmen
- to identify sponsors
- to organize local arrangements activities for the Aquatic Toxicity Workshop including: location, accommodation, meeting rooms, meals, socials
- to plan the scientific programme of the Aquatic Toxicity Workshop including themes, programmes, formats, speakers, tours
- to schedule and post correspondence to the general mailing list recipients, including the announcement of the meeting, call for papers,etc.
- to establish a budget and bank account for the annual meeting and levy and collect fees to meet costs
- to prepare according to format and edit manuscripts for the annual proceedings
- to forward completed manuscript to the Continuity Chairman for publication and distribution
- to arrange publicity for the meeting
- to prepare progress reports and an annual review for the steering committee appraisal
- to revise the mailing list and inform the Continuity Chairman of changes.

The Continuity Chairman: Keith Marshall
Fish Habitat Protection Branch, D.F.O.
240 Sparks Street
Ottawa, Ontario K1A OE6

shall provide a fixed address for inquiries related to the Aquatic Toxicity Workshops and "Proceedings". The incumbent shall maintain an updated mailing list and provide same to host committees as required. He shall further be responsible for the maintenance of a file of reprints of the "Proceedings" and distribution of same upon request. Moreover, he shall be responsible for the printing of these "proceedings", annually.

ACTION: P. HODSON
Explore the options for financing the publication of the proceedings; following this, establish the "seed" money for an Aquatic Toxicity Workshop Bank Account. Report these findings to the steering committee and general mailing list recipients.

ACTION: ALL MEMBERS OF THE STEERING COMMITTEE

Submit existing revisions, additions, and deletions to the mailing list to the Continuity Chairman, Keith Marshall on or before December 1, 1980. Forward advice on planning and organisational materials from previous workshops to the 1981 Workshop Chairman, Gordon Craig or Keith Solomon. Maintain a file of above materials and bring it to the next workshop

to be retained in the "Archives" of the Aquatic Toxicity Workshop". (This responsibility remains to be delegated. Would the continuity chairman consider maintaining such a file?)

The proposed locations for future meetings of the Aquatic Toxicity Workshop are:

1981 - Ontario (confirmed)
1982 - Alberta (confirmed)
1983 - Nova Scotia
1984 - British Columbia

Respectfully submitted,

Sharon L. Leonhard

To: Members of the steering committee
From: S. L. Leonhard
For your consideration.

INCORPORATION OF A CORPORATION WITHOUT SHARE CAPITAL UNDER PART II OF THE CANADA CORPORATIONS ACT

1.0 INTRODUCTION

This kit is intended to provide clients with the basic instructions for fulfilling the filing requirements in respect of an incorporation under PART II of the Canada Corporations Act; it does not contain a comprehensive list of provisions which may be included in the application or by-laws if desired.

2.0 REQUIRED DOCUMENTS

In order to obtain letters patent, you must file certain documents with the Corporations Branch of Consumer and Corporate Affairs. These are:

2.1 An application in complete and proper form:

Enclosed is a model of a completed application which complies with all the requirements set out in Section 155(1) of the Act. Adherence to the form and content of the model (with substitution of certain information) should prevent substantial errors in the application.

2.2 A statutory declaration:

This must be made out by one of the applicants and attest to the truth of the facts set out in the application. A sample is enclosed.

2.3 By-laws of the proposed corporation:

The sample by-laws which are attached hereto contain all the provisions required to be included therein under Section 155(2) of C.C.A. and many extra clauses which are optional. As long as you cover the points set out in items (a) through (g) of S. 155(2) of the Act, you may add any other clause you wish as long as it does not conflict with any provision of the law.

3.0 CORPORATE NAME

The proposed name of the corporation must be searched and approved before letters patent may be issued. It is recommended that you have a name approved before you file the application if at all possible and to do this

.../2

you may

a) forward requests for reserving a proposed corporate name to the private firms noted on the enclosed list, who directly access the database and automated search system administered by the Minister.

b) if you are unable to clear the proposed name in advance, it will be searched when the application is received in the Corporations Branch. However, if the proposed name is not available and you have not previously indicated your willingness in this event to accept a numeric name, then the application will be rejected and the original engrossing date will be lost.

If approved, the name will be reserved for a period of 90 days.

4.0 FEES

A complete schedule of fees is to be found on page 3.

4.1 Name Fee

The fee for searching by the Corporations Branch is $15.00 for each proposed name requested.

4.2 Filing Fee

The charge for processing an application for letters patent under C.C.A. Part II is $200.00. As no service may be rendered in advance of payment, the fee should accompany the application.

If the application must be returned to you for additions or corrections, the fee will be retained pending return of the documents. Should you wish to withdraw your application, you will be granted a full refund upon receipt of a written notice from you to the effect that you are abandoning the application.

CANADA CORPORATIONS ACT FEES

1. Item 1 of Schedule 2 to the Canada Corporations Regulations is revoked and the following substituted therefor:

a) Each direct request to the Minister to search one proposed corporate name, including the reservation of the name..... $ 15.00

b) Each request for access to the database and automated name search system administered by the Minister for the purpose of making a search of one proposed corporate name, made by a government agency or a private sector firm that offers name search services..... $ 2.00

c) Each request for a name search or an analysis of the trade name database that cannot be effected by a standard computer search..... Cost Plus 20%

2. An application to the Minister for:

a) letters patent issued under sections 137, 154 and 159 ..... $200.00

b) supplementary letters patent issued under sections 13, 20, 29, 60 and 135..... $ 50.00

c) a certificate of increase in consideration for shares without par value..... $ 50.00

3. On the issuance by the Minister of any certificate..... $ 10.00

4. On filing a summary under section 133..... $ 30.00

5. For uncertified copies of or extract from documents supplied by the Minister under subsection 129(2), per document if in excess of nine (9) documents..... $ 1.00

6. Certified copies of or extract from documents supplied by the Minister under subsection 129(2)..... $ 10.00

7. On registering particulars of mortgages or charges under subsection 68(6)..... $ 10.00

8. On any search of the register under section 68..... $ 10.00

9. On registering an order for the appointment of a receiver or receiver and manager under section 69..... $ 10.00

10. On the inspection of documents under sections 100.1 and 108.4..... $ 10.00

1. L'alinéa 1 de l'annexe 2 du Règlement sur les corporations canadiennes est abrogé et remplacé par ce qui suit:

a) Chaque demande, adressée directement au Ministre, concernant la recherche d'une dénomination sociale projetée, y compris la réservation de cette dénomination..... $ 15.00

b) Chaque demande d'accès au fichier et au système de recherche automatisée de dénomenations, concernant la recherche d'une dénomination sociale et soumise par une agence gouvernementale ou par une entreprise privée offrant des services de recherche de dénominations..... $ 2.00

c) Chaque demande pour une recherche de nom ou d'analyse du fichier des noms commerciaux ne pouvant être satisfaite par une recherche normale par ordinateur..... Coût Plus 20%

2. Demande au Ministre pour:

a) une émission de lettres patentes en vertu des articles 137, 154 et 159..... $200.00

b) une émission de lettres patentes supplémentaires en vertu des articles 13, 20, 29, 60 et 135... $ 50.00

c) une émission d'un certificat confirmant une augmentation du montant auquel peuvent être émises des actions sans valeur au pair..... $ 50.00

3. Délivrance d'un certificat par le Ministre..... $ 10.00

4. Dépôt d'un sommaire en vertu de l'article 133..... $ 30.00

5. Fourniture par le Ministre de copies non certifiées de documents, en vertu du paragraphe 129(2), par document, s'il y en a plus de 9 .... $ 1.00

6. Fourniture par le Ministre de copies certifiées de documents, en vertu du paragraphe 129(2)..... $ 10.00

7. Enregistrement des détails d'hypothèques ou de charges en vertu du paragraphe 68(6)..... $ 10.00

8. Recherche dans le registre en vertu de l'article 68..... $ 10.00

9. Enregistrement d'une ordonnance pour la nomination d'un séquestre ou d'un séquestre-et-gérant en vertu de l'article 69..... $ 10.00

10. Consultation de documents en vertu des articles 100.1 et 108.4..... $ 10.00

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APPLICATION FOR INCORPORATION OF A CORPORATION WITHOUT SHARE CAPITAL UNDER PART II OF THE CANADA CORPORATIONS ACT

To the Minister of Consumer and Corporate Affairs of Canada.

I

The undersigned hereby apply to the Minister of Consumer and Corporate Affairs for the grant of a charter by letters patent under the provisions of Part II of the Canada Corporations Act constituting the undersigned, and such others as may become members of the Corporation thereby created, a body corporate and politic under the name of

XYZ CORPORATION - LA CORPORATION XYZ*

The undersigned have satisfied themselves and are assured that the proposed name under which incorporation is sought is not the same or similar to the name under which any other company, society, association or firm, in existence is carrying on business in Canada or is incorporated under the laws of Canada or any province thereof or so nearly resembles the same as to be calculated to deceive (** and, where required by the circumstances: except that of ..... which has signified its consent to the use of the said name) and that it is not a name which is otherwise on public grounds objectionable.

II

The applicants are individuals of the full age of twenty-one years with power under law to contract. The name, the place of residence and the calling of each of the applicants are as follows:

JOHN DOE,	100 Dominic Street, Bytown, Ontario Barrister;
JAMES SMITH,	200 Dominic Street, Bytown, Ontario Office Manager;
ANN JONES,	300 Dominic Street, Bytown, Ontario Secretary.

The said JOHN DOE, JAMES SMITH and ANN JONES will be the first directors of the Corporation.

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* If the name has not been cleared in advance, it is suggested to leave a blank space so that the name which is finally found to be acceptable may be inserted by the officials of the Department.

** When drafting please insert only applicable wording where choices are provided for in this form.

III

The objects of the Corporation are:

(The objects should be set forth in the infinitive form, in general terms and be as brief as possible; they should also be compatible with the type of business implied by any descriptive word which may form part of the corporate name.)

IV

The operations of the Corporation may be carried on throughout Canada and elsewhere.

V

The place within Canada where the head office of the Corporation is to be situated is: *

VI

(The following clause should be inserted if the Corporation is to be registered as a Canadian charitable organization with the Department of National Revenue - Taxation.)

It is specially provided that in the event of dissolution or winding-up of the Corporation all its remaining assets after payment of its liabilities shall be distributed to one or more recognized charitable organizations in Canada.

(If the Corporation is not intending to be registered as a Canadian charitable organization the following wording is required:)

It is specially provided that in the event of dissolution or winding-up of the Corporation all its remaining assets after payment of its liabilities shall be distributed to one or more organizations in Canada having cognate or similar objects.

(See also subsections 16(3) and (4) and subsection 155(3) of the Canada Corporations Act.)

VII
(Optional)

In accordance with Section 65 of the Canada Corporations Act, it is provided that, when authorized by by-law, duly passed by the directors

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* Need only specify municipality and province here. Please advise us of postal address by covering letter.

and sanctioned by at least two-thirds of the votes cast at a special general meeting of the members duly called for considering the by-law, the directors of the Corporation may from time to time

a) borrow mony upon the credit of the Corporation;

b) limit or increase the amount to be borrowed;

c) issue debentures or other securities of the Corporation;

d) pledge or sell such debentures or other securities for such sums and at such prices as may be deemed expedient; and,

e) secure any such debentures, or other securities, or any other present or future borrowing or liability of the Corporation, by mortgage, hypothec, charge or pledge of all or any currently owned or subsequently acquired real and personal, movable and immovable, property of the Corporation, and the undertaking and rights of the Corporation.

Any such by-law may provide for the delegation of such powers by the directors to such officers or directors of the Corporation to such extent and in such manner as may be set out in the by-law.

Nothing herein limits or restricts the borrowing of money by the Corporation on bills of exchange or promissory notes made, drawn, accepted or endorsed by or on behalf of the Corporation.

VIII

The by-laws of the Corporation shall be those filed with the application for letters patent until repealed, amended, altered or added to. (Please ensure that by-laws deal with each matter set out in subsection 155(2) of the Canada Corporations Act.)

IX

The Corporation is to carry on its operations without pecuniary gain to its members and any profits or other accretions to the Corporation are to be used in promoting its objects.

DATED at the City of Bytown in the Province of Ontario, this _____ day of _____, 19 .

_____

_____

_____

Signature of Applicants

CANADA

PROVINCE OF _____

REGIONAL MUNICIPALITY

OF _____

TO WIT:

IN THE MANNER OF the application

of _____ and others

for incorporation under Part II of the

Canada Corporations Act under the name

of _____
(corporate name)

I, _____, of the City of _____ in the

Province of _____, do solemnly declare this:

1. I am one of the applicants herein.

2. I have knowledge of the matter, and that the statements in the annexed application contained are, to the best of my knowledge and belief, true in substance and in fact.

3. I am informed and believe that each applicant signing the said application is of the full age of twenty-one years and has power under law to contract and that his or her name and description have been accurately set out in the preamble thereto.

4. The proposed corporate name of the company is not on any public grounds objectionable and that it is not that of any known company, incorporated or unincorporated, or of any partnership or individual, or any name under which any known business is being carried on, or so nearly resembling the same as to deceive.

5. I have satisfied myself and am assured that no public or private interest will be prejudicially affected by the incorporation of the company aforesaid.

AND I make this solemn declaration conscientiously believing it to be true and knowing that it is of the same force and effect as if made under oath and by virtue of the Canada Evidence Act.

SWORN before me at _____

in the Regional Municipality of

_____ this _____ day of _____

_____, 19 .

_____

A commissionner, etc.

SAMPLE BY-LAWS FOR A C.C.A. PART II CORPORATION

CORPORATE SEAL

1. The seal, an impression whereof is stamped in the margin hereof, shall be the seal of the _____ (corporate name).

CONDITIONS OF MEMBERSHIP

2. Membership in the corporation shall be limited to persons interested in furthering the objects of the corporation and shall consist of anyone whose application for admission as a member has received the approval of the board of directors of the corporation.

3. There shall be no membership fees or dues unless otherwise directed by the board of directors.

4. Any member may withdraw from the corporation by delivering to the corporation a written resignation and lodging a copy of the same with the secretary of the corporation.

5. Any member may be required to resign by a vote of three-quarters (3/4) of the members at an annual meeting.

HEAD OFFICE

6. * The Head Office of the corporation shall be in the (City - Municipality) of _____, _____ (province).

* (Please do not include a postal address here; only municipality and province should be noted).

BOARD OF DIRECTORS

7. The property and business of the corporation shall be managed by a board of _____ directors of whom _____ shall constitute a quorum. The board of directors may on literature of the corporation be designated as a board of governors.

8. The applicants for incorporation shall become the first directors of the corporation whose term of office on the board of directors shall be for _____.

9. Successors to the first directors shall be elected for a term of one (1) year by the members at an annual meeting of members.

10. The office of director shall be automatically vacated:

a) if a director shall resign his office by delivering a written resignation to the secretary of the corporation;

b) if he is found to be a lunatic or become of unsound mind;

c) if he becomes bankrupt or suspends payment or compounds with his creditors;

d) if at a special general meeting of members a resolution is passed by _____ of the members present at the meeting that he be removed from office;

e) on death;

provided that if any vacancy shall occur for any reason in this paragraph contained, the board of directors by majority vote, may, by appointment, fill the vacancy with a member of the corporation.

11. Meetings of the board of directors may be held at any time and place to be determined by the directors provided that seven (7) clear days notice of such meeting shall be sent in writing to each director, provided there shall be at least one (1) meeting per year of the board of directors. No error or omission in giving notice of any meeting of the board of directors or any adjourned meeting of the board of directors of the corporation shall invalidate such meeting or make void any proceedings taken thereat and any director may at any time waive notice of any such meeting and may ratify, approve and confirm any or all proceedings taken or had thereat.

12. Directors and executive committee members, as such, shall not receive any stated remuneration for their services, but, by resolution of the board of directors, expenses of their attendance may be allowed for their attendance at each regular or special meeting of the board of directors. Nothing herein contained shall be construed to preclude any director from serving the corporation as an officer or in any other capacity and receiving compensation therefor. The directors shall serve as such without remuneration and no director shall directly or indirectly receive any profit from his position as such; provided that a director may be paid reasonable expenses incurred by him in the performance of his duties; and provided futher that any director who is engaged in or is a member of a firm engaged in any business or profession may act in and be paid the usual professional costs and charges for any professional business required to be done in connection with the administration of the affairs of the corporation.

.../10

13. A retiring director shall remain in office until the dissolution or adjournment of the meeting at which his retirement is accepted and his successor is elected. A director shall hold office until the next annual meeting of members following his election or appointment.

14. At the first meeting of members, the board of directors then elected shall replace the provisional directors named in the Letters Patent of the corporation as provided for in paragraph 8 hereof.

15. The board of directors may appoint such agents and engage such employees as it shall deem necessary from time to time and such persons shall have such authority and shall perform such duties as shall be prescribed by the board of directors at the time of such appointment.

16. The remuneration of all officers, agents and employees and committee members shall be fixed by the board of directors by resolution. Such resolution shall have force and effect only until the next meeting of members when such resolution shall be confirmed by resolution of the members, or in the absence of such confirmation by the members, then the remuneration to such officers, agents or employees shall cease to be payable from the date of such meeting of members.

INDEMNITIES TO DIRECTORS AND OTHERS

17. Every director or officer of the corporation or other person who has undertaken or is about to undertake any liability on behalf of the corporation or any company controlled by it and their heirs, executors and administrators, and estate and effects, respectively, shall from time to time and at all times, be indemnified and saved harmless out of the funds of the corporation, from and against;

a) all costs, charges and expenses whatsoever which such director, officer or other person sustains or incurrs in or about any action, suit or proceedings which is brought, commenced or prosecuted against him, or in respect of any act, deed, matter or thing whatsoever, made, done or permitted by him, in or about the execution of the duties of his office or in respect of any such liability;

b) all other costs, charges and expenses which he sustains or incurrs in or about or in relation to the affairs thereof, except such costs, charges or expenses as are occasioned by his own wilful neglect or default.

EXECUTIVE COMMITTEE

18. The board of directors may provide for the creation of an executive committee comprising, who shall be appointed by the board of directors and which committe shall exercise such powers as are authorized by the board of directors. Any executive committee member may be removed by a majority vote of the board of directors.

19. Meetings of the executive committee may be held at any time and place to be determined by the members of such committee provided that forty-eight (48) hours notice of such meeting shall be sent in writing to each member of such committee. Members of such committee shall constitute a quorum. No error or omission in giving notice of any meeting of the executive committee or any adjourned meeting of the executive committee of the corporation shall invalidate such meeting or make void any proceedings taken thereat and any member of such committee may at any time waive notice of any such meeting and may ratify, approve and confirm any or all proceedings taken or had thereat.

POWERS OF DIRECTORS

20. The directors of the corporation may administer affairs of the corporation in all things and make or cause to be made for the corporation, in its name, any kind of contract which the corporation may lawfully enter into and, save as hereinafter provided, generally, may exercise all such other powers and do all such other acts and things as the corporation is by its charter or otherwise authorized to exercise and do.

21. The directors shall have power to authorize expenditures on behalf of the corporation from time to time and may delegate by resolution to an officer or officers of the corporation the right to employ and pay salaries to employees. The directors shall have the power to make expenditures for the purpose of furthering the objects of the corporation. The directors shall have the power to enter into a trust arrangement with a trust company for the purpose of creating a trust fund in which the capital and interest may be made available for the benefit of promoting the interest of _____ (corporate name) in accordance with such terms as the board of directors may prescribe.

22. The board of directors shall take such steps as they may deem requisite to enable the corporation to acquire, accept, solicit or receive legacies, gifts, grants, settlements, bequests, endowments and donations of any kind whatsoever for the purpose of furthering the objects of the corporation.

OFFICERS

23. The officers of the corporation shall be a president, vice-president, secretary and treasurer and any such other officers as the board of directors may by by-law determine including, but without restricting the foregoing, an executive secretary. Any two offices may be held by the same person.

24. The president shall be elected at the annual meeting of the members. Officers other than president of the corporation shall be appointed by resolution of the board of directors at the first meeting of the board of directors following each annual meeting of the members.

25. The officers of the corporation shall hold office for one (1) year from the date of appointment or election or until their successors are elected or appointed in their stead.

DUTIES OF OFFICERS

26. All officers shall be directors of the corporation and they shall cease to be officers if they cease to be directors or if they are removed by a majority of the board of directors.

27. The president shall be the chief executive officer of the corporation. He shall preside at all meetings of the corporation and of the board of directors. He shall have the general and active management of the affairs of the corporation. He shall see that all orders and resolutions of the board of directors are carried into effect.

28. The vice-president shall, in the absence or disability of the president, perform the duties and exercise the powers of the president and shall perform such other duties as shall from time to time be imposed upon him by the board of directors.

29. The treasurer shall have the custody of the funds and securities of the corporation and shall keep full and accurate accounts of all assets, liabilities, receipts and disbursements of the corporation in the books belonging to the corporation and shall deposit all monies, securities and other valuable effects in the name and to the credit of the corporation in such chartered bank or trust company, or, in the case of securities, in such registered dealer in securities as may be designated by the board of directors from time to time. He shall disburse the funds of the corporation as may be directed by proper authority taking proper vouchers for such disbursements, and shall render to the president and directors at the regular meeting of the board of directors, or whenever they may require it, an accounting of all the transactions and a statement of the financial position, of the corporation. He shall also perform such other duties as may from time to time be directed by the board of directors.

30. The executive secretary may be empowered by the board of directors, upon resolution of the board of directors, to carry on the affairs of the corporation generally under the supervision of the officers thereof and shall attend all meetings and act as clerk thereof and record all votes and minutes of all proceedings in the books to be kept for that purpose. He shall give or cause to be given notice of all meetings of the members and of the board of directors, and shall perform such other duties as may be prescribed by the board of directors or president, under whose supervision he shall be. He shall be custodian of the seal of the corporation, which he shall deliver only when authorized by a resolution of the board of directors to do so and to such person or persons as may be named in the resolution.

31. The duties of all other officers of the corporation shall be such as the terms of their engagement call for or the board of directors required of them.

EXECUTION OF DOCUMENTS

32. Contracts, documents or any instruments in writing requiring the signature of the corporation, shall be signed by any two officers and all contracts, documents and instruments in writing so signed shall be binding upon the corporation without any further authorization or formality. The directors shall have power from time to time by resolution to appoint an officer or officers on behalf of the corporation to sign specific contracts, documents and instruments in writing. The directors may give the corporation's power of attorney to any registered dealer in securities for the purposes of the transferring of and delaing with any stocks, bonds, and other securities of the corporation. The seal of the corporation when required may be affixed to contracts, documents and instruments in writing signed as aforesaid or by any officer or officers appointed by resolution of the board of directors.

MEETINGS

33. The annual or any other general meeting of the members shall be held at the head office of the corporation or at any place as the board of directors may determine and on such day as the said directors shall appoint.

34. At every annual meeting, in addition to any other business that may be transacted, the report of the directors, the financial statement and the report of the auditors shall be presented and a board of directors elected and auditors appointed for the ensuing year. The members may consider and transact any business either special or general at any meeting of the members. The board of directors or the president or vice-president shall have power to call, at any time, a general meeting of the members of the corporation.

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35. Fourteen (14) days' prior written notice shall be given to each member of any annual or special general meeting of members. Members present in person at a meeting shall constitute a quorum. Each member present at a meeting shall have the right to exercise one vote.

36. No error or omission in giving notice of any annual or general meeting or any adjourned meeting, whether annual or general, of the members of the corporation shall invalidate such meeting or make void any proceedings taken thereat and any member may at any time waive notice of any such meeting and may ratify, approve and confirm any or all proceedings taken or had thereat. For purpose of sending notice to any member, director or officer for any meeting or otherwise, the address of the member, director or officer shall be his last address recorded on the books of the corporation.

MINUTES OF BOARD OF DIRECTORS AND EXECUTIVE COMMITTEES

37. The minutes of the board of directors or the minutes of the executive committee shall not be available to the general membership of the corporation but shall be available to the board of directors, each of whom shall receive a copy of such minutes.

VOTING OF MEMBERS

38. At all meetings of members of the corporation every question shall be determined by a majority of votes unless otherwise specifically provided by statute or by these by-laws.

FINANCIAL YEAR

39. Unless otherwise ordered by the board of directors the fiscal year-end of the corporation shall be _____.

40. The board of directors may appoint committees whose members will hold their offices at the will of the board of directors.

AMENDMENT OF BY-LAWS

41. The by-laws of the corporation may be repealed or amended by by-law enacted by a majority of the directors at a meeting of the board of directors and sanctioned by an affirmative vote of at least two-thirds (2/3) of the members at a meeting duly called for the purpose

of considering the said by-law, provided that the enactment, repeal or amendment of such by-law shall not be enforced or acted upon until the approval of the Minister of Consumer & Corporate Affairs has been obtained.

AUDITORS

42. The members shall at each annual meeting appoint an auditor to audit the accounts of the corporation to hold office until the next annual meeting provided that the directors may fill any casual vacancy in the office of auditor. The remuneration of the auditor shall be fixed by the board of directors.

BOOKS AND RECORDS

43. The directors shall see that all necessary books and records of the corporation required by the by-laws of the corporation or by any applicable statute or law are regularly and properly kept.

RULES AND REGULATIONS

44. The board of directors may prescribe such rules and regulations not inconsistent with these by-laws relating to the management and operation of the corporation as they deem expedient, provided that such rules and regulations shall have force and effect only until the next annual meeting of the members of the corporation when they shall be confirmed, and failing such confirmation at such annual meeting of members shall at and from that time cease to have any force and effect.

INTERPRETATION

45. In these by-laws and in all other by-laws of the corporation hereafter passed unless the context otherwise requires, words importing the singular number or the masculine gender shall include the plural number or the feminine gender, as the case may be, and vice versa, and references to persons shall include firms and corporations.

IN WITNESS WHEREOF we have hereunto set out hands at _____ _____, _____ on the _____ day of _____ _____, _____.

_____
(signatures)