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par

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Effet du filtrat d'un champignon B-89 (Deuteromycotina) sur les Cyprinidae et la faune non-cible.

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À mes parents

à Michel

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Problématique et résumé

L'introduction de nouvelles espèces de poissons entraîne souvent des impacts néfastes pour les populations ichtyologiques indigènes. Les Cyprinidae, représentés par 44 espèces dans les eaux canadiennes, ont été introduits par les pêcheurs sportifs les utilisant comme appâts-vivants. Ces poissons se sont rapidement propagés dans plusieurs réseaux hydrographiques du bouclier canadien où l'omble de fontaine (*Salvelinus fontinalis* Mitchill), espèce la plus exploitée par les pêcheurs sportifs au Québec, était originellement retrouvé en allopatrie. La présence d'espèces introduites se traduit par un changement de niche alimentaire de l'omble de fontaine passant du zoobenthos au zooplancton, une modification de la distribution spatiale des individus et principalement par une diminution marquée du rendement de pêche annuel moyen, ce qui entraîne des pertes financières considérables au niveau de la gestion de la pêche sportive au Québec. En raison de l'ampleur du phénomène, la restauration du potentiel perdu dans les lacs et les rivières est devenue une nécessité.

Afin d'éliminer les espèces de poissons non-désirées, on emploi couramment toute une série de méthodes et de produits. Les agents chimiques, à large spectre d'action, ont été largement utilisés en aménagement piscicole. Cependant, plusieurs de ces agents avaient déjà été employés pour contrôler d'autres organismes. Ainsi, on a vu apparaître des insecticides-piscicides, des molluscicides-piscicides, des herbicides-piscicides, des stérilisants-piscicides et des piscicides proprement dits. L'empoisonnement à l'aide de produits non-sélectifs entraîne l'éradication de toutes les espèces de poissons en plus d'occasionner des dommages substantiels aux populations

d'invertébrés benthiques et de zooplancton. L'utilisation de ces piscicides présente certains risques et suscite beaucoup de controverses environnementales compte tenu de leurs toxicités sur l'ensemble des organismes présents ainsi que de leurs rémanences dans le milieu. C'est pourquoi, des techniques présentant des impacts minimes pour l'environnement ont été développées.

Les contrôles physiques, qui englobent un vaste ensemble de techniques (filets, verveux, barrières, pêches...), font appel à une récolte directe des individus non-désirés ou encore à la restriction de leurs déplacements. Bien que seule l'espèce nuisible soit généralement affectée, la main-d'oeuvre et le temps requis pour mener à bien une telle opération constituent un facteur limitant l'utilisation de ces techniques.

Dans la recherche de contrôles efficaces, économiques et sans dangers pour l'environnement, les contrôles biologiques ont connu un regain de popularité. Les méthodes dites biologiques privilégient l'introduction de prédateurs, de pathogènes, les manipulations génétiques et l'utilisation de toxines produites par les organismes vivants. Ce sont les toxines biologiques qui ont suscité le plus d'attention au cours des dernières années. Très peu de tentatives se sont avérées fructueuses dans le domaine aquatique avec l'introduction de pathogènes, de prédateurs et les manipulations génétiques qui présentent des inconvénients majeurs, comme par exemple, la destruction incomplète de la peste et/ou l'imprévisibilité de l'effet du prédateur ou du pathogène en milieu naturel. Les deux toxines biologiques les plus utilisées présentement en aménagement piscicole sont l'antimycine A et la roténone.

L'antimycine A, produite par la bactérie *Streptomices* sp, a été utilisée pour détruire toutes les espèces de poissons dans les lacs et les étangs et aussi comme piscicide sélectif. La majorité des organismes benthiques des milieux lacustres ne sont pas affectés par un traitement à l'antimycine A, cependant, un traitement en milieu lotique occasionne une augmentation immédiate de la dérive des organismes et une réduction marquée de la population d'invertébrés. De plus, le coût d'un traitement à l'antimycine A est près du double de celui de la roténone.

La roténone, produite par des racines de légumineuse comme le *Derris* sp, le *Lonchocarpus* sp et le *Tephrosia* sp, est actuellement le piscicide le plus utilisé en aménagement piscicole. Le traitement d'un lac à la roténone affecte toutes les espèces de poissons présentes en plus d'avoir comme effet direct et immédiat la réduction marquée des populations de zooplancton, ce que la plupart des chercheurs qualifie de désastreux. Par contre, l'effet immédiat de la roténone sur le benthos est variable et moins important que sur le zooplancton. Les études démontrent cependant, que le benthos en eaux courantes est plus sensible et que le temps de recolonisation est beaucoup plus long que celui des espèces lacustres.

Cette disparition temporaire des invertébrés aquatiques et du zooplancton entraîne des répercussions importantes sur la restauration des lacs, car ces organismes sont la base alimentaire des espèces de poissons désirées. L'utilisation de piscicides non-sélectifs doit être suivie d'un ensemencement de poissons recherchés après des délais de plusieurs mois, étant donné qu'on doit attendre que les populations de zooplancton et autres invertébrés benthiques se soient rétablies.

Par conséquent, le recours à un produit hautement spécifique serait la réponse aux nombreux problèmes que soulèvent les pratiques d'aménagement piscicoles actuelles.

Un champignon B-89 a été découvert dans le cadre du projet "isolation et évaluation de mycètes pour le contrôle biologique des moustiques et des mouches noires". Des essais biologiques avaient alors démontré que cet hyphomycète présentait un potentiel intéressant pour le contrôle des insectes piqueurs. Cependant, lors d'études subséquentes sur la faune non-cible, les Cyprinidae avaient de plus démontré une grande sensibilité au filtrat de culture.

L'objectif premier de notre travail était donc de vérifier le potentiel piscicide du filtrat brut du B-89 sur les Cyprinidae en relation avec la concentration du filtrat, la durée d'exposition des poissons et la température. On retrouve les résultats correspondant à cet objectif, de même que l'évaluation des pertes d'activité du filtrat brut en présence des Cyprinidae dans un premier article intitulé "Effect of temperature and dose on the piscicidal activity of a fungal (Deuteromycotina) extract" rédigé en conformité avec le "Journal canadien des sciences halieutiques et aquatiques". Cette étude démontre que le filtrat brut du B-89 présente un effet piscicide très rapide pour les Cyprinidae (Figures 1, 2 et 3). La mortalité, se produisant dans les premières heures d'exposition, croît avec la concentration (exprimée en dilution), le temps d'exposition et la température. Lorsque les effets toxiques ne se produisent pas assez rapidement, on obtient alors des arrêts de mortalités (Figures 1 et 3). La vitesse de pénétration des toxines étant plus lente à basse température (Figure 1), nous croyons que ces arrêts de mortalités peuvent être corrélés, d'une part, avec une détoxification rapide de la toxine par le poisson. D'autre part, ces arrêts de

mortalités (Figure 3) peuvent être directement associés avec la perte d'activité du filtrat brut en présence des Cyprinidae. En effet, si la quantité nécessaire de toxine pour engendrer des effets létaux n'est pas atteinte, il y a possibilité de survie pour les poissons.

Les essais sur larves de moustiques, avec des filtrats récupérés après différents temps de contact des poissons, indiquent qu'il existe une corrélation entre l'augmentation des temps de contact et la perte d'activité résiduelle du filtrat brut (Figure 6). La diminution rapide et marquée de la mortalité des moustiques suggère que les toxines sont enlevées du filtrat par pénétration rapide dans les poissons. Les pertes mesurées sont de 70% dans les 20 premières minutes, soit jusqu'aux premiers signes d'intoxication et de 9% entre 20 et 73 minutes d'exposition.

En se basant sur les résultats obtenus précédemment, nous avons vérifié l'existence d'une relation isodose. Cette relation isodose a été établie à la Figure 4 et elle nous indique que la mortalité des Cyprinidae est proportionnelle à la dose. La dose en contact avec le poisson est exprimée par le produit de la concentration (dilution) et du temps d'exposition. Pour l'ensemble de nos tests, le temps d'exposition correspond au temps de la mort des individus. La relation isodose permet donc de dire qu'une concentration de 1 mg/L injectée pendant 10 minutes donnerait le même pourcentage de mortalité que 10 mg/L injecté pendant 1 minute. En effet, peu importe la dilution et le temps d'exposition utilisés, nos résultats indiquent qu'à 13°C (Figure 4 A) une dose de 2,8 est nécessaire pour tuer 50% de la population et cette dose diminue avec l'augmentation de la température. La relation de température permet de voir que la dose létale 50% (DL_{50}) diminue par un facteur de 3,5 lorsque la température s'élève de 13 à 25°C alors que les pentes sont

semblables (Figure 5). Ceci indique que l'augmentation de température ne modifie pas le mécanisme d'action de la toxine.

L'ensemble des résultats de cette première partie de recherche indique que le filtrat brut du B-89 possède un pouvoir piscicide certain sur les Cyprinidae. Cela fait donc du filtrat brut un agent potentiel pour l'éradication des espèces non-désirées. Puisque le temps d'exposition ressort comme un facteur important, notre deuxième objectif était d'étudier le potentiel toxique en condition d'eaux courantes où le temps de contact est d'une durée limitée. Nous avons de plus, évalué les effets du filtrat brut sur les invertébrés benthiques. Les résultats de cette deuxième partie sont présentés dans l'article intitulé "Effect of a fungal (Deuteromycotina) extract with piscicidal activity on non-target benthic invertebrates" dont la rédaction se conforme aussi aux exigences de la revue "Journal canadien des sciences halieutiques et aquatiques".

Afin d'évaluer un temps de contact et une température qui nous seraient idéal, des tests de temps de contact variant entre 2,5 et 80 minutes ont été effectués à deux températures. À partir de ces résultats (Figures 1 et 2), nous avons décidé d'utiliser un temps de contact de 30 minutes à une température de 18°C. Les bioessais ont été effectués dans un système de gouttières avec 3 dilutions (exprimée en dose). Nous avons observé que la mortalité des Cyprinidae se produisait très rapidement après le début du traitement et même avant la fin du traitement avec l'utilisation d'une dose de 3,0. Les mortalités finales des Cyprinidae (Fig. 3 colonne A) sont de 21, 73 et 100% avec l'utilisation d'une dose de 0,75, 1,5 et 3,0 respectivement. L'augmentation des

pourcentages de mortalité (augmentation de 50% entre la dose 0,75 et 1,5) nous laisse croire que la dose de 3,0 correspondrait à une surdose pour les organismes de la faune non-cible étudiés.

En dépit de l'utilisation d'une forte dose qui tue 100% des Cyprinidae, il n'y a aucun groupe d'invertébrés benthiques (Figure 3; colonne B à N) qui manifeste une grande sensibilité au traitement du filtrat brut du B-89. Vingt-quatre heures après le traitement, aucune mortalité n'est enregistrée pour au moins 12 groupes d'invertébrés tandis que 13 autres groupes démontrent des mortalités variant entre 1 et 21% à la plus forte dose utilisée. Le filtrat brut a donc peu ou pas d'effet sur la mortalité des invertébrés benthiques associés au Cyprinidae. L'échantillonnage de la dérive (Table 1) ne démontre pas d'augmentation notable chez les éphémères, les plécoptères, les mégaloptères, les trichoptères et les odonates après l'injection du filtrat brut à l'exception des coléoptères et des diptères où l'on remarque des augmentations respectives de 4 et 8 fois avec l'utilisation d'une dose de 3,0. L'effet sur les diptères n'est pas surprenant car, le filtrat brut démontrait au départ un effet insecticide sur les moustiques et les mouches noires. L'augmentation de la dérive chez les diptères est principalement occasionnée par la présence de mouches noires. Parmi les différents groupes étudiés, la seule mortalité notable de la dérive est de 8,6% chez les éphémères exposés à la plus forte dose. Pour tous les ordres d'insectes étudiés, les moyennes des mortalités et des dérives indiquent qu'il n'y a pas de différence marquée entre le témoin et les trois doses utilisées. Considérant l'absence d'impact évident sur les invertébrés benthiques, le filtrat brut présente une forte sélectivité envers les Cyprinidae. Ceci fait donc du filtrat brut, un agent fort désirable pour les programmes de contrôles des Cyprinidae.

Afin de compléter l'étude sur la faune non-cible, notre troisième objectif était d'évaluer les effets du filtrat brut dans des conditions d'exposition continue, sur différents groupes de zooplancton et quelques larves d'insectes. Ces expériences ont été réalisées en laboratoire et les résultats se retrouvent dans l'article intitulé "Effect of a fungal (Deuteromycotina) extract with piscicidal activity on zooplankton and insect larvae" rédigé suivant les exigences de la revue "Journal canadien des sciences halieutiques et aquatiques". Les résultats de la Figure 1 confirment que les différents groupes de zooplancton étudiés ne sont pas affectés par l'action du filtrat brut. Comparativement aux Cyprinidae, nous notons pour les rotifères, les cladocères, les ostracodes et les copépodes, des augmentations respectives de la DL_{50} de 5, 26, 27 et 37 fois, alors que les pentes sont semblables. Le groupe le plus sensible est celui des rotifères mais il n'est pas affecté par la dose qui tue 100% des Cyprinidae. Par conséquent, le filtrat brut n'a pas d'effet sur les différents groupes de zooplancton que nous avons étudiés. Par contre, les plécoptères démontrent une légère sensibilité lorsque la population de Cyprinidae est tuée à 100%. Comparativement aux Cyprinidae, les éphémères ne sont pas affectés démontrant une DL_{50} et une DL_{95} de 9,5 et 24,8 fois plus élevée. Finalement, on n'obtient aucune mortalité chez les trichoptères pendant le traitement. Ces résultats viennent confirmer que le filtrat brut du B-89 démontre une spécificité remarquable sur les Cyprinidae.

L'ensemble des travaux effectué a permis de faire avancer les connaissances sur la toxicité du filtrat brut du B-89 en apportant des indications sur les effets et sur l'efficacité de ce filtrat. Nous avons démontré dans un premier article les effets du filtrat brut sur les Cyprinidae. Les taux élevés de mortalité observés et la vitesse à laquelle l'activité toxique se manifeste font du B-89 un

agent piscicide prometteur. Conséquemment, dans un deuxième article, nous avons vérifié la spécificité d'action du filtrat envers les Cyprinidae en déterminant ses effets sur les invertébrés benthiques. Suite à un temps d'exposition de 30 minutes, les faibles pourcentages de mortalité observés comparativement aux Cyprinidae et l'absence d'une dérive marquée, nous démontre que le filtrat brut possède une forte sélectivité sur les Cyprinidae. Enfin, nous avons observé dans un troisième article que les différents groupes de zooplancton n'étaient pas affectés par un traitement continu (24 h) avec le filtrat brut du B-89. Cependant, un groupe d'invertébré benthique, les plécoptères, a démontré une certaine sensibilité après un traitement continu (8 h) avec la dose éliminant les Cyprinidae.

Finalement nous avons mis en évidence que le filtrat brut de B-89 possède une activité toxique certaine pour les Cyprinidae affectant que très légèrement les organismes de la faune non-cible. Enfin, ces travaux ouvrent des perspectives de recherches en ce qui concerne la spécificité d'action du B-89 au niveau des différentes espèces de poissons et les effets à long terme du filtrat brut, comme par exemple sur l'émergence des insectes.

EFFECT OF TEMPERATURE AND DOSE ON THE PISCICIDAL ACTIVITY OF A FUNGAL (DEUTEROMYCOTINA) EXTRACT

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Abstract

A fungal extract obtained from a deuteromycete B-89 was tested in field conditions to assess the effects of temperature, concentration and exposure time on the mortality of a cyprinid population. Mortality increases with a rise in exposure time, concentration and temperature. The toxicity data suggest that B-89 crude extract would be adequate to kill cyprinid fish within 3 h from 13°C to 25°C. The exposure time and quantity of B-89 crude filtrate required to induce mortality decreased 3.5 fold from 13°C to 25°C. The amount of toxin that penetrates into the fish is dependent on maximum exposure time to induce damage, otherwise there is detoxification of the toxin. Penetration of the toxin is extremely quick as 70% of the residual activity is gone after 20 min of contact. Our results suggest that the crude filtrate of B-89 could be an alternative to rotenone as a tool for management of non-desirable fish populations.

Introduction

Cyprinids, the largest family of fish in the world, are represented by 44 species in Canadian waters (Scott and Crossman 1974). These fish have been introduced by bait fishermen in many brook trout (*Salvelinus fontinalis* Mitchill) lakes of Quebec and Ontario and may naturally overlap in distribution with brook trout (Scott and Crossman 1973). This involuntary introduction brought potential competitors of brook trout in Laurentian Shield lakes (Blais and Beaulieu 1992; Lacasse and Magnan 1994). Some species of cyprinids can force brook trout to shift their food habits from benthic organisms to zooplankton in some oligotrophic lakes (Magnan and FitzGerald 1982, 1984), causing reduced growth and a large decrease in productivity of brook trout populations (Magnan and FitzGerald 1982; Magnan 1988). Consequently, fishing yields of brook trout, the most desired species in sport fishing in Eastern Canada, were reduced by 30-70 % since the introduction of the non-desirable species (Magnan et al. 1990). Based on the magnitude of the socio-economic consequences and the fact that native populations are on the brink of disappearance, restoration of brook trout populations in lakes and rivers became a necessity.

Various programs were aimed at reducing or eliminating the non-desirable species by using piscicides. Chemicals such as endrin, toxaphene, Guthion®, Thiodan® and copper sulfate have been used in fishery management to control non-desirable species. Being non-selective, these piscicides eliminated and caused substantial damages not only to desirable fish species but also to zooplankton (Johnston 1972 *In* Magnan et al. 1990; Anderson 1970) and aquatic invertebrate populations (Becker 1975 *In* Magnan et al. 1990). Except for the use of Squoxin® and TFM (3-

trifluoromethyl-4-nitrophenol), not much research has been done to develop selective piscicides. In addition to being expensive and use-regulated, chemical piscicides present risks (e.g., persistence) to the environment (Magnan et al. 1990). Although chemical controls remain important in control programs, the problems they have caused in the past have stimulated interest in alternative control agents.

Without offering a final solution, physical control methods offer some advantages. Controls consist of massive withdrawal of non-desirable fish species, or restriction of their movements. Different techniques are used such as fishing devices (e.g., net, trap, seine and hoop net), dynamiting of lakes, commercial and electrical fishing as well as various types of barriers. These methods have minor environmental and social impacts and no effect on non-target organisms. The major drawbacks in carrying out these operations are crop burden, manpower and time. Moreover, total elimination is difficult to obtain with these methods (see review by Magnan et al. 1990).

In the search for safe, effective and economical control agents, biological methods have shown growing popularity. These methods favour the introduction of agents such as predators, pathogens, genetic manipulations and toxins produced by living organisms to control non-desirable fish species. The introduction of predators, pathogens and genetic manipulations has received a lot of attention over the last 20 yr, particularly with respect to high control rates at a low cost (once a program is started) and to the lack of harmful effects on man and non-target fauna. However, researchers have found incomplete destruction of the pest, unforeseeable effects of

predators or pathogens in the field, expensive cost to elaborate good programs and time limit to have tangible results. To date, few attempts have been fruitful (see review by Magnan et al. 1990). Biological toxins are substances produced by living organisms and their use has gained acceptance as a promising alternative. Among those substances, antimycin A produced by *Streptomyces* sp presents certain advantages with few effects on non-target organisms (Walker et al. 1964; Houf and Campbell 1977), irreversible effects (Gilderhus 1972), no repulsive effects (Gilderhus et al. 1969 *In* Magnan et al. 1990; Lennon and Berger 1970), and selectivity on certain fish species (Helms 1967; Burress 1968, 1970; Burress and Luhning 1969). However, treatment cost is twice that of rotenone treatment (Leduc et al. 1973).

At present, rotenone is the most popular product for removing or reducing non-desirable fish populations in fishery management. Rotenone is derived from the roots of certain leguminous plants such as *Derris* sp, *Lonchocarpus* spp and *Tephrosia* spp (Sousa et al. 1987; Blais and Beaulieu 1992). On account of the plants growing in a wild state, rotenone supply is limited (Blais and Beaulieu 1992; Marking 1992). Rotenone treatments involve the elimination of all fish species, both non-desirable and desirable (Burdick et al. 1955; Marking and Bills 1976). Most researchers agree that rotenone has disastrous effects on zooplankton (Almquist 1959; Kiser et al. 1963; Anderson 1970; Bandow 1980) but less impact on the benthic fauna (Almquist 1959; Bandow 1980). Temporary disappearance of these organisms will have important repercussions on the restoration of lakes because they are the feeding base of desirable species (Scott and Crossman 1974). In addition amphibians can be indirectly affected because their food supply is composed of fish and/or aquatic insects (Hodge 1983). According to Millis (1985), other

drawbacks include the extreme light sensitivity of rotenone, temperature and water characteristics affecting the efficacy of rotenone as well as the fish species to eliminate.

It can thus be concluded that, among the non-mechanical control methods presented, the major problems are a lack of selectivity on fish species and the elimination of zooplankton and benthic fauna populations. The use of a more specific product would be the response to the environmental drawbacks that affect present fishery management.

A fungi B-89[1] was discovered during a search for isolation and estimation of fungal extracts to control mosquito and black fly larvae. Although culture filtrates of B-89 have presented interesting larvicidal activity on mosquitoes (Nadeau 1990), subsequent studies (Escudero 1996; Lachance 1997) have demonstrated an important sensitivity of cyprinid fish treated with the crude filtrate.

The purpose of this study was to assess the efficiency of B-89 crude filtrate on cyprinids, to determine the relationship between mortality and concentration (expressed as dilution of the extract), exposure time and temperature. The activity loss of B-89 filtrate toxicity according to time in the presence of fish was also assessed .

[1] This deuteromycete is in the process of receiving a patent.

Materials and Methods

Fungus strain

In 1988, a fungus was isolated by the Groupe de Recherche sur les Insectes Piqueurs (GRIP) at the Université du Québec à Trois-Rivières from infected black fly eggs (Diptera: Simuliidae) collected at the Réserve Faunique du Saint-Maurice (Québec, Canada) (Nadeau 1990). This deuteromycete identified as B-89 is maintained by serial transfer on oat medium agar (4% oatmeal; 1.5% agar). Cultures are incubated at 25°C until sufficient sporulation occurs (about 3-4 wk) and then transferred at 4°C to ensure preservation. The isolate is maintained by subcultures every 4-6 mo.

Filtrate production

Crude filtrates were obtained from liquid medium M-1 (2% glucose; 4% yeast extract). One liter flasks containing 400 mL of M-1 medium were inoculated with 7.5×10^5 spores.mL^{-1}. Spores were obtained by scratching 4 wk old fungal cultures with 8-10 mL of sterile distilled water. The liquid was transferred into a sterile test tube and the spore concentration was determined with a haemocytometer after 1/10 dilution. The flasks were incubated at 23°C on a rotary shaker (180 rpm) with an angular support (30°). After 14 d, the crude filtrate was obtained by eliminating mycelium and spores by successive filtrations first on a filter paper and then on 3 and 0.45 μm porosity membranes.

To compare the toxicity level of different filtrate pools, we carried out bioassays on *Aedes triseriatus* (Say) neonate larvae to establish comparison standards between filtrates. This allowed us to readjust all filtrate pools at the same LD_{50}. The filtrates were kept frozen (-20°C) until used in field experiments. Escudero (1996) showed that after 13 mo of storage at -20°C, the activity of the solution was not modified and that repeated freezing and thawing did not impair the toxic activity of the crude filtrates.

Bioassays on *Aedes triseriatus* (Say)

Bioassays were performed using *Ae. triseriatus* (Say) neonate larvae to assess the crude filtrate toxicity. The method used was based essentially on that described by Ibarra and Federici (1987) in which single neonate larvae are placed in individual wells of a microtiter plate (96 holes) and then exposed to serial dilutions of the samples to be tested. To minimize experimental variations, all samples were assayed with 48 larvae in triplicate. Mortalities were counted after 48 h (Tousignant et al. 1993). Each bioassay was analysed using a probit analysis (Finney 1971). All the data obtained were corrected for controls using Abbott's formula (Abbott 1925).

Fish samples

Studies were conducted in the Réserve Saint-Maurice, approximately 75 km northwest of Trois-Rivières. Cyprinids were collected in the Boitel lake (46°57' N; 73°02' W) with a 1 m^2 dipping net. Selection was carried out to get homogenous size of *c.a.* 7 cm. The fish were kept in plastic buckets containing freshly collected lake water and used within 1 h for toxic activity of the crude filtrate. Cyprinid population was made up of 95% of common shiner (*Notropis cornutus*

Mitchill), 3% of northern redbelly dace (*Chrosomus eos* Cope) and 2% of creek chub (*Semotilus atromaculatus* Mitchill). The terminology of Scott and Crossman (1974) was used to identify cyprinids.

Bioassays on cyprinids

To assess sensitivity of cyprinids to B-89 crude filtrate, freshly collected fish were placed in polypropylene containers with a minimum of 20 fish per container, in 2L of diluted filtrate. Cyprinids were exposed continuously to dilutions of 1/10, 1/20 and 1/40 at temperatures of 13°C ±1 and 18°C ±1 in the first and second bioassay trials, while exposed to dilutions 1/20, 1/40 and 1/80 at a temperature of 25°C ±1 in the third trial. One polypropylene container was kept untreated and used as control. All containers were covered with a screen top. To keep a constant temperature, all containers were placed in a bath supplied with water from a nearby stream. The treated fish were observed continuously for up to 6 h and when a single mortality occurred, the number of dead fish within the next minute and the time of the first death were recorded. This time is expressed as exposure time. Thus, the time of death corresponds to an exposure time necessary to accumulate a lethal dose. Dead fish were immediately removed and placed in a 4% formaldehyde solution for later identification. Fish were presumed dead when no gill movement and/or muscular spasms were observed. All experiments were stopped when a single mortality occurred in the control container.

To determine the effect of contact time on the toxic activity of the crude filtrate and to evaluate the residual activity left in the filtrate, we compared toxicity at different contact times on

both cyprinids and *Ae. triseriatus* (Say). A minimum of 20 fish were exposed, in separate containers, for 2.5, 5, 10, 20, 40 and 80 min to a 1/10 dilution of the crude extract (2L) at 13°C. At the end of each contact period, the fish were removed (i.e., each container was inverted over a sieve) and transferred into a new plastic container with fresh stream water. One container was kept untreated to serve as control. Mortalities were counted immediately after transfer. All experiments were stopped when a single mortality occurred in the control container. To estimate residual toxic activity of the filtrate after contact with fish, samples were collected from the 2L filtrate after each exposure period and analysed for mortality on *Ae. triseriatus* (Say) neonate larvae in the laboratory.

Statistical analysis

Each set of bioassays was analysed using probit analysis (Finney 1971). All mortalities were corrected for controls using Abbott's formula (Abbott 1925). Each bioassay was individually analysed and grouped as recommended by Finney (1971). The different groups were then compared together by means of the Pearson statistical test (Goodness-of-fit Chi-square), of the parallelism test (Chi-square) and by the estimate of the relative median potencies (SPSS-X 1986).

Results

Figs. 1, 2 and 3 near here

The progression in mortality of cyprinids induced by B-89 crude filtrate is shown in Figs. 1, 2 and 3. Mortality occurred very rapidly after the beginning of the experiment. At 18°C (Fig. 2),

mortality of 100% was obtained after 16 and 26 min with dilutions of 1/10 and 1/20 respectively while at 25°C (Fig. 3), we reached a mortality of 100% after 17 min with a 1/20 dilution. For all temperatures and dilutions, the mortality reached its maximum before 180 min of exposure time. The onset of death is reduced with increases in temperature (Figs. 1, 2 and 3). When 100% was not attained (Figs. 1 and 3), a plateau was reached and stayed level for up to 6 h. The highest percentage of cumulative mortality observed at 13°C with a 1/40 dilution (Fig. 1) was of 70%, as with 25°C (Fig. 3) where the plateau reached a maximum mortality of 71%, but with half the amount of crude filtrate (1/80 dilution). However, the cumulative mortality was elicited faster at 25°C. Moreover, with the same 1/40 dilution, 70% mortality was observed at 13°C (Fig. 1) whereas a mortality of 100% was reached at 18°C (Fig. 2) and at 25°C (Fig. 3). But at 1/40 dilution there was little temperature effect on the mortality rate at either 18°C and 25°C. These results indicate that mortality occurred at the same speed. These experiments confirmed the efficacy of B-89 crude filtrate.

Based on these results, we analysed the relationship between concentration (expressed as dilution) and exposure time (time of death). From data of the regression lines for each dilution and temperature, we plotted dilution versus exposure time to obtain 50% mortality. Using a logarithmic representation for dilution and exposure time, we obtained a straight line called an isodose. The actual dose in contact with the fish is obtained by using the product of the dilution of the crude filtrate times the duration of exposure (min). This dose is represented by the area under the curve. As seen in Fig. 4 (A), at 13°C, a dilution of 1/10 with an exposure time of 28 min (area x) gives the same mortality as a dilution of 1/20 for 55 min (area y) and as a dilution of 1/40 after

Fig. 4 near here

an exposure time of 111 min (area z). No matter the exposure time and dilution used, the results indicate that at 13°C a dose 2.8 ±0.035 is needed to kill 50% of the cyprinid population. However, the dose of the crude filtrate required for LD_{50} decreases with an increase in temperature. For example, doses of 1.37 ±0.125 and 0.72 ±0.115 were necessary at 18°C (B) and 25°C (C), respectively.

Fig. 5 near here

The rate at which temperature influences mortality is shown in Fig. 5. Doses were calculated for each set of data and placed on a probit graph to establish temperature-efficacy relationship. The dose required to induce a specific mortality decreased as temperature increased. Cyprinid mortality at 13, 18 and 25°C gave regression slopes of 5.63, 8.37, and 5.57 respectively. This indicates that an increase from 13 to 25°C does not seem to modify the mode of action of the toxin.

Fig. 6 near here

The loss of activity of the crude filtrate in the presence of fish is shown in Fig. 6. An exposure time of 20 min was necessary to induce mortality. This mortality increased over the amount of time that cyprinids were exposed to crude filtrate, reaching 100% after 73 min. Bioassay of the crude filtrate recovered at the end of the different exposure periods made on *Ae. triseriatus* (Say) neonate larvae, showed a rapid decline in the first 20 min and leveled off between 20 to 73 min. The measured loss of B-89 was 70% in the first 20 min of exposure time of cyprinids, whereas 9% was removed between 20 and 73 min. When 100% mortality was attained, only 21% of the toxicity was left. The mortality recorded in *Ae. triseriatus* (Say) after 2.5 min of exposure is unexpected and unexplained.

Discussion

The effectiveness of B-89 crude filtrate against cyprinids is evidenced by the rapid mortality observed following treatment. The toxicity data suggest that B-89 crude filtrate would be adequate to kill cyprinid fish within 3 h from 13 to 25°C (Figs. 1, 2 and 3). Compared to the exposure time necessary for antimycin A and rotenone to eliminate certain freshwater fish, Gilderhus (1972) has determined that inducing 100% mortality in carp and white suckers required shorter exposures to antimycin A (6 h) than to rotenone (18-24 h) at field-use concentrations of 5 and 50 $\mu g.L^{-1}$ (active ingredient) respectively. However, the long exposure time necessary in colder water (25 h at 100 $\mu g.L^{-1}$ in 12°C water) made elimination of bullheads with rotenone difficult in cold seasons. Meadows (1973) noted that mirror carp were killed within 20 h at 2.0 $mg.L^{-1}$ of a 5% rotenone formula at a temperature of 11°C while the perch, the least resistant species tested, was killed in about 6.5 h at an initial concentration of 0.1 $mg.L^{-1}$. B-89 crude filtrate showed a piscicidal effect faster than antimycin A or rotenone, currently used to reduce or eliminate populations of non-desirable freshwater fish, considering that the majority of treatments occurred in the fall (Blais and Beaulieu 1992). Moreover, since the exposure time at which the filtrate induced total mortality is short, the use of B-89 crude filtrate has an advantage over rotenone or antimycin A in the context of limited time and manpower saved, thus reducing the cost to carry out a treatment.

Since fish cannot regulate their body temperature, their metabolic rate is dependent on the water temperature. Generally, the effects are for vital processes to be accelerated by warm

temperatures and decelerated by cold ones. Indeed, toxic activity is faster at high temperatures with lethal time reduced compared to lower temperatures. We noticed that for a same dilution (1/40), the response varied according to an increase in temperature (Figs. 1, 2 and 3). The variation in percentage of mortality between different temperatures can be explained in part by reduced metabolic activity in cold water. The speed at which toxins penetrate into the fish being slowest at 13°C, there is presumably a quick detoxification of the toxins by the fish. A minimal quantity is necessary to generate a lethal effect, and if not reached, there is a possibility that some fish may survive. As detoxification is greater than the toxic effect after a certain time, the response of toxicity approaches a plateau (Fig. 1). So, for a given dilution, there is a maximum contact time necessary to accumulate a lethal dose, in other words, a limit over which there is detoxification of toxins by the fish. Escudero (1996) reported that the filtrate toxic activity was inactivated at pH 8.0 or lower. Consequently, the toxin of crude filtrate having penetrated into the fish bloodstream might be detoxified by a pH of 8.0 (M.L. Escudero, Département de biologie, Université du Québec à Trois-Rivières, Trois-Rivières, unpublished data).

The same effect was observed at 25°C with a 1/80 dilution (Fig. 3) when the time needed to accumulate a sufficient quantity of toxin to produce mortality is long, introducing the possibility of detoxification. After a certain contact time, the quantity of toxin that remains in the filtrate is not sufficient to reach a lethal dose in the fish because of the rapid decrease of the initial concentration. Indeed, an increase in exposure time of cyprinids to B-89 crude extract induces a rapid decrease in residual toxic activity (Fig. 6). Marked decline of mortality of *Ae. triseriatus* (Say) neonate larvae shows that the toxin was removed from the crude filtrate by penetrating into

the fish, thus accounting for the loss of toxic activity observed in *Ae. triseriatus* (Say). A quick decrease of residual toxic activity in the first 20 min of exposure time indicates that the toxin rapidly penetrates until obtaining a first poisoning indication, after which the loss of activity is weak.

Considering the results above, the isodose relationship (Fig. 4) between dilution (concentration) and contact time should be used with caution. Since, the amount of toxin that penetrates into the fish is related to contact time, the efficacy of B-89 crude filtrate is not only dependent on the probability of encounter between toxin and fish, but is also dependent on a maximum exposure time to reach a lethal dose and to induce irreversible damage, otherwise there is detoxification of the toxin and survival of the fish. Contact time thus becomes an important consideration in lentic conditions, since it is possible that the toxicant will lose its activity before the lethal dose is reached. Therefore, the isodose relationship established with B-89 crude filtrate could be used in running-water treatment where the length of exposure is brief because the toxicant moves away from the site of application. The crude filtrate would be an alternative tool to actual piscicides since it induces mortality in less than 3 h. According to Gilderhus (1972), some stream reclamations using rotenone or antimycin A toxicants have failed, probably due to the fish not being exposed long enough to the chemical.

The exposure duration and concentration (dilution) of B-89 crude extract required to induce mortality decreases as temperature increases. However, as with rotenone and antimycin A, required exposure time to induce mortality was affected more by water temperature than by the

toxicant concentration (Fig. 4). Indeed, a fourfold decrease in exposure time is observed when the temperature is raised from 13 to 25°C, while doubling the concentration of the filtrate produces only a twofold decrease in the exposure time. Our results are similar to those of Gilderhus (1972) where mortalities induced by antimycin A and rotenone were more affected by temperature than concentration. This author found that doubling the concentration shortened the exposure time by an average of 38.3% for antimycin A and by 36.6% for rotenone. Increases of 5°C decreased the exposure time by an average of 61.7 and 60.9% for antimycin A and rotenone, respectively. But contrary to our results, the temperature effect was less.

The toxin affecting cyprinids appears different than other known products used to kill fish. Indeed, 100% mortality is reached very rapidly even at low temperatures, and when 100% is not attained, fish stop dying (Figs. 1 and 3), contrary to rotenone were mortality can occur over a period of 96 h. Moreover, the toxin action of antimycin A and rotenone on most fish species elicits no striking response from the fish and the death is slow, contrary to B-89 crude filtrate that gives rise to a spectacular response exhibiting frenzied activity.

We believe that the high rate of mortality observed in this study and the speed at which toxic activity occurred are interesting enough so that B-89 crude filtrate deserves more consideration for use as a piscicidal agent. Further investigations should be performed to determine the effects on non-target organisms and to evaluate the specificity on different non-desirable fish species.

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References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ Entomol. **18**: 265-267.

Almquist, E. 1959. Observations on the effect of rotenone emulsives on fish food organisms. Fish. Board Sweden. Inst. Fresh. Res. Drott. Rep. **40**: 146-160.

Anderson, R. S. 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. Jour. Fish. Res. Board Can. **27** (8): 1335-1356.

Bandow, F. 1980. Effects of winterkill and chemical eradication of fish on lake ecosystem. Minn. Dep. Nat. Resour. Div. Fish Wildl. Completion Rep., Study 303, D.J. Projet F-26-R, Invest. Rep. **369**: 1-40.

Becker, G. 1975. Fish toxification: biological sanity or insanity ? *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Blais, J.-P. et G. Beaulieu. 1992. La roténone comme outil pour la restauration des populations d'Omble de fontaine (*Salvelinus fontinalis*): revue de littérature et exemple d'application pour le Québec. Ministère du Loisir, de la Chasse et de la Pêche, Direction régionale de Montréal, Direction de la gestion des espèces et des habitats. 290 p.

Burdick, G. E., H. J. Dean and E. J. Harris. 1955. Toxicity of emulsifiable rotenone to various species of fish. N. Y. Fish Game Journal. **2** (1): 36-67.

Burress, R. M. 1968. Antimycin for controlling sunfish populations in ponds. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Burress, R. M. 1970. Improved method of treating ponds with antimycin A to reduce sunfish populations. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Burress, R. M. and C. W. Luhning. 1969. Field trials of antimycin as a selective toxicant in channel catfish ponds. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Escudero, M. L. 1996. Recherches sur les métabolites toxiques du champignon B-89 et de leurs effets biologiques. Thèse de doctorat, Université de Montpellier, France.

Finney, D. J. 1971. Probit Analysis. 3rd ed. Cambridge University Press, New York, NY. 333 p.

Gilderhus, P. A. 1972. Exposure times necessary for antimycin and rotenone to eliminate certain freshwater fish. Jour. Fish. Res. Board Can. **29** (2): 199-202.

Gilderhus, P. A., B. L. Berger and R. E. Lennon. 1969. Field trials of antimycin A as a fish toxicant. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Helms, D. R. 1967. Experimental use of antimycin A to control spawning carp in Backbone Lake. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Hodge, R. P. 1983. Washington amphibians and reptiles: check list and habitat guide. Wash. Dep. Game, Nongame Program brochure 8 p.

Houf, L. J. and R. S. Campbell. 1977. Effects of antimycin A and rotenone on macrobenthos in ponds. U.S. Fish and Wildlife Service, Investigations in Fish Control. No. **80**. 29 p.

Ibarra, J. E. et B. A. Federici. 1987. An alternative bioassay employing neonate larvae for determining the toxicity of suspended particles to mosquitoes. J. Am. Mosq. Control Assoc. **3**: 187-192.

Johnston, J. M. 1972. Tolerance of juvenile sockeye salmon and zooplankton to the selective squawfish toxicant 1, 1'-methylenedi-2-naphthol. *In* Magnan P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Kiser, R. W., J. R. Donalson and P.R. Olson. 1963. The effect of rotenone on zooplankton population in freshwater lakes. Trans. Am. Fish. Soc. **92**(1): 17-24.

Lacasse, S. et P. Magnan. 1994. Distribution post-glacière de l'Omble de fontaine dans le bassin hydrographique du fleuve Saint-Laurent: Impact des interventions humaines. Université du Québec à Trois-Rivières, pour le Ministère de l'Environnement et de la Faune du Québec. 83 p.

Lachance, L. 1997. Effet du filtrat d'un champignon B-89 (Deuteromycotina) sur les Cyprinidae et la faune non-cible. Mémoire de Maîtrise, Université du Québec à Trois-Rivières, Trois-Rivières.

Leduc, G., Y. Gravel, L.-R. Seguin, B. Vincent and F. Guilbert. 1973. The use of sodium cyanide as a fish eradicant in some Quebec lakes. Naturaliste Can. **100**: 1-10.

Lennon, R. E. and B. L. Berger. 1970. A resume on field applications of antimycin A to control fish. U. S. Fish and Wildlife Service, Investigations in fish control. No. **40**. 19 p.

Magnan, P. 1988. Interactions between brook charr, *Salvelinus fontinalis*, and nonsalmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. Can. J. Fish. Aquat. Sci. **45**: 999-1009.

Magnan, P., and G. J. FitzGerald. 1982. Resource partitioning between brook trout (*Salvelinus fontinalis* Mitchill) and creek chub (*Semotilus atromaculatus* Mitchill) in selected oligatrophic lakes of southern Quebec. Can. J. Zool. **60**: 1612-1617.

Magnan, P., and G. J. FitzGerald. 1984. Mechanisms responsible for the niche shift of brook charr, *Salvelinus fontinalis* Mitchill, when living sympatrically with creek chub, *Semotilus atromaculatus* Mitchill. Can J. Zool. **62**: 1548-1555.

Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Marking, L. L. 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. Fisheries. **17** (6): 6-13.

Marking, L. L. and T. D. Bills 1976. Toxicity of rotenone to fish in standardized laboratory tests. U. S. Fish Wildl. Serv. Invest. Fish Control **72**.

Meadows, B. S. 1973. Toxicity of rotenone to some species of coarse fish and invertebrates. Jour. Fish Biol. **5**: 155-163.

Millis, R. L. 1985. Determination of rotenone concentrations in fish toxicants from various commercial suppliers. Wyoming Game Fish Dep. Project 7085-17-8301, Adm. Rep. 7 p.

Nadeau, M. 1990. Isolation et évaluation de mycètes pour le contrôle biologique des moustiques (Diptera: Culicidae) et de mouches noires (Diptera: Simuliidae) en zone tempérée. Mémoire de Maîtrise, Université du Québec à Trois-Rivières, Trois-Rivières.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Scott, W. B., and E. J. Crossman. 1974. Poissons d'eau douce du Canada. Ministère de l'Environnement, Service des pêches et des sciences de la mer, Office des recherches sur les pêcheries du Canada, Ottawa. Bulletin 184. 1 026 p.

Sousa, R. J., F. P. Meyer and R. A. Schnick. 1987. Re-registration of rotenone: A State Federal cooperative effort. Bull. Am. Fish. Soc. **12** (4): 9-13.

SPSS-X. 1986. SPSS-X user's guide. 2nd ed. SPSS inc. McGraw-Hill, New York.

Tousignant, M. E., J. L. Boisvert and A. Chalifour. 1993. Loss of *Bacillus thuringiensis* var. *israelensis* larvicidal activity and its distribution in benthic substrates and hyporheic zone of streams. Can. J. Fish. Aquat. Sci. **50**: 443-451.

Walker, C. R., R. E. Lennon and B. L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U. S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2 (Circular No. 186). 18 p.

Fig. 1. Effect of dilutions of B-89 crude filtrate on cyprinid mortality at 13°C. Exposure time is the time when death occurred.

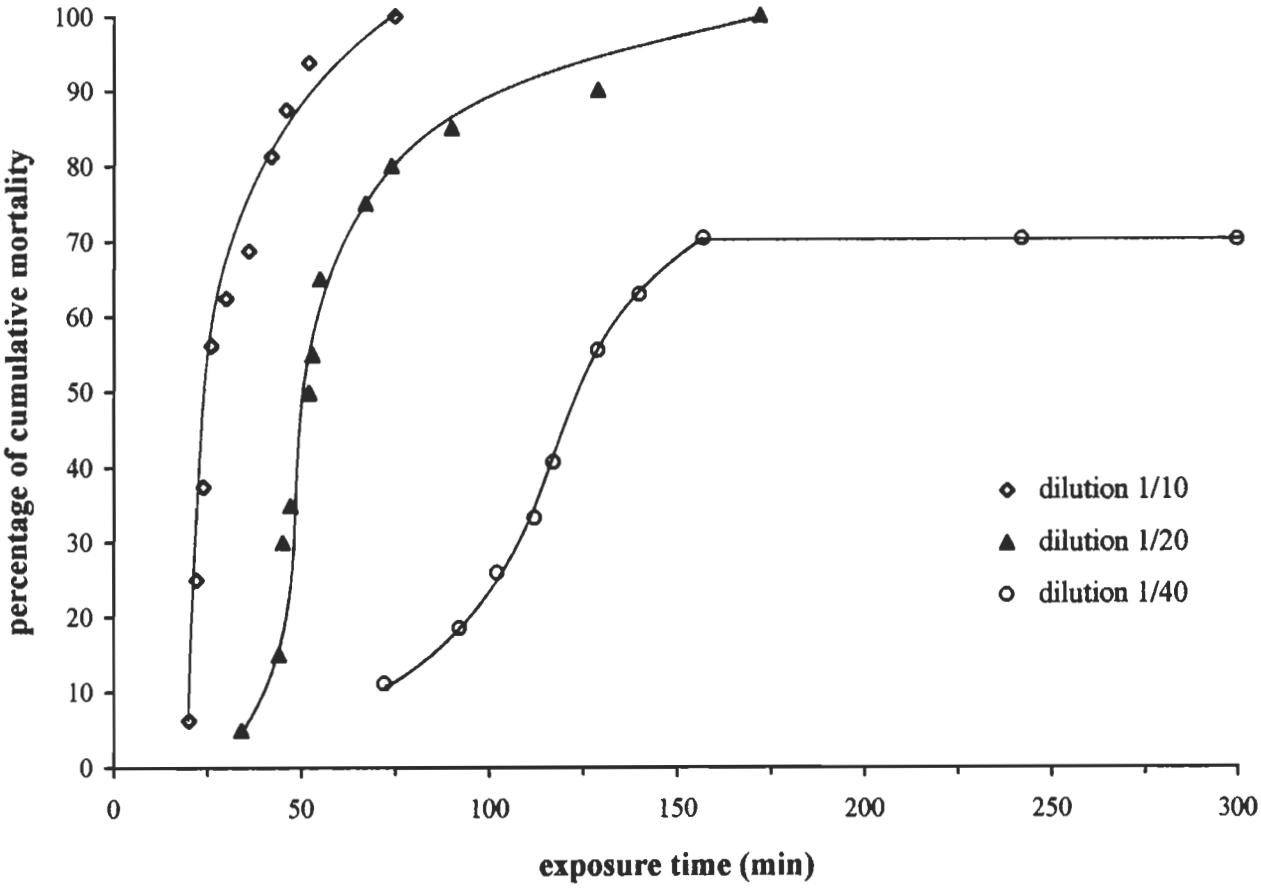
Fig. 2. Effect of dilutions of B-89 crude filtrate on cyprinid mortality at 18°C. Exposure time is the time when death occurred.

Fig. 3. Effect of dilutions of B-89 crude filtrate on cyprinid mortality at 25°C. Exposure time is the time when death occurred.

Fig. 4. Logarithmic representation existing between dilution of B-89 crude filtrate and exposure time after which a mortality of 50% was reached. A: 13°C; B: 18°C; C: 25°C.

Fig. 5. Effect of temperature on B-89 efficacy on cyprinids.

Fig. 6. Percentage of mortality of cyprinids after different contact times to B-89 crude filtrate showing the loss of activity when the crude filtrate recovered was tested on *Aedes triseriatus* (Say) neonate larvae.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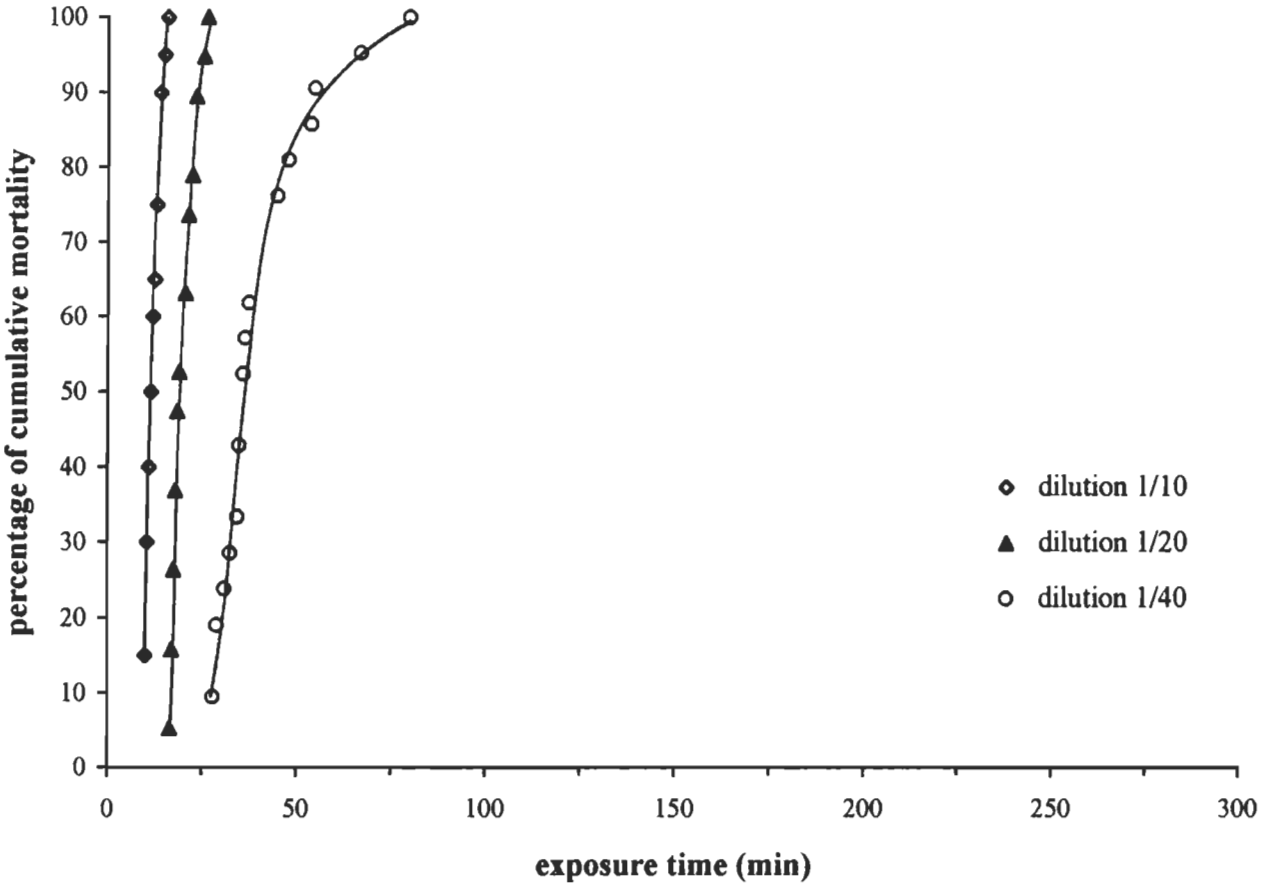
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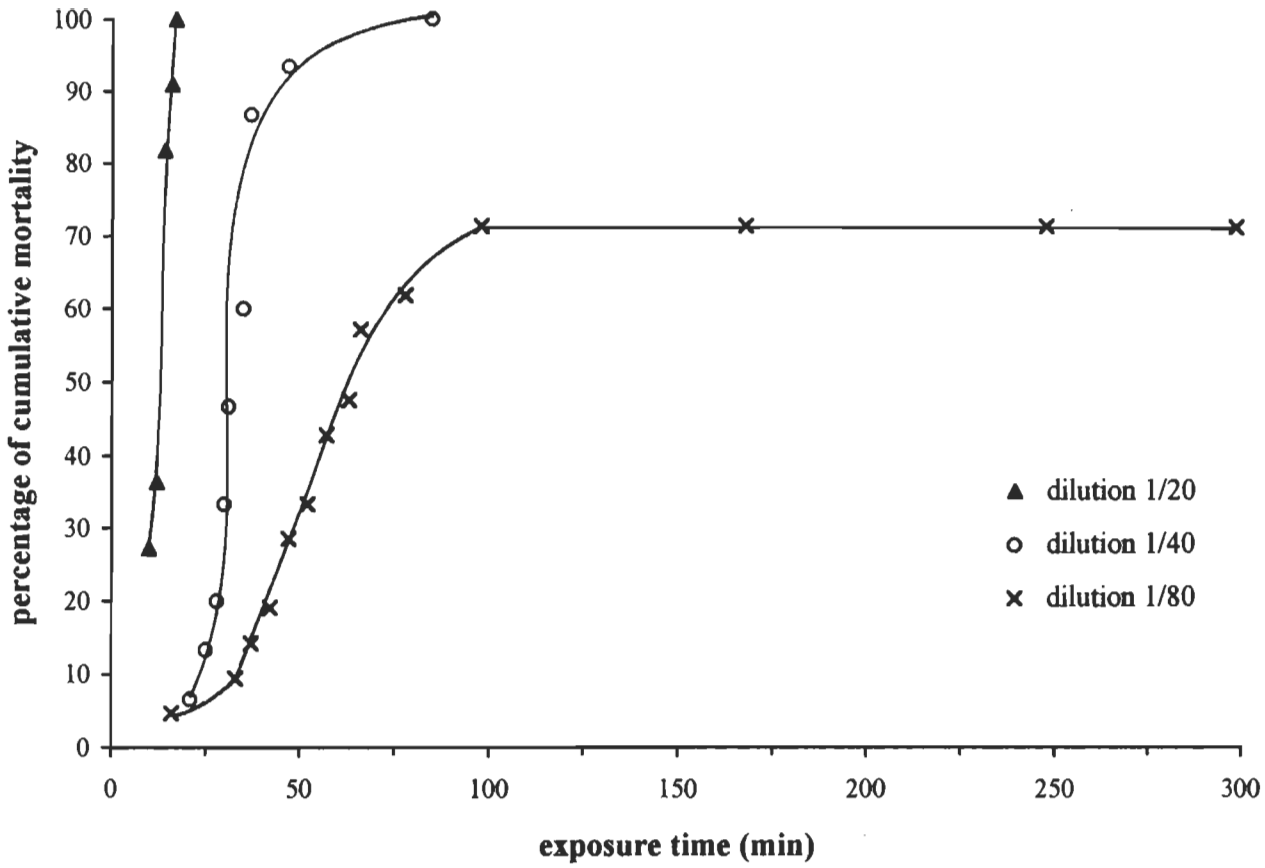
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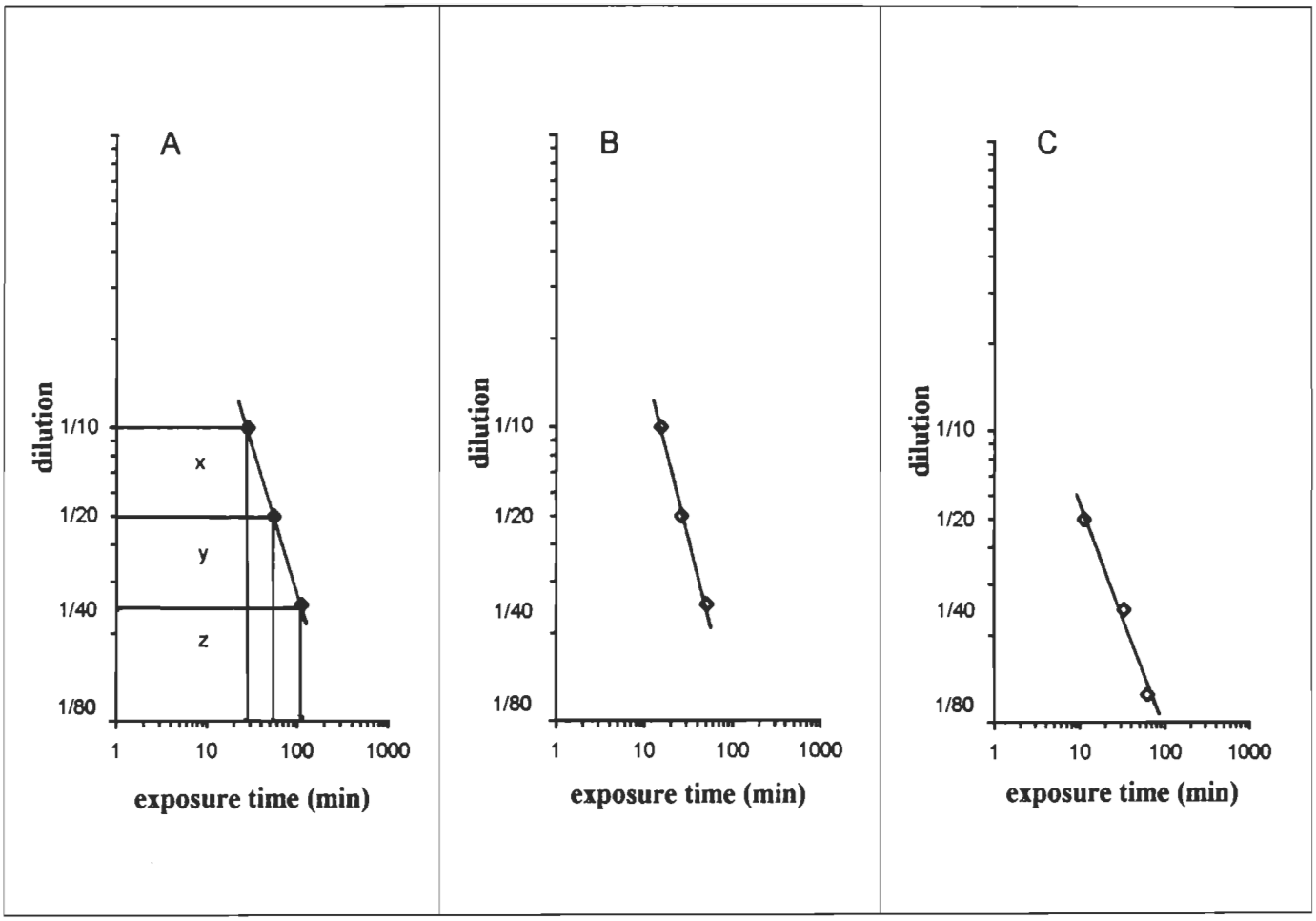
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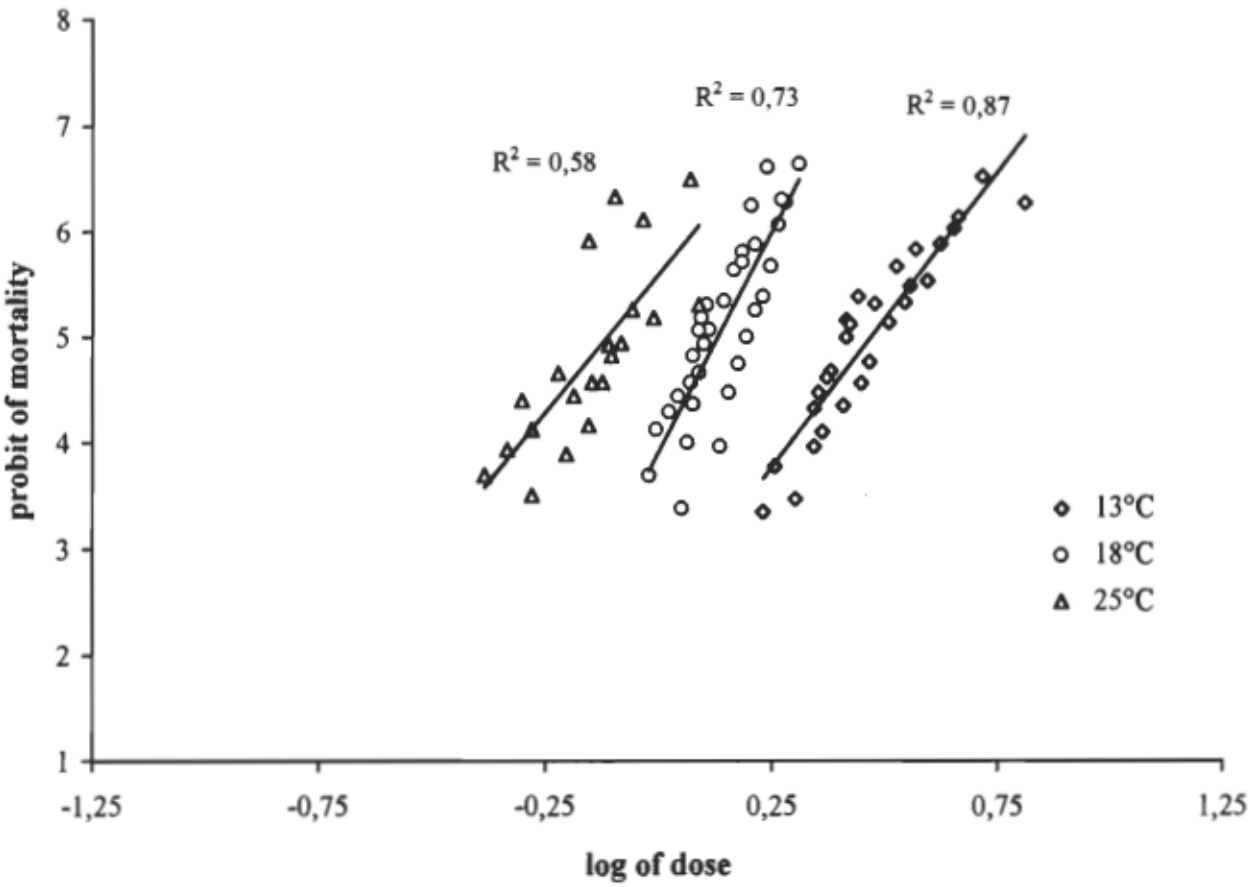
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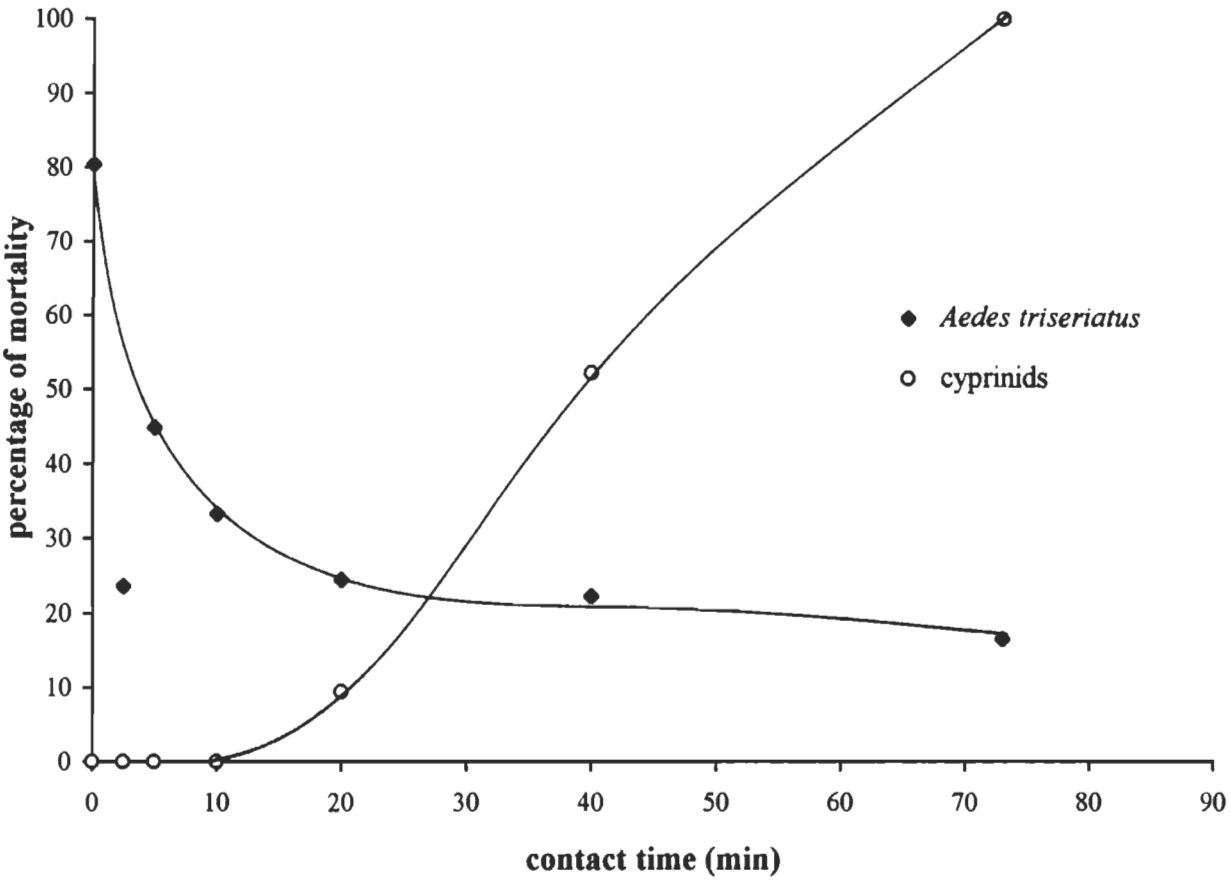
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Aedes triseriatus
cyprinids

EFFECT OF A FUNGAL (DEUTEROMYCOTINA) EXTRACT WITH PISCICIDAL ACTIVITY ON NON-TARGET BENTHIC INVERTEBRATES

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Abstract

A fungal extract obtained from a deuteromycete (B-89) and showing piscicidal activity was tested in field conditions to assess an ideal temperature and contact time for running-water treatments. To evaluate short-term efficiency on cyprinids and the impact on non-target benthic invertebrates, three different doses were tested in a multi-gutter system at 18°C using an exposure time of 30 min. The highest dosage used, caused 100% mortality of cyprinid fish before the end of the 30 min exposure time. No mortalities were recorded in the gutters for at least 12 invertebrate groups while 13 showed mortalities between 0.8 and 21% only at the highest dosage, twenty four hours post-treatment. Compared to controls, there was no evident drift increase in larvae of Ephemeroptera, Odonata, Plecoptera, Megaloptera or Trichoptera, but Coleoptera (adults and larvae) and Diptera were affected as their drift increased 4 and 8 times respectively at the highest dosage. Mortality in the drift samples was found only with Ephemeroptera (8.6%) at the highest dosage. Our results suggest that crude filtrate of B-89 at high dosage does not cause a major disturbance in benthic fauna associated with cyprinid populations.

Introduction

The involuntary introduction of some species of cyprinids is currently considered as a very serious threat to the preservation of native brook trout (*Salvelinus fontinalis* Mitchill) populations in Eastern Canada (Blais and Beaulieu 1992; Lacasse and Magnan 1994). The invasion of hydrographic basins by non-desirable species involve predation and competition of habitats and food partitioning between both native and the introduced populations (Magnan and FitzGerald 1982; 1984). Consequently, this ecological imbalance induces a decrease in growth and productivity of brook trout populations (Magnan 1988), causing a reduction in fishing yield by up to 70% (Magnan et al. 1990). For both economical and ecological reasons, as well as the fact that in many areas native populations are on the brink of disappearance, restoration of brook trout populations in lakes and rivers became inevitable.

Several programs have been set up to control non-desirable fish populations. Chemical controls with copper sulfate, endrin and toxaphene have widely been used to control non-desirable fish species, but these methods are becoming less and less common because of environmental concerns (Magnan et al. 1990). Moreover, broad spectrum chemical agents cause substantial damage not only to desirable fish but also to zooplankton (Anderson 1970; Johnston 1972 *In* Magnan et al. 1990) and benthic invertebrate populations (Becker 1975 *In* Magnan et al. 1990). These adverse effects have prompted the development of agents expected to have little or no toxicity towards non-target organisms.

In fishery management, large numbers of techniques such as nets, traps, dynamite, electroshocking, predators, pathogens and even complete drainage of lakes have been tried in order to eliminate non-desirable fish species. Although safe for the environment and non-target fauna, complete eradication is difficult to obtain (see review by Magnan et al. 1990), and creates economically unacceptable maintenance costs.

Unacceptable environmentally control methods, non selective chemical fish toxicants use or other methods technically non effective have been conducted to the development of biological toxins as an alternative control. To date, the most used control agents in fishery management are antimycin A and rotenone. Antimycin A, an antifungal antibiotic isolated from *Streptomyces* sp has been used to destroy all fish species, in lakes and ponds, and also as selective toxicant for channel catfish (Lennon and Berger 1970). According to Walker et al. (1964) and Houf and Campbell (1977), most benthic organisms, in ponds and pools, appeared to be unaffected by antimycin A treatment at concentrations applied for fish eradication. However, Jacobi and Degan (1977) showed that in a small stream, an application of antimicyn A resulted in an immediate increase in drift rates and a drastic reduction of populations of macroinvertebrates. Mayflies, scuds, caddisflies and craneflies were the taxa most affected. Total benthic biomass almost reached or, in some cases, exceeded the level before treatment between the first and second year.

Rotenone is produced from the roots of leguminous plants such as *Derris* sp, *Lonchocarpus* spp and *Tephrosia* spp in subtropical and tropical zones (Blais and Beaulieu 1992). At present, various rotenone based formulations are used in fishery management. As result of

rotenone treatment, all non-desirable and desirable fish species are touched by this piscicide (Burdick and al. 1955; Marking and Bills 1976). According to the results of Almquist (1959), Wollitz (1962), Bandow (1980) and Chandler and Marking (1982), it appeared that rotenone immediate effects on benthic animals in lakes and ponds varies, but it doesn't affect them as drastically as it does to zooplankton. Researchers unanimously agree that immediate effect of rotenone on zooplankton is catastrophic. Immediate reduction in total numbers of benthic fauna ranged from a low of 0% (Houf and Campbell 1977) to a high of 71% (Wollitz 1962). The most affected taxa, in ponds and lakes, were true midges (Almquist 1959; Wollitz 1962; Bandow 1980) and mayflies (Burress 1982). In general, these studies demonstrated that rotenone has a far more drastic initial impact on stream benthos than on lake benthos.

While stream invertebrate communities do recover from rotenone, it takes more time in streams than in standing water. Binns (1967), Cook and Moore (1969) and Helfrich (1978) found that rotenone treatment of streams eliminated most aquatic insects while some species re-established within a month or two (Cook and Moore 1969) and full restoration of the original fauna might require as long as 2 yr (Binns 1967). Populations of stoneflies, dipterans, caddisflies and mayflies were the most affected by rotenone treatment in streams (Cook and Moore 1969; Helfrich 1978). Engstrom-Heg et al. (1978) concluded that there is no level of rotenone application at which rough (non-desirable) fish can be eliminated without at least minor damage to the bottom fauna. However Dudgeon (1990) found that rotenone induced immediate catastrophic drift, especially in beatid mayflies, but did not produce large-scale mortality in stream benthic invertebrates. Up to now, no technical alternatives or agents were developed to eliminate only the

species aimed by the piscicide while being safe for zooplankton and benthic invertebrate populations. Because of the many different formulation available and because the performance of rotenone is greatly affected by many biological and physico-chemical factors (pH, dissolved oxygen, temperature and light), it is nearly impossible to predict or quantify the amount of damage caused during a treatment.

A fungi B-89 [1] (Deuteromycotina) was discovered at the time of study for isolation and estimation of fungal extract to control mosquitoes and black flies. While culture filtrates of B-89 have presented interesting larvicidal activity on mosquitoes (Nadeau 1990), subsequent studies on non-target fauna (Escudero 1996; Lachance et al. 1997) have demonstrated an important sensitivity of cyprinid fish treated with this crude filtrate.

The purpose of the present study was to determine temperature and contact time effects of B-89 crude filtrate on cyprinids in order to establish optimal conditions for testing the selectivity of the filtrate on stream benthic invertebrates in a multi-gutter system.

[1] This deuteromycete is in the process of receiving a patent.

Materials and Methods

Fungus strain

In 1988, a fungus was isolated by the Groupe de Recherche sur les Insectes Piqueurs (GRIP) at the Université du Québec à Trois-Rivières, from infected black fly eggs (Diptera: Simuliidae) collected at the Réserve Faunique du Saint-Maurice (Québec, Canada) (Nadeau 1990). This deuteromycete identified as B-89 was maintained by serial transfers on oat medium agar (4% oatmeal; 1.5% agar).

Extract production

Crude filtrates were obtained from liquid medium M-1 (2% glucose; 4% yeast extract). One-liter flasks containing 400 mL of M-1 medium were inoculated with 7.5×10^5 spores.mL^{-1}. The flasks were incubated at 23°C on a rotary shaker (180 rpm) with an angular support (30°). After 14 d, the crude filtrate was obtained by eliminating mycelium and spores by successive filtrations on a filter paper and then, on 3 and 0.45 µm porosity membranes (Lachance et al. 1997). The filtrates were kept frozen until used.

Field site

Studies were conducted in the Réserve Saint-Maurice, a wildlife reserve bordered by the Rivière Mattawin and the Rivière Saint-Maurice, located approximately 75 km northwest of Trois-Rivières (Québec, Canada). All samples were collected during summer from the Boitel lake area

(46°57' N; 73°02' W). This small oligotrophic lake has 2 small inlets (Aubin and Pluvier streams) and one outlet (Boitel stream).

Field samples

Cyprinids were collected from the Boitel lake using a 1 m^2 dipping net. Selection was carried out to get homogenous fish size of *c.a.* 7 cm. The fish were kept in plastic buckets containing stream water and used within 1 h. Cyprinid population was made up of 95% of common shiner (*Notropis cornutus* Mitchill), 3% of northern redbelly dace (*Chrosomus eos* Cope) and 2% of creek chub (*Semotilus atromaculatus* Mitchill). The terminology of Scott and Crossman (1974) was used to identify cyprinids. The Limnephilidae Trichoptera were hand collected in the Aubin stream and the other benthic organisms were captured by kick samples in the Aubin, Pluvier and Boitel streams. The mixed population of organisms was kept in polypropylene containers filled with stream water and used within 1 h. The non-target fauna was made up of thirty-five invertebrate taxa, where most were the larvae. The terminology of Merritt and Cummins (1978) as well as of Pennack (1978) was used to identify benthic fauna after the experiments were concluded.

Bioassays on cyprinids

To determine optimum conditions for gutter treatments, we studied the effects of temperature and contact time on cyprinids. In the first bioassay trial, a minimum of 20 fish were exposed, in separate polypropylene containers (30 cm long x 16 cm wide x 10 cm deep), for 2.5; 5; 10; 20; 40 and 80 min to a 1/10 dilution of the crude filtrate (2L) at 13°C and covered with a

screen top. Knowing that mortalities at 13°C occurred fairly quick, in the second trial, cyprinids were exposed for 2.5; 5; 10 and 20 min to a 1/40 dilution at 25°C. At the end of each exposure period, the fish were removed (i.e. each container was inverted over a sieve) and transferred into a new plastic container with fresh stream water. One polypropylene container was kept untreated and used as control. To keep the temperature constant, all containers were placed in a bath supplied with water from a nearby stream. The samples were observed continuously for a period of up to 2.5 h and when a single mortality occurred, the number of dead fish within the next minute and the time of the first death were recorded. This time is expressed as exposure time. Dead fish were removed and placed in a 4% formaldehyde solution for later identification. Fish were presumed dead when no gill movement and/or muscular spasms were observed. All experiments were stopped when a single mortality occurred in the control container.

Bioassays in gutter system

To determine B-89 crude filtrate effectiveness on cyprinids and its effects on stream invertebrates, we carried out bioassays in an artificial river system. The tests were realized in a multi-gutter system similar to that described by Troubat (1981). The system was made up of six wood gutters (120 cm long x 16.5 cm wide x 15 cm deep) waterproofed with a polyethylene sheet. The water was dispatched in each gutter by a pipe supplied with water from Boitel stream. The Boitel stream water was filtered through two 1 mm^2 mesh screens before reaching the gutters. This combination with the fact that the water was collected in the upper part of the water column prevented natural drift in the Boitel stream from entering our gutters. Water flow to each gutter was individually regulated. The bottom of gutters was covered with stones (less than 10

cm) and/or sand to mimic a natural dwelling. Gutters were covered and stopped at one end with a screen to prevent escape of cyprinids and Limnephilidae Trichoptera. For other organisms, a drift net (250 μm mesh) was placed at the end of the gutter to recover drifting organisms. Cyprinids (a minimum of 40), Limnephilidae Trichoptera (about a hundred) and the mixed benthic invertebrates were transferred directly into the gutters. Species that drifted were trapped in nets, at the end of the gutters, and replaced in the gutters until stability of system (insignificant drift). After a minimum of 2 h of adaptation in the gutters, the tests were started. Cyprinids, salmonids and benthic invertebrates can be maintained in gutters during many days without showing any sign of disturbance (death, excessive drift).

Injections of the crude filtrate were performed using a calibrated Mariotte bottle to add the filtrate at a rate of 0.1 $L.min^{-1}$ during an exposure time of 30 min. During injections, flow rate was reduced in each gutter to 0.01 $L.s^{-1}$ to minimize the quantity of filtrate used and, increased afterwards to 0.15 $L.s^{-1}$. Cyprinids, Limnephilidae Trichoptera and the mixed population benthic invertebrates were separately tested with three dilutions corresponding to 0.75, 1.5 and 3.0 doses. The dose is expressed as the product of the dilution of the filtrate in the gutters during treatment by the time of contact (30 min). At 0.01 $L.s^{-1}$ a total of 18 L of stream water went through the gutter, thus 1.8 L of filtrate was injected over 30 min to create a 1/10 dilution. Then by multiplying the 1/10 dilution by 30 min a dose of 3.0 was obtained. A dose of 0.75 would require 450 mL of the filtrate to create a final dilution of 1/40. During the treatment of 30 min, water temperature was at 18°C, with a minimum and maximum of 15 and 20°C during the 24 h observation period. Three gutters not exposed to B-89 crude filtrate served as controls: one for

cyprinids, one for Limnephilidae Trichoptera and the other for the benthic invertebrates. A plastic gutter with no substrate, equiped with a drift net was used to verify the presence of any drifting organisms from the Boitel stream. The temperature, pH and dissolved oxygen were recorded every minute during a treatment.

Mortalities of cyprinids and Limnephilidae Trichoptera were recorded 24 h post-treatment. Drift nets were sampled after 2 and 24 h. The organisms were sorted out, and ascertained for mortality. At the end of the experiment (24 h), the stones in the gutters were removed and individually cleaned to collect attached invertebrates. The other organisms were removed by gently moving the sand. The manual disturbance carried the organisms in the water column which allowed the insect collection in the drift nets. This operation was repeated until no more drift occurred. Organism mortality was determined by the absence of reflex motion after touching each individual with tweezers. All organisms (dead and live) were separately preserved in 95% ethanol (for benthic invertebrates) and 4% formaldehyde solution (for fish) for subsequent identification.

Results

Static tests on cyprinids

Figs. 1 and 2 near here

The toxicity of the crude filtrate on cyprinids was field tested to determine optimum temperature and contact time, for later running-water treatments (Figs. 1 and 2). At 13°C (Fig. 1), contact times of 2.5, 5 and 10 min did not induce any mortality whereas contact times of 20 and 40 min produced final mortalities of 9.5 and 56.5% after 22 and 45 min respectively.

Mortality of 100% was obtained before the end of treatment at 73 min. At the end of the 20 and 40 min contact times, the fish were transferred in freshwater, causing an immediate suspension of mortality. At 25°C (Fig. 2) contact times of 10 and 20 min gave mortalities of 77.8 and 100% after 10 and 20 min, whereas for 2.5 and 5 min contact times, no mortality was registered before removal of the fish. However, contrary to what was observed at low temperature (Fig. 1), 30 min after the transfer in freshwater, some mortalities occurred to reach at 50% for a contact time of 5 min and 39% for a contact time of 2.5 min after 2 h.

Based on these results, we chose a contact time of 30 min with a temperature of 18°C to pursue the experiments. The bioassays in the multi-gutter system were carried out using three doses (dilution x 30 min exposure) calculed to cause from 20% to 100% mortality of cyprinids.

Dynamics tests in gutter system

Mortality of target group

Fig. 3 near here

The mortality of cyprinids induced by B-89 crude filtrate is shown in Fig. 3 column A. Final mortalities of 21.7, 73.3 and 100% were recorded at 0.75, 1.5 and 3.0 doses respectively. In all cases, final mortalities were reached shortly after treatment or before the end of treatment (dose 3.0). Control gutter showed 0% mortality after 24 h.

Mortality of non-target organisms

Mean mortalities (24 h after treatment) for different taxa are shown in Fig. 3. There are ten groups which are not included in the figure because they were present in low numbers (less

than 5 individuals); these were: **Siphlonuridae**, (Ephemeroptera); **Cordulegaster**, (Odonata); Perlodidae, **Chloroperlidae** (Plecoptera); **Sialidae**, (Megaloptera); **Blephariceridae**, (Diptera); **Glossossomatidae**, **Polycentropodidae** (Trichoptera); Pyralidae (Lepidoptera) and Hemiptera. Although in low numbers, families in bold did not show any mortality.

Twelve groups showing no mortality at all doses but not included in Fig. 3 were: Leptophlebiidae (Ephemeroptera); Aeshnidae, Gomphidae, unidentified (Odonata); Elmidae (larvae and adults) (Coleoptera); Corydalidae (Megaloptera); Tipulidae, Ceratopogonidae (Diptera); Limnephilidae, Hydroptilidae (Trichoptera); Hydracarina and Nematode.

When compared to the mortality in the control gutters, no taxa were found to be highly susceptible to the introduction of B-89 crude filtrate at the selected doses (Fig. 3). At the highest dosage (dose = 3.0) which killed 100% of cyprinids in less than 30 min, the highest mortalities observed after 24 h were 13.2% in Heptageniidae (E), 14.9% in Beatidae (G) mayflies, 13.7% in Perlidae (H), 21.2% in Nemouridae (J) stoneflies.

At the intermediate dose of 1.5 which killed 73.3% of cyprinids, the highest mortality was seen in Rhyacophilidae (L) caddisflies at 7.96% after 24 h. For all the other groups, the mortalities were under 4%. The mortality (2 individuals) observed for Hydropsychidae (M) at a dose of 0.75 is believed to be more a reflection of natural mortality than true mortality because the percentage of mortality was 0% at dose 3.0. Thus, 25 groups of benthic invertebrates with more than 5 specimens/group showed mortalities from 0-21% even at the highest dosage.

Drift samples

The percentage of drift for the 0-2 and 0-24 h periods, after the introduction of B-89 crude filtrate is shown in Table 1. In this table Heptageniidae, Ephemereliidae, Beatidae, Siphlonuridae and Leptophlebiidae were grouped as Ephemeroptera; Cordulegaster, Aeshnidae, Gomphidae and unidentified were grouped as Odonata; Perlidae, Leuctridae, Nemouridae, Perlodidae and Chloroperlidae were grouped as Plecoptera; Psephenidae and Elmidae were grouped as Coleoptera; Sialidae and Corydalidae were grouped as Megaloptera; Simuliidae, Chironomidae, Emphididae, Blephariceridae and Tipulidae were grouped as Diptera, and Rhyacophilidae, Hydropsychidae, Philopotamidae, Glossossomatidae, Polycentropodidae, Limnephilidae and Hydroptilidae were grouped as Trichoptera.

Table 1 near here

We observed that the drift increased much in the first 2 h following treatment. However, the patterns of drift in control and treated gutters appeared almost similar, indicating that increases (except Diptera and Coleoptera) in drift could not be attributed directly to the introduction of crude filtrate but rather to the variation of flow rate associated with the treatment.

No evident increases in drift were found with Ephemeroptera, Odonata, Plecoptera, Megaloptera or Trichoptera larvae after the application of the crude extract. Only two taxa showed a significant increase in drift. As expected, drift in Diptera increased by 3.7 times (3.67% versus 13.57%) after application of 1.5 dose and 8 times (3.67% versus 29.81%) at dose 3.0 in the first 2 h. Drift of Coleoptera also increased within the first 2 h of 3.6 times (5% versus 18.18%) and 5 times (5% versus 25%) at dose 1.5 and 3.0 respectively. Elevated drift did not persist past

the 2 h sampling period. Of the non-target groups examined, only a very small amount of individuals had died in the drift. Compared to control, the only significant mortality observed was 8.57% with Ephemeroptera larvae exposed to the highest dosage. When we look at the average drifts and mortalities for all insect orders examined, there is no significant differences between the control and the 3 dosages used.

Monitoring of pH and dissolved oxygen showed that the values were relatively uniform during the treatment between control and treated gutters indicating, that they were not responsible for mortalities or drifts in these experiments.

Discussion

The response of cyprinid fish to different contact times of B-89 crude filtrate was studied and a proportional relationship was found between mortalities and contact times (Figs. 1 and 2). After the contact time, the transfer of cyprinids in freshwater caused mortality to stop. Since a minimal quantity of toxins is necessary to generate a lethal effect, there is a possibility that some fish may survive when this quantity is not reached. Comparable results have been reported by Escudero (1996) who mentioned that the response of toxicity reached a plateau rapidly after treatment. The increase in mortality after the transfer in freshwater that extended for more or less 1 h was unusual (Fig. 2). According to Escudero (1996), total mortality was quickly reached and the toxic activity of B-89 crude filtrate appeared in 30 min or less. Although the 2.5 and 5 min

curves reached a plateau as for others curves, these results were intriguing. In spite of these unexpected results, the data indicated that an ideal contact time for crude filtrate application could be achieved using 30 min of contact time against cyprinid populations.

The effectiveness of B-89 crude filtrate against cyprinid fish in the multi-gutter system was evident by the mortality observed following the treatments (Fig. 3; column A). As the mortality of 100% was reached before the end of treatment with the 3.0 dose, this corresponds to an overdose for non-target organisms. Although the high dosage used in this experiment, the mortality of non-target organisms (Fig. 3; column B to N) clearly shows that crude filtrate does not cause a marked reduction in benthic invertebrates associated with cyprinids. It is interesting to note that we obtained a maximum of 21% mortality, even with an overdose.

The drift patterns were similar in untreated and treated gutters for most aquatic invertebrates other than Coleoptera and Diptera (Table 1). The increases in drift, in the first 2 h, could be ascribed directly to the variation of flow rate associated with treatment. Even if the organisms drifted early, they were all still subjected to crude filtrate action that flows out by the drift nets. According to Waters (1972), it would seem that water current velocity would have a direct positive effects. Floods and higher discharges usually have the expected effect of increasing the drift sometimes to the point of catastrophic results. On the other hand, unusually low discharges with reduced current velocities have also been observed to increase the drift. This result is apparently due to a swimming response of the organism seeking greater currents for respiratory facilitation.

The introduction of B-89 crude filtrate only caused a low increase in drift of Diptera and Coleoptera as well as some mortalities in drift of Ephemeroptera larvae at highest dosage. The drift of Diptera is not surprising, since the bioassays evaluating the insecticidal effects of B-89 crude filtrate (Nadeau 1990; Escudero 1996) indicated that the dipterans (Culicidae and Simuliidae) showed a high level of susceptibility when submitted to a continuous contact with the crude extract in laboratory conditions, whereas with a contact time of 30 min (at a 0.5 dose) in field trials, mortality rates of 20 and 25% were obtained after 7 and 12 d respectively (Escudero 1996). Our results showed that the increase in drift by 8 times of the dipteran population was mainly due to the presence of Simuliidae.

Within the 35 groups of non-target benthic invertebrates investigated, we found almost no effect of B-89 crude filtrate on mortality, percentage of drift, or drift mortality in treated gutters when compared to controls. According to Merritt's classification (1978), no evident effect was observed in the functional and habit group composition of the samples that could be associated with the application of B-89 crude filtrate. Considering the absence of evident impact on non-target groups, this confirms that B-89 crude filtrate is a highly selective piscicide.

Contrary to our results, Jacobi and Degan (1977) have determined that the application of antimycin A caused an immediate increase in drift rates and a temporary reduction in macroinvertebrate population. Cook and Moore (1969) and Helfrich (1978) reported similar short-term effects with rotenone where insect populations suffered mortalities greater than 95%

after the treatment. The populations of mayflies, midges, stoneflies and blackflies were most affected (Cook and Moore 1969; Jacobi and Degan 1977; Helfrich 1978). Even if mayfly and stonefly populations showed the highest mortalities with B-89 crude filtrate, these groups exhibited the greatest tolerance compared to antimycin A and rotenone toxicant. Moreover, Helfrich (1978) and Dudgeon (1990) noted a dramatic increase in drift dominated by mayflies up to 500 times (Helfrich 1978) during a rotenone treatment. On the contrary, our results revealed only a slight increase in drift (8 times) of dipterans.

From an ecological standpoint, Binns (1967) mentioned that the introduction of rotenone into the complex ecosystem of the river was catastrophic. The complete destruction of some species and the reduction of others, at all trophic levels, undoubtedly influenced the entire ecosystem. On the basis of the data obtained in this study, it is obvious that stream reclamation programs designed to eliminate cyprinid fish with B-89 crude filtrate cannot seriously reduce populations of non-target benthic invertebrates, many of which are the important fish food organisms.

In summary, this short-term study found no evident effects of B-89 crude filtrate on non-target invertebrates. The high selectivity obtained showed that crude filtrate has good potential for use as a biological control agent against cyprinids. Further research should be performed to determine the long-term effects and evaluate the specificity on different non-desirable fish species.

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References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ Entomol. **18**: 265-267.

Almquist, E. 1959. Observations on the effect of rotenone emulsives on fish food organisms. Fish. Board Sweden. Inst. Fresh. Res. Drott. Rep. **40**: 146-160.

Anderson, R.S. 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. J. Fish. Res. Board Can. **27** (8): 1335-1356.

Bandow, F. 1980. Effects of winterkill and chemical eradication of fish on lake ecosystem. Minn. Dep. Nat. Resour. Div. Fish Wildl. Completion Rep., Study 303, D.J. Projet F-26-R, Invest. Rep. **369**: 1-40.

Becker, G. 1975. Fish toxification: biological sanity or insanity ?. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Binns, N.A. 1967. Effects of rotenone treatment on the fauna of the Green River, Wyoming. Wyoming Game Fish Comm. Fish. Res. Bull. 1, 114 p.

Blais, J.-P. et G. Beaulieu. 1992. La roténone comme outil pour la restauration des populations d'Omble de fontaine (*Salvelinus fontinalis*): revue de littérature et exemple d'application pour le Québec. Ministère du Loisir, de la Chasse et de la Pêche, Direction régionale de Montréal, Direction de la gestion des espèces et des habitats. 290 p.

Burdick, G. E., H. J. Dean and E. J. Harris. 1955. Toxicity of emulsifiable rotenone to various species of fish. N. Y. Fish Game Journal. **2** (1): 36-67.

Burress, R.M. 1982. Effects of synergized rotenone on nontarget organisms in ponds. U.S. Fish Wildl. Serv. Invest. Fish Control. **91**: 7 p.

Chandler, J.H. and L.L. Marking. 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. Prog. Fish-Cult. **44**(2): 78-80.

Cook, S.F. Jr. and R.L. Moore. 1969. The effects of rotenone treatment on the insect fauna of a California stream. Trans. Am. Fish. Soc. **98**(3): 539-544.

Dudgeon, D. 1990. Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks in two Papua New Guinea streams. Arch. Hydrobiol. **119**(1): 35-53.

Engstrom-Heg, R., R.T. Colesante and E. Silco. 1978. Rotenone tolerances of stream-bottom insects. N.Y. Fish Game Jour. **25**(1): 31-41.

Escudero, M. L. 1996. Recherches sur les métabolites toxiques du champignon B-89 et de leurs effets biologiques. Thèse de doctorat, Université de Montpellier, France.

Helfrich, L.A. 1978. Effects of rotenone on macrobenthic invertebrates of a Pennsylvania stream. Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agencies **32**: 401-408.

Houf, L. J. and R. S. Campbell. 1977. Effetcs of antimycin A and rotenone on macrobenthos in ponds. U.S. Fish and Wildlife Service, Investigations in Fish Control. No. **80**. 29 p.

Jacobi, G. Z. and D. J. Degan. 1977. Aquatic macroinvertebrates in a small Wisconsin trout stream before, during, and two years after treatment with the fish toxicant antimicyn. U. S. Fish and Wildlife Service, Invertigations in fish control. No. **81**. 23 p.

Johnston, J. M. 1972. Tolerance of juvenile sockeye salmon and zooplankton to the selective squawfish toxicant 1, 1'-methylenedi-2-naphthol. *In* Magnan P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Lacasse, S. et P. Magnan. 1994. Distribution post-glacière de l'Omble de fontaine dans le bassin hydrographique du fleuve Saint-Laurent: Impact des interventions humaines. Université du Québec à Trois-Rivières, pour le Ministère de l'Environnement et de la Faune du Québec. 83 p.

Lachance, L., J.O. Lacoursière and J.L. Boisvert. 1997. Effect of temperature and dose on the piscicidal activity of a fungal (Deuteromycotina) extract. Submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

Lennon, R.E. and B. L. Berger. 1970. A resume on field applications of Antimycin A to control fish. U. S. Fish and Wildlife Service, Investigations in fish control. No. **40**. 19 p.

Magnan, P. 1988. Interactions between brook charr, *Salvelinus fontinalis*, and nonsalmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. Can. J. Fish. Aquat. Sci. **45**: 999-1009.

Magnan, P., and G.J. FitzGerald. 1982. Resource partitioning between brook trout (*Salvelinus fontinalis* Mitchill) and creek chub (*Semotilus atromaculatus* Mitchill) in selected oligatrophic lakes of southern Quebec. Can. J. Zool. **60**: 1612-1617.

Magnan, P., and G. J. FitzGerald. 1984. Mechanisms responsable for the niche shift of brook charr, *Salvelinus fontinalis* Mitchill, when living sympatrically with creek chub, *Semotilus atromaculatus* Mitchill. Can J. Zool. **62**: 1548-1555.

Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Marking, L. L. and T. D. Bills 1976. Toxicity of rotenone to fish in standardized laboratory tests. U. S. Fish Wildl. Serv. Invest. Fish Control No.**72**.

Merritt, R.W. and K.W. Cummins. *(editors)* 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa. 441 p.

Nadeau, M. 1990. Isolation et évaluation de mycètes pour le contrôle biologique des moustiques (Diptera: Culicidae) et de mouches noires (Diptera: Simuliidae) en zone tempérée. Mémoire de Maîtrise, Université du Québec à Trois-Rivières, Trois-Rivières.

Pennack, R.W. 1978. Freshwater invertebrates of the United States. *Edited by* John Wiley & Sons, Inc. New York. 803 p.

Scott, W.B., and E.J. Crossman. 1974. Poissons d'eau douce du Canada. Ministère de l'Environnement, Service des pêches et des sciences de la mer, Office des recherches sur les pêcheries du Canada, Ottawa. Bulletin 184. 1 026 p.

Troubat, J.J. 1981. Dispositif à gouttières multiples destiné à tester *in situ* la toxicité des insecticides vis-à-vis des invertébrés benthiques. Rev. Hydrobiol. Trop. **14**: 149-152.

Walker, C. R., R. E. Lennon and B. L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U. S. Bureau of Sport Fisheries and Wildlife, Invertigations in Fish Control No. 2 (Circular No. 186). 18 p.

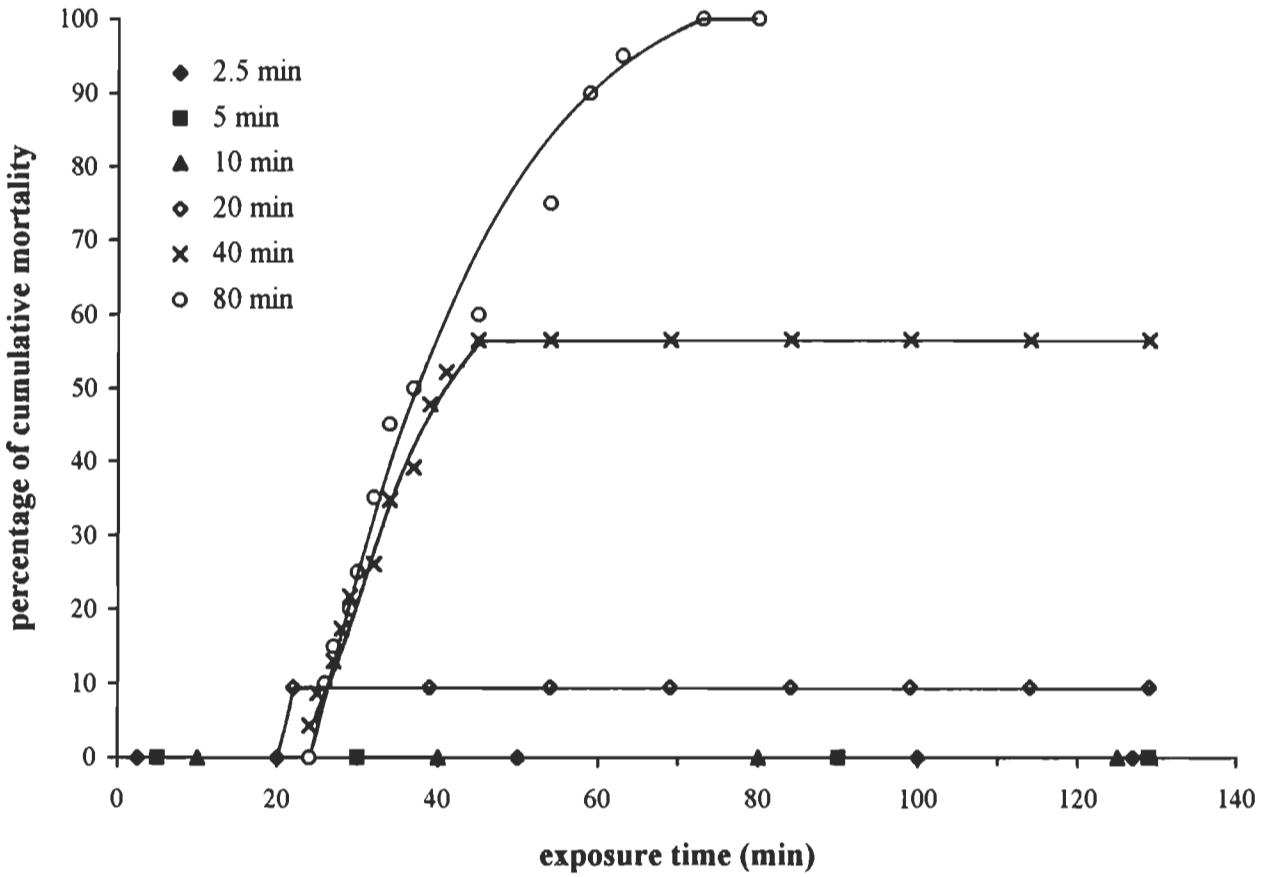
Waters, T. F. 1972. The drift of stream insects. Annu. Rev. Entomol. 17:253-272.

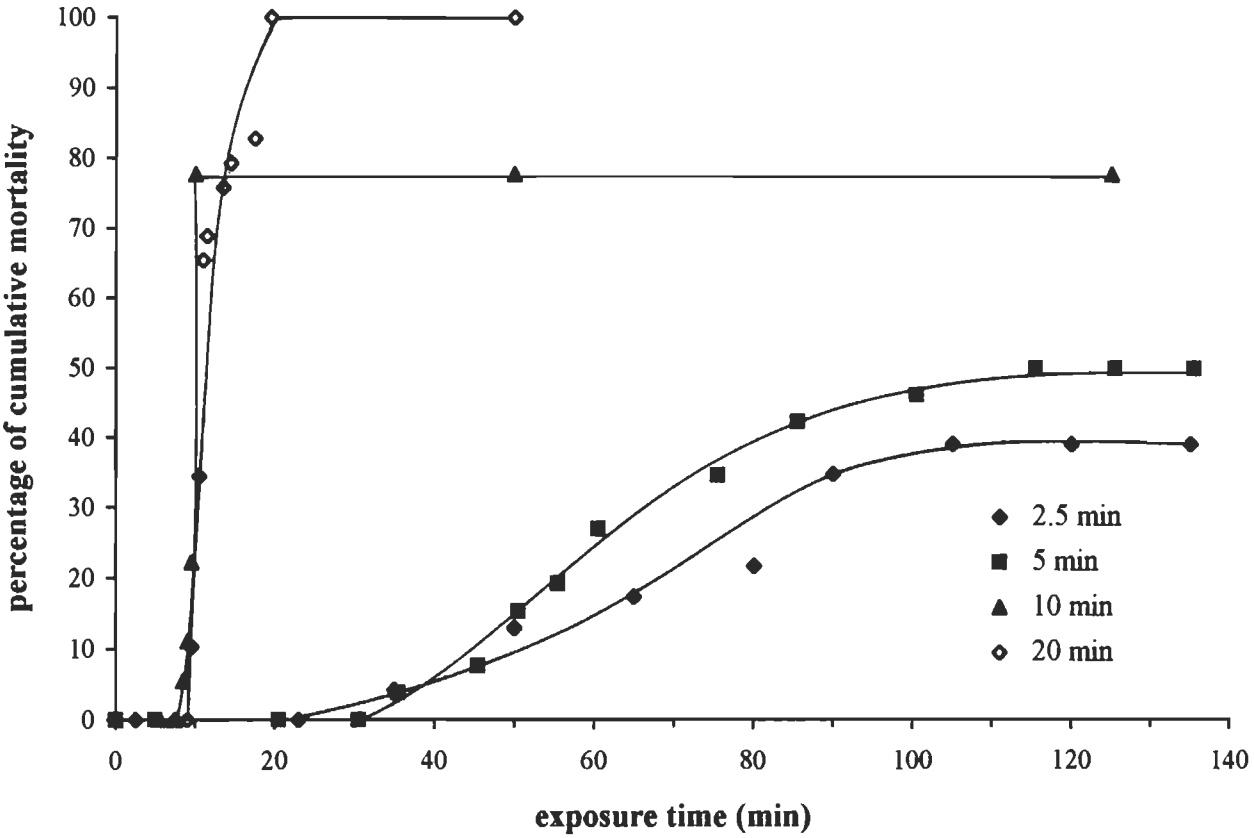
Wollitz, R. E. 1962. Effects of certain commercial fish toxicants on the limnology of three cold-water ponds, Montana. Proceedings of the Montana Academy of Sciences **22**: 54-81.

Fig. 1. Cumulative mortality of cyprinids during different exposure times to B-89 crude filtrate (1/10 dilution) at 13°C.

Fig. 2. Cumulative mortality of cyprinids during different exposure times to B-89 crude filtrate (1/40 dilution) at 25°C.

Fig. 3. Percentage of mortality in the gutters plus the drift of different families treated for 30 min with B-89 crude filtrate in the gutter system at 18°C. [a] corrected for control using Abbott's (1925) formula. ( ) individual numbers. * data excluded because of low individual numbers ($n<5$).


2.5 min
5 min
10 min
20 min
40 min
80 min
percentage of cumulative mortality
exposure time (min)
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
140


percentage of cumulative mortality
100
90
80
70
60
50
40
30
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10
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140
exposure time (min)
2.5 min
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20 min

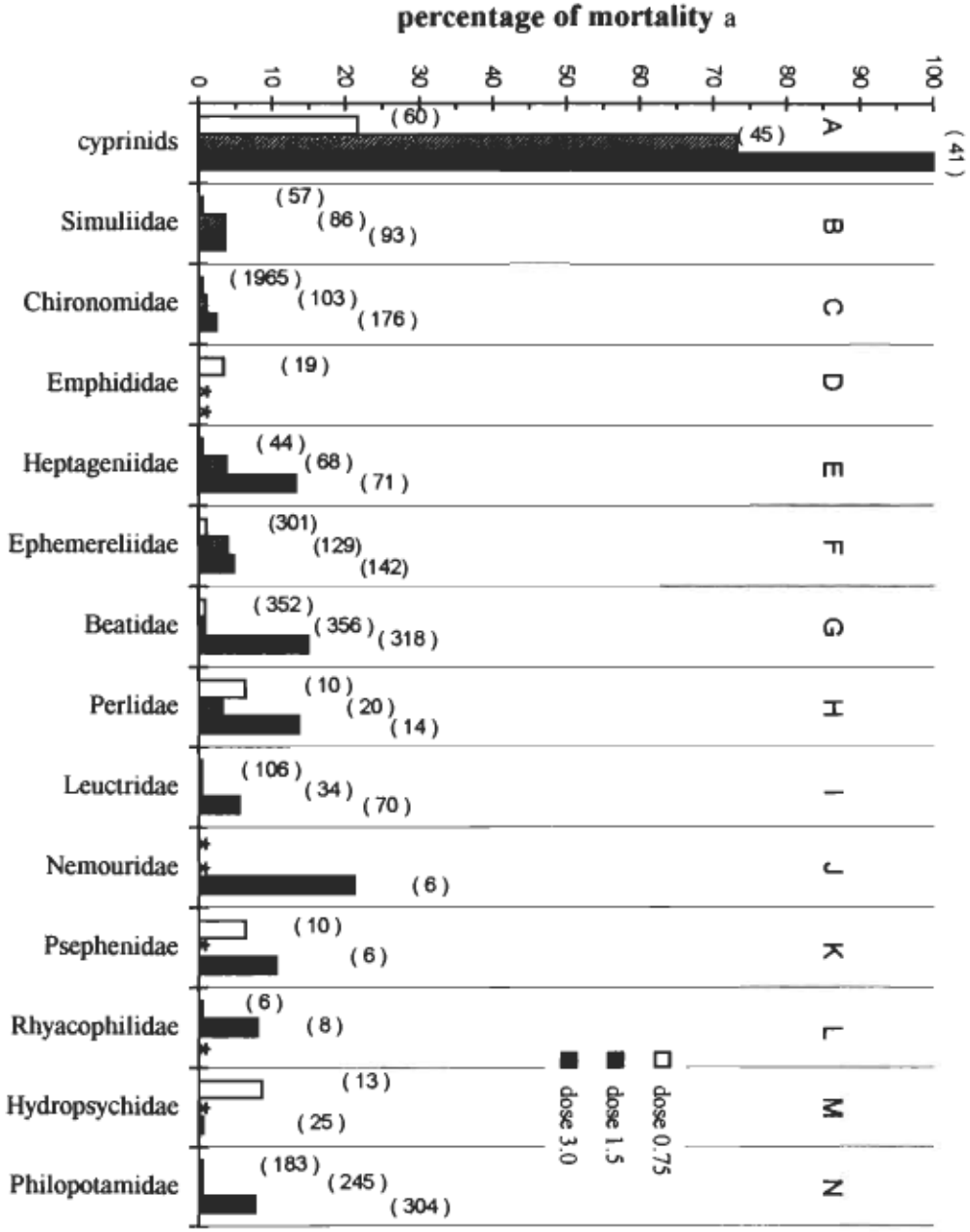
percentage of mortality a
0
10
20
30
40
50
60
70
80
90
100
cyprinids
Simuliidae
Chironomidae
Emphididae
Heptageniidae
Ephemereliidae
Beatidae
Perlidae
Leuctridae
Nemouridae
Psephenidae
Rhyacophilidae
Hydropsychidae
Philopotamidae
(60)
(45)
(41)
(57)
(86)
(93)
(1965)
(103)
(176)
(19)
(44)
(68)
(71)
(301)
(129)
(142)
(352)
(356)
(318)
(10)
(20)
(14)
(106)
(34)
(70)
(6)
(10)
(6)
(6)
(8)
(13)
(25)
(183)
(245)
(304)
A
B
C
D
E
F
G
H
I
J
K
L
M
N
dose 0.75
dose 1.5
dose 3.0

Table 1. Percentage of drift and mortality of the insect orders in themulti-gutter system after treatment with B-89 crude filtrate at 18°C.

Orders[a]	Control			Treated								
				dose 0.75			dose 1.5			dose 3.0		
	% drift [b]		% dead	% drift		% dead	% drift		% dead	% drift		% dead
	0-2 h	0-24 h[c]	0-24 h[d]	0-2 h	0-24 h	0-24 h	0-2 h	0-24 h	0-24 h	0-2 h	0-24 h	0-24 h
Ephemeroptera	51.49	53.26	0.95	25.66	34.53	1.15	58.91	61.86	1.98	43.06	61.09	**8.57**
Odonata	50.00	50.00	0.00	46.15	61.54	0.00	6.67	53.33	0.00	0.00	22.22	0.00
Plecoptera	4.67	6.54	0.00	2.48	6.61	0.00	9.68	24.19	2.05	6.38	17.02	3.39
Coleoptera	**5.00**	20.00	15.00	15.15	21.21	0.00	**18.18**	27.27	0.00	**25.00**	43.75	3.98
Megaloptera	0.00	10.00	0.00	0.00	7.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diptera	**3.67**	4.61	0.19	1.52	1.95	0.15	**13.57**	17.19	0.86	**29.81**	33.85	2.97
Trichoptera	23.53	38.45	12.61	44.09	49.61	1.76	41.18	45.33	3.31	21.35	26.80	9.84
Average for all orders	**19.77**	**26.12**	**4.11**	**19.29**	**26.08**	**0.44**	**21.17**	**32.74**	**1.17**	**17.94**	**30.68**	**4.11**

(a) total, including families with less than 5 individuals
(b) includes dead and living organisms
(c) cummulated total for 0-2 h plus 2-24 h period
(d) total dead after 24 h

EFFECT OF A FUNGAL (DEUTEROMYCOTINA) EXTRACT WITH PISCICIDAL ACTIVITY ON ZOOPLANKTON AND INSECT LARVAE

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Abstract

The toxicity of B-89 crude extract was tested for 4 groups of zooplankton and 3 insect families. The tests in continuous exposure were conducted in laboratory conditions. No groups of zooplankton were killed by a dose causing 100% mortality of cyprinid fish. Zooplankton were 5 to 37 times most resistant than cyprinids, copepods had the greatest resistance and rotifers the least. There were no effects in larvae of Ephemeroptera and Trichoptera, but Plecoptera larvae were slightly affected at a dose of crude filtrate used in cyprinid eradication. Our results suggest that no impact could occur on insect larvae and zooplankton populations after a treatment with B-89 crude filtrate except for one group, Plecoptera.

Introduction

The introduction of some species of cyprinids in many oligotrophic brook trout (*Salvelinus fontinalis* Mitchill) lakes of Eastern Canada poses a serious threat to native brook trout population (Blais and Beaulieu 1992; Lacasse and Magnan 1994). This involuntary introduction brought potential competitors of brook trout, causing in some oligotrophic lakes, a shift of food habits from benthic organisms to zooplankton (Magnan and FitzGerald 1982, 1984), thus reducing population growth (Magnan and FitzGerald 1982; Magnan 1988). Consequently, the productivity of brook trout, in terms of annual yield, was significantly lower when living in sympatry with these introduced species (Magnan 1988). Judging by a reduction in fishing yield by up to 70% (Magnan et al. 1990) and the fact that many brook trout populations are on the brink of disappearance, restoration of native brook trout populations in lakes and rivers became inevitable.

Different strategies have been used to control non-desirable fish species. Broad spectrum chemical agents cause substantial damage not only to desirable fish but also to zooplankton (Anderson 1970; Johnston 1972 *In* Magnan et al. 1990) and benthic invertebrate populations (Becker 1975 *In* Magnan et al. 1990). In addition, these synthetic chemicals present risks of environmental contamination. In seeking replacements for chemicals, biological control methods have received considerable attention, particularly with respect to toxins produced by living organisms. Biological toxins most used to date for eliminating or reducing non-desirable fish species are antimicyn A and rotenone.

Antimicyn A produced by *Streptomyces* sp has proven to be very effective against all non-desirable species and also, in certain cases, has been used as selective piscicide (Helms 1967; Burress 1968, 1970; Burress and Luhning 1969). Moreover, antimycin A has no effect on zooplankton and benthic organisms in ponds and lakes (Walker et al. 1964; Houf and Campbell 1977). Treatment cost, however, is twice that of rotenone treatment, limiting its use (Leduc et al. 1973).

Rotenone is derived from the roots of leguminous plants such as *Derris* sp, *Lonchocarpus* spp and *Tephrosia* spp (Sousa et al. 1987; Blais and Beaulieu 1992). A rotenone treatment causes an eradication of all fish species (both non-desirable and desirable) (Burdick et al. 1955; Marking and Bills 1976) as well as zooplankton and benthic invertebrate populations. Almquist (1959), Wollitz (1962), Kiser et al. (1963), Anderson (1970) and Bandow (1980) all observed a dramatic decrease in zooplankton with 95-100% mortality within a few days. There is a general agreement that planktonic crustaceans, especially the cladocerans, are the group most quickly and thoroughly eliminated (Almquist 1959; Wollitz 1962; Anderson 1970; Bandow 1980). Generally, most lakes are repopulated by zooplankton between 2-12 mo.

Benthic organisms are not impacted by rotenone as severely as zooplankton. Immediate reduction in total numbers of benthic fauna in lakes and ponds ranged from a low of 0% (Houf and Campbell 1977) to a high of 71% (Wollitz 1962). The benthic community recovers to at least prerotenone levels within 2 mo after the treatment, and the groups most affected were true midge (Almquist 1959; Wollitz 1962; Bandow 1980) and mayfly larvae (Burress 1982).

To our knowledge, no control agents have been developed to eliminate only the non-desirable species without affecting zooplankton and benthic fauna populations. A fungi B-89[1] (Deuteromycotina) was discovered at the time of study for isolation and estimation of fungal extract to control mosquitoes and black flies. While culture filtrates of B-89 have presented interesting larvicidal activity on mosquitoes (Nadeau 1990), subsequent studies on non-target fauna (Escudero 1996; Lachance et al. 1997a) have demonstrated a high piscicidal activity on cyprinid fish treated with this crude filtrate.

The purpose of this study was to determine the susceptibility of zooplankton and some insect groups submitted to a continuous contact with the crude filtrate.

[1] This deuteromycete is in the process of receiving a patent.

Materials and Methods

Fungus strain

In 1988, a fungus was isolated by the Groupe de Recherche sur les Insectes Piqueurs (GRIP) at the Université du Québec à Trois-Rivières, from black fly eggs (Diptera: Simuliidae) collected at the Réserve Faunique du Saint-Maurice (Québec, Canada) (Nadeau 1990). This deuteromycete identified as B-89 is maintained by serial transfers on oat medium agar (4% oatmeal; 1.5% agar) at 25°C.

Extract production

To produce mycelium elements, cultures in liquid medium M-1 (2% glucose; 4% yeast extract) were carried out. One-liter flasks containing 400 mL of M-1 medium were inoculated with 7.5 x 10^5 spores.mL^{-1}. The flasks were incubated at 23°C on a rotary shaker (180 rpm) with an angular support (30°). After 14 d, the crude filtrate was obtained by eliminating mycelium and spores by successive filtrations on a filter paper and then, on 3 and 0.45 µm pore-size membranes (Lachance et al. 1997a). The filtrates were kept frozen until used.

Field samples

Zooplankton were collected in the Saint-Quentin island pond in the Trois-Rivières area (Québec, Canada). The organisms were collected by dipping a polypropylene container (30 cm long x 16 cm wide x 10 cm deep) in the pond. Samples were taken to the laboratory and used within 24 h. The organisms were roughly sorted out to keep out copepods, ostracods, cladocerans and rotifers and maintained in environmental chambers at 18°C ±1 until the start of the experiment.

The Trichoptera larvae were hand collected in the Aubin stream in the Réserve Saint-Maurice (Québec, Canada). The Trichoptera were kept in polypropylene containers filled with stream water and used within 1 h. Ephemeroptera and Plecoptera larvae were captured by kick samples in a small stream near Trois-Rivières. The organisms were kept, in plastic buckets filled with stream water, in environmental chambers at 18°C ±1 and used within 24 h. Trichoptera population was made up of 100% of Limnephilidae family, Ephemeroptera population was made

up of 99% of Ephemerella and 1% of Beatidae families, and the Plecoptera population of 92% of Leuctridae and 8% of Perlodidae families. The terminology of Merritt and Cummins (1978) as well as of Pennack (1978) was used to identify benthic fauna after the experiments were concluded.

Bioassays on zooplankton

To assess the sensitivity of different groups of zooplankton to B-89 crude filtrate, we carried out bioassays according to the methods of Ibarra and Federici (1987). Rotifers were placed in the individual wells of a microtiter plate (96 holes) with 0.2 mL of serially diluted filtrate per well. For cladoceran females, copepode females and ostracods, two individuals per well were placed in a microtiter plate (24 holes) with 2 mL per well. One microtiter plate for each group was kept untreated and used as control. The water used for dilution was pond water filtered through a 3 µm porosity membrane. All samples were assayed with 30 individuals in triplicate with each different dilutions. Plates were maintained at 18°C ±1 in an environmental chamber with a photoperiod of 18:6 (L:D) and mortalities were checked during a 24 h period. Organisms were presumed dead when a gentle probing with a sharp instrument elicited no response. All organisms (dead and live) were separately preserved in 95% ethanol. All mortalities were corrected for controls using Abbott's formula (Abbott 1925).

Bioassays on insect larvae

To determine the sensitivity of some benthic invertebrates to B-89 crude filtrate, Ephemeroptera and Plecoptera were placed in polypropylene containers with a minimum of 20

specimens per container, in 1L of serially diluted filtrate. Ephemeroptera were exposed continuously to dilutions of 1/10 and 1/20, while the Plecoptera were exposed continuously to a dilution of 1/40 at a temperature of 18°C ±1 in laboratory trials. Oxygen level was maintained with a small pump, and all containers were placed in a bath supplied with ice, if necessary, to keep a constant temperature.

The Trichoptera were put in glass containers (10 per container) with 100 mL of diluted filtrate. Trichoptera were exposed to 1/10 and 1/20 dilutions in a field trial. To keep a constant temperature, all containers were placed in a bath supplied with water from a nearby stream. For each group, one container was kept untreated and used as control. All treated larvae were observed continuously for up to 8 h and when a single mortality occurred, the number of dead insects within the next minute and the time of the first death were recorded. Organism mortality was determined by the absence of reflex motion after touching each individual with tweezers. All organisms (dead and live) were separately preserved in 95% ethanol. All mortalities were corrected for controls using Abbott's formula (Abbott 1925).

The mortality of cyprinid fish in B-89 crude filtrate is proportional to the dose (Lachance et al. 1997a), given by the product of the concentration (dilution) times the exposure time. Therefore, the mortality of zooplankton and insect larvae is expressed as a function of the dose.

Results and Discussion

For comparison purposes, we used a curve mortality on cyprinids obtained in Lachance et al. (1997a). The experiments were carried out in field conditions at 18°C in polypropylene containers where cyprinids were exposed continuously to three dilutions. The results showed that mortality occurred very rapidly after the beginning the experiment. Cyprinid mortality gave a regression slope of 8.37 with a LD_{50} of 1.37.

Effects on zooplankton

The sensitivity of zooplankton to B-89 crude filtrate in laboratory conditions is shown in Fig. 1. No groups were found susceptible to the crude filtrate even at the highest dosage which killed 100% of cyprinids. When compared to cyprinids, increases of 5, 26, 27 and 37 times in the LD_{50} and increases of 5.4, 21, 26 and 29 times in the LD_{95} were observed for rotifers, cladocerans, ostracods and copepods respectively. Thus, the group most sensitive was rotifer, but it was not affected by the dose being used to kill 100% of cyprinids. Mortalities in all control plates were less than 7% after 24 h. We observed no evident effect with respect to zooplankton reproduction based on comparisons of the presence of offspring in both control and treated plates at the doses used.

Fig. 1 near here

These results clearly show that B-89 crude filtrate did not disturb the zooplankton populations. Our results are similar to those of Almquist (1959) and Wollitz (1962) where the ostracods seemed unaffected by the rotenone treatment. Contrary to our results, Bandow (1980),

Almquist (1959) and Anderson (1970) noted that rotifers are generally considered to be more tolerant than the cladocerans and copepods to rotenone treatment. The immediate effect of rotenone on the crustacean zooplankton was catastrophic (Almquist 1959; Wollitz 1962; Kiser et al. 1963; Anderson 1970; Bandow 1980). In contrast, Burress (1982) reported that no significant deleterious effects from the treatments of the Pro-Noxfish formulation were observed in zooplankton populations. Since these organisms directly or indirectly constitute the fish food, the temporary reduction of zooplankton had important repercussions on lake management. On the basis of these laboratory toxicity tests, it is obvious that zooplankton would be safe during treatment of B-89 crude filtrate.

Effects on insect larvae

Fig. 2 near here

Mortalities on some benthic insects is reported in Fig. 2. Trichoptera larvae were not included in this figure because no mortality was observed at all doses used. When compared to cyprinids, no evident mortality was found in Ephemeroptera larvae, the LD_{50} and LD_{95} being 9.5 and 24.8 times higher respectively. The only other group conspicuously affected by B-89 crude filtrate was Plecoptera larvae. When 100% mortality in cyprinids was reached, the mortality observed in Plecoptera group was around 50%. Control containers showed 0% mortality in the Ephemeroptera and Plecoptera groups after 8 h.

These results show that B-89 crude filtrate did not cause a major disturbance in insect larvae when submitted to continuous contact. The mortality in stonefly larvae is unexpected, since

the bioassays with a brief contact time of 30 min (Lachance et al. 1997b) showed that Leuctridae and Ephemerella larvae were not highly susceptible at a dose killing 100% of cyprinids.

Contrary to our results, Burress (1982) reported that mayflies were very sensitive and significantly reduced 3 d post-treatment. According to Claffey and Ruck (1967) there was a slight reduction (12.5%) in the caddisfly larvae after the rotenone treatment. In contrast, Houf and Campbell (1977) reported that rotenone treatment produced no immediate effect on abundance of benthic organisms in a shallow, mud-bottomed pond that was heavily vegetated.

It appears that the application of B-89 crude filtrate has no effect upon the zooplankton and benthic invertebrates except for Plecoptera larvae. On the basis of the data obtained in this study, it is obvious that B-89 crude filtrate has a large margin of safety for non-target organisms.

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References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ Entomol. **18**: 265-267.

Almquist, E. 1959. Observations on the effect of rotenone emulsives on fish food organisms. Fish. Board Sweden. Inst. Fresh. Res. Drott. Rep. **40**:146-160.

Anderson, R.S. 1970. Effects of rotenone on zooplankton communities and a study of their recovery patterns in two mountain lakes in Alberta. Jour. Fish. Res. Board Can. **27**(8): 1335-1356.

Bandow, F. 1980. Effects of winterkill and chemical eradication of fish on lake ecosystem. Minn. Dep. Nat. Resour. Div. Fish Wildl. Completion Rep., Study 303, D.J. Projet F-26-R, Invest. Rep. **369**: 1-40.

Becker, G. 1975. Fish toxification: biological sanity or insanity ?. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Blais, J.-P. et G. Beaulieu. 1992. La roténone comme outil pour la restauration des populations d'Omble de fontaine (Salvelinus fontinalis): revue de littérature et exemple d'application pour le Québec. Ministère du Loisir, de la Chasse et de la Pêche, Direction régionale de Montréal, Direction de la gestion des espèces et des habitats. 290 p.

Burdick, G. E., H. J. Dean and E. J. Harris. 1955. Toxicity of emulsifiable rotenone to various species of fish. N. Y. Fish Game Journal. **2** (1): 36-67.

Burress, R.M. 1982. Effects of synergized rotenone on nontarget organisms in ponds. U.S. Fish Wildl. Serv. Invest. Fish Control. **91**: 7 p.

Burress, R. M. 1968. Antimycin for controlling sunfish populations in ponds. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Burress, R. M. 1970. Improved method of treating ponds with antimycin A to reduce sunfish populations. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Burress, R. M. and C. W. Luhning. 1969. Field trials of antimycin as a selective toxicant in channel catfish ponds. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Claffey, F.J. and J.E. Ruck. 1967. The effect of rotenone on certain fish food organisms. Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm. **20**: 278-283.

Escudero, M. L. 1996. Recherches sur les métabolites toxiques du champignon B-89 et de leurs effets biologiques. Thèse de doctorat, Université de Montpellier, France.

Helms, D.R. 1967. Experimental use of antimycin A to control spawning carp in Backbone Lake. *In* Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Houf, L. J. and R. S. Campbell. 1977. Effects of antimycin A and rotenone on macrobenthos in ponds. U.S. Fish and Wildlife Service, Investigations in Fish Control. No. 80. 29 p.

Ibarra, J.E. et B.A. Federici. 1987. An alternative bioassay employing neonate larvae for determining the toxicity of suspended particles to mosquitoes. J. Am. Mosq. Control Assoc. **3**:187-192.

Johnston, J. M. 1972. Tolerance of juvenile sockeye salmon and zooplankton to the selective squawfish toxicant 1, 1'-methylenedi-2-naphthol. *In* Magnan P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Kiser, R.W., J.R. Donalson and P.R. Olson. 1963. The effect of rotenone on zooplankton population in freshwater lakes. Trans. Am. Fish. Soc. **92**(1): 17-24.

Lacasse, S. et P. Magnan. 1994. Distribution post-glacière de l'Omble de fontaine dans le bassin hydrographique du fleuve Saint-Laurent: Impact des interventions humaines. Université du Québec à Trois-Rivières, pour le Ministère de l'Environnement et de la Faune du Québec. 83 p.

Lachance, L., J.O. Lacoursière and J.L. Boisvert. 1997a. Effect of temperature and dose on piscicidal activity of a fungal (Deuteromycotina) extract. Submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

Lachance, L., J.O. Lacoursière and J.L. Boisvert. 1997b. Effect of a fungal (Deuteromycotina) extract with piscicidal activity on nontarget benthic invertebrates. Submitted to the Canadian Journal of Fisheries and Aquatic Sciences.

Leduc, G., Y. Gravel, L.-R. Seguin, B. Vincent and F. Guilbert. 1973. The use of sodium cyanide as a fish eradicant in some Quebec lakes. Naturaliste Can. **100**: 1-10.

Magnan, P. 1988. Interactions between brook charr, *Salvelinus fontinalis*, and nonsalmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. Can. J. Fish. Aquat. Sci. **45**: 000-1009.

Magnan, P., and G.J. FitzGerald. 1982. Resource partitioning between brook trout (*Salvelinus fontinalis* Mitchill) and creek chub (*Semotilus atromaculatus* Mitchill) in selected oligatrophic lakes of southern Quebec. Can. J. Zool. **60**: 1612-1617.

Magnan, P., and G. J. FitzGerald. 1984. Mechanisms responsible for the niche shift of brook charr, *Salvelinus fontinalis* Mitchill, when living sympatrically with creek chub, *Semotilus atromaculatus* Mitchill. Can J. Zool. **62**: 1548-1555.

Magnan, P., P. East et M. Lapointe. 1990. Modes de contrôle des poissons indésirables: revue et analyse critique de la littérature. Université du Québec à Trois-Rivières, pour le ministère du Loisir de la Chasse et de la Pêche du Québec et la Fondation de la faune du Québec. Rapp. tech. 198 p.

Marking, L. L. and T. D. Bills 1976. Toxicity of rotenone to fish in standardized laboratory tests. U. S. Fish Wildl. Serv. Invest. Fish Control 72.

Merritt, R.W. and K.W. Cummins. (*editors*).1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa. 441 p.

Nadeau, M. 1990. Isolation et évaluation de mycètes pour le contrôle biologique des moustiques (Diptera: Culicidae) et de mouches noires (Diptera: Simuliidae) en zone tempérée. Mémoire de Maîtrise. Université du Québec à Trois-Rivières. Trois-Rivières. Canada.

Pennack, R.W. 1978. Freshwater invertebrates of the United States. *Edited by* John Wiley & Sons, Inc. New York. 803 p.

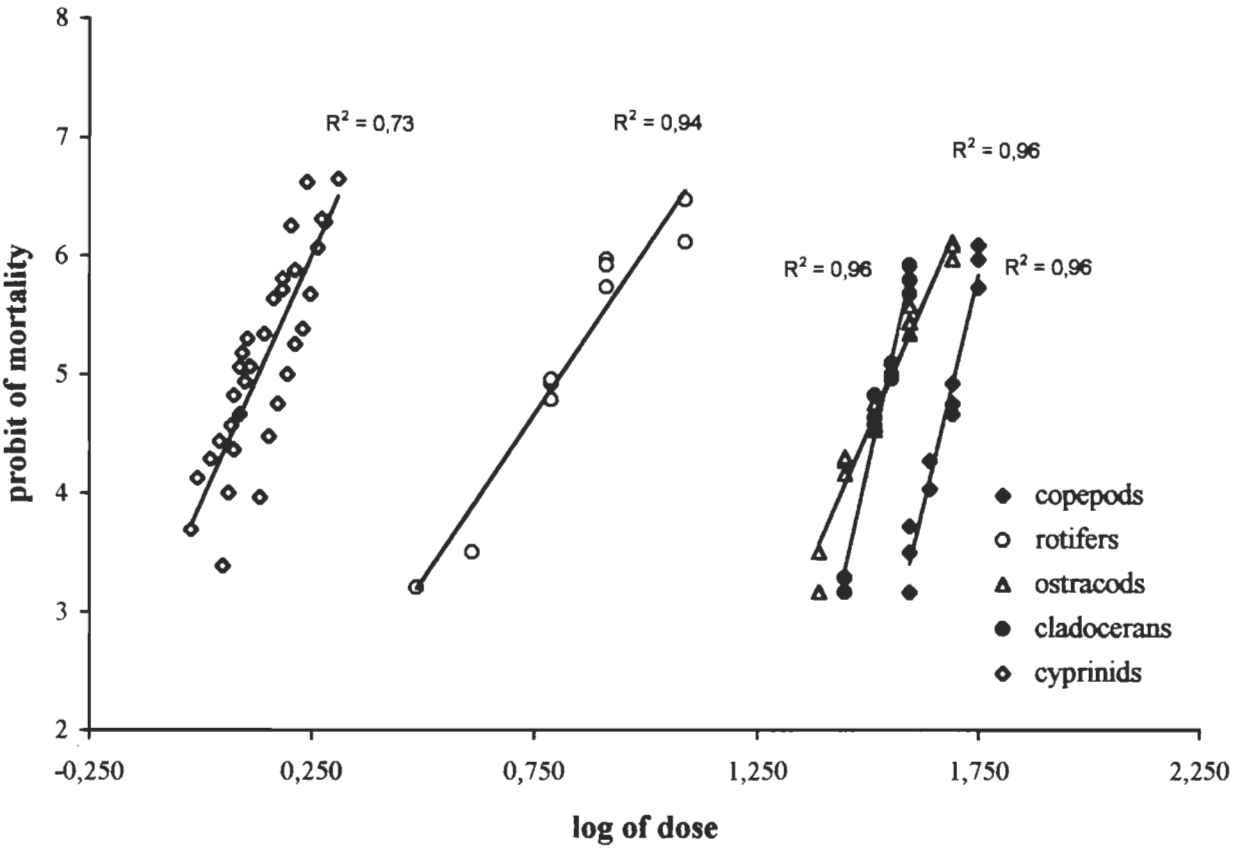
Sousa, R. J., F. P. Meyer and R. A. Schnick. 1987. Re-registration of rotenone: A State Federal cooperative effort. Bull. Am. Fish. Soc. **12** (4): 9-13.

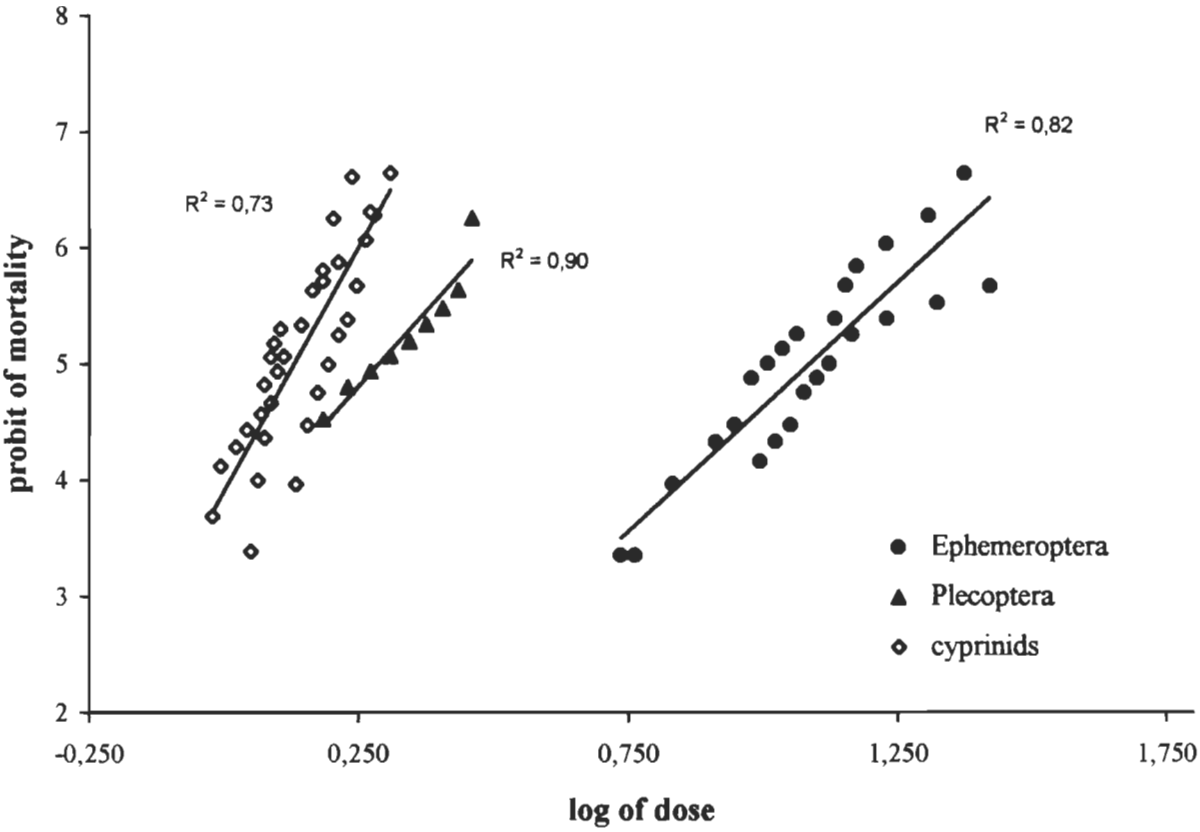
Walker, C. R., R. E. Lennon and B. L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U. S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2 (Circular No. 186). 18 p.

Wollitz, R.E. 1962. Effects of certain commercial fish toxicants on the limnology of three cold-water ponds, Montana. Proceedings of the Montana Academy of Sciences **22**: 54-81.

Fig. 1. Effect of B-89 crude filtrate on zooplankton and cyprinids at 18°C.

Fig. 2. Effect of B-89 crude filtrate on benthic invertebrates and cyprinids at 18°C.


8
7
6
5
4
3
2
probit of mortality
R² = 0,73
R² = 0,94
R² = 0,96
R² = 0,96
R² = 0,96
copepods
rotifers
ostracods
cladocerans
cyprinids
-0,250
0,250
0,750
1,250
1,750
2,250
log of dose


8
7
6
5
4
3
2
probit of mortality
R² = 0,73
R² = 0,90
R² = 0,82
Ephemeroptera
Plecoptera
cyprinids
-0,250
0,250
0,750
1,250
1,750
log of dose

Annexe

Recommandations aux auteurs

Journal canadien des sciences halieutiques et aquatiques

Instructions to Authors

Canadian Journal of Fisheries and Aquatic Sciences

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together.

The *Journal* considers the following types of contributions:

Articles — Studies of broad scope that are original contributions to science.

Reviews — Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives — Essays of opinion or hypothesis on aquatic science of concern to the professional and layperson. References are desirable; informal style is welcome.

Comments — Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript submission

Submit manuscripts in triplicate to the Editor, *Canadian Journal of Fisheries and Aquatic Sciences*, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada, accompanied by a covering letter that (1) states the main points and significance of the work, (2) avows that all coauthors fully participated in and accept responsibility for the work and that it is not being considered for publication elsewhere, (3) suggests potential referees, (4) identifies other manuscripts containing the same or similar information, and (5) includes the telephone and facsimile numbers and e-mail address of the corresponding author. Original typescripts and figures (except halftones) are best retained by the author until requested. **Packaging of manuscripts and illustrations should be robust enough to resist damage in transit.**

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal*'s editorial policy or scope.

Editorial practices

The *CBE Manual for Authors, Editors, and Publishers: Scientific Style and Format* (6th ed. 1994) published by the Council of Biology Editors, Inc., Chicago, IL 60603, U.S.A., is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103, U.S.A.). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed. 1991. Spec. Publ. No. 20, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990. Trans. Am. Fish. Soc. 119: 2–15). All measurements must be metric, use SI symbols in most instances (exceptions noted in the editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Etobicoke, ON M9W 1R3, Canada).

Authors are requested to submit diskettes with *accepted* manuscripts only, prepared on Macintosh or IBM-compatible personal computers. Include files in both a word-processing format (WordPerfect or Word is preferable) and ASCII file. Please identify the word-processing software and version number used.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. **Alterations to the content must be avoided.**

Copyright transfer — All authors are required to complete a copyright transfer form assigning all rights to NRC. Requests for permission to republish the paper, in whole or in part, should be sent to NRC Research Press.

Preparation of manuscripts

General guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. General guidelines follow.

Type the manuscript on white paper (8.5 X 11 in.) on only one side of the page. Leave all margins at least 1 in. wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference

list. Use italic font if available; when not available, underline material that is to be set in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what readers need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

(1) Limit abstract to one paragraph of about 175 words.

(2) Double-space all elements of the manuscript, including references, table captions, and figure legends.

(3) Avoid exclusive use of capitals anywhere in the manuscript, including headings, table captions, and figure legends.

(4) Italicize or underline only Latin names of organisms and appropriate statistical and mathematical notations.

(5) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

(6) Avoid ambiguous forms such as g C/m²/d; use g $C \cdot m^{-2} \cdot d^{-1}$.

(7) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

(8) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

(9) Include acknowledgements section at manuscript stage, not when page proofs arrive.

(10) Provide the person's initials and mailing address when referring to personal communications.

(11) Delete unnecessary references that do not apply directly to the problem.

(12) Check references carefully against text citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g., available from Department of Economics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

(13) Delete commas between name and date in citations such as (Smith 1990) and do not underline "et al."

(14) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g., thousands).

(15) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints or laser printouts of figures, or originals if they do not exceed 8.5 X 11 in., on request for publication. Use the same type of lettering throughout if possible.

Parts of the manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g., X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . . " or "Investigations on . . . " Good titles greatly assist scientists and librarians in using scientific literature and aid indexers in preparing titles for keyword indexes. Series titles should be avoided.

Abstract

An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant

findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as " . . . is discussed" or " . . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents and be completely self-contained (i.e., any *essential* references) in a retrievable form (e.g., R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. 37: 268–282).

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and methods

Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . . " If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence.** Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1 ½-, or 2-column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra space instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript lowercase letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed within the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions, laser printouts, or the original artwork (no larger than 8.5 X 11 in.) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide four photocopies of line illustrations and four sets of any photographs for review purposes.

Design figures to fit a 1- or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures produced on laser printers are acceptable; figures produced on dot matrix printers are not acceptable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

CorelDraw! is our preferred graphics program, which can accommodate a variety of IBM-compatible formats (see electronic graphics list at NRC Research Press home page). Clearly label the disk with the program, version number, and the figure numbers with the corresponding filenames and extensions. Use the correct extension(s) for the program(s) used.

or otherwise our system may not be able to read or open the files.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are charged to the author. Further details may be obtained from Jennifer McColl, Publication Officer, NRC Research Press (tel.: 613-993-4500; fax: 613-952-7656; e-mail: jennifer.mccoll@nrc.ca).

Submit only photographs of the highest quality for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

For the electronic version of the *Journal*, colour graphics may be considered even though the print version is black and white; authors may provide colour electronic files for the electronic version along with black and white illustrations for the print version.

Preparing Photographs and Illustrations for Reproduction, a pamphlet prepared by NRC Research Press, is available from the Editorial Office.

Mathematical expressions, equations, and formulae

Leave three spaces above and below lines that have much superscript material, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between lowercase *l* and the numeral *one*, and between capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	(Bq)
calorie	(cal)
centimetre	(cm)
centimetre, square	(cm^2)
centimetre, cubic	(cm^3)
centimetres per gram per second	($cm \cdot g^{-1} \cdot s^{-1}$)
coulomb	(C)
day	(d)
decimetre	(dm)
degree Celcius	(°C)
degrees of freedom	(df)
gram	(g)
hectare	(ha)
hertz	(Hz)
hour	(h)
joule	(J)
kilometre	(km)
litre	(L)
lumen	(lm)
lux	(lx)
metre	(m)
metre, square	(m^2)
metre, cubic	(m^3)
micrometre	(μm)
milligram	(mg)
millilitre	(mL)
millimetre	(mm)
millimetre, square	(mm^2)
minute	(min)
molar mass	(M)
mole	(mol)
moles per litre	(mol/L, M)
month	(mo)
Pascal	(Pa)
second	(s)
standard deviation	(SD)
standard error	(SE)
tonne (metric ton)	(t)
volt	(V)
volume	(vol)
watt	(W)
week	(wk)
year	(yr)

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be used with the year offset by commas (On October 9, 1983, the . . .).

Reference citations in text

Name-and-year system

The Journal uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . . " or " . . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . . " or " . . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "personal communication."

Unpublished data

If an unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "In press." Only those manuscripts that are in galley or page proof stage or for which there is an acceptance letter can be considered in press. If an article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpublished data" in the text and do not include it in the reference list.

Reference lists

References should be listed at the end of the paper in alphabetical order according to surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. **39**: 1444–1452.

Entire issue of journal

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Book in a series

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Book not in a series

LeBlond, P.H., and Mysak, L.A. 1978. Waves in the ocean. Elsevier, New York.

Part of book

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. Neil and D.C. Himsworth. Oregon State Unversity Press, Corvallis, Oreg. pp. 203–229.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C.

Theses

Kutty, M.N. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can.Tech. Rep. Fish. Aquat. Sci. No. 1032.

Translation

Koike, A., and Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. (Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word list

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)