TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

Introduction générale

L'Amérique du Nord représente le plus important bassin du globe en terme de diversité de moules d'eau douce (Embranchement : Mollusca; Classe : Bivalvia; Superfamille : Unionoidea); le terme mulette est aussi employé dans les pays francophones. Au sein de la superfamille des Unionoidés, deux familles sont bien représentées en Amérique du Nord, soit les Margaritiferidea et les Unionoidea, pour un total de 297 espèces et sous-espèces connues (Williams *et al.* 1993). De ces 297 espèces, 55 sont présentes au Canada (Metcalfe-Smith & Cudmore-Vokey 2004) et 12 au Nouveau-Brunswick (Williams *et al.* 1993, Metcalfe-Smith & Cudmore-Vokey 2004).

Au cours du dernier siècle, les moules d'eau douce ont connu un déclin sévère en terme de diversité et d'abondance (Metcalfe-Smith *et al.* 1997). Soixante-douze pourcent des espèces d'Amérique du Nord sont considérées comme étant en danger de disparition, menacées ou à statut préoccupant à travers leur aire de distribution (Williams *et al.* 1993). Williams *et al.* (1993) stipulent que ce n'est que récemment que la sévérité de ce déclin a été reconnue. Évidemment, une telle situation suscite un intérêt croissant de la part des chercheurs en malacologie en ce qui a trait à la dynamique des populations d'unionoidés. Au Canada, suite à la mise sur pied en 1995 d'un groupe de travail sur les mollusques (Mollusc Working Group), le Comité sur la Situation des Espèces en péril au Canada (COSEPAC) a officiellement reconnu le statut précaire des unionoidés en les considérant comme un des groupes d'invertébrés les plus menacés au Canada (Metcalfe-Smith *et al.* 1997). Plusieurs auteurs sont d'accord pour dire que cette chute d'abondance et de diversité (jusqu'à 58% des espèces canadiennes pourraient être en péril, menacées, à statut préoccupant ou extirpées) (Metcalfe-Smith & Cudmore-Vokey 2004) des unionoidés est principalement le résultat de destruction et de dégradation importantes de l'habitat directement associées aux activités anthropiques (Fuller 1974; Bogan 1993; Williams *et al.* 1993; Richter *et al.* 1997; Brim Box & Mossa 1999). Toutefois, les introductions indirectes d'espèces exotiques, telles la moule zébrée (*Dreissena polymorpha*), la moule quagga (*Dreissena bugensis*) et la moule asiatique (*Corbicula fluminea*) peuvent également être des causes de perturbations majeures des communautés benthiques, affectant notamment les populations d'unionoidés (Fuller 1974; Neves 1997; Richter *et al.* 1997; Haltuch & Berkman 2000; Martel *et al.* 2001).

Les unionoidés sont des invertébrés généralement sédentaires, dont la durée de vie peut atteindre plusieurs décennies et ayant un faible taux de recrutement impliquant la participation de poissons hôtes, une composante unique de leur cycle de vie. Les larves d'unionoidés, appelées glochidies, sont éjectées dans l'eau par les femelles et à ce moment, elles doivent s'attacher à un poisson hôte (Fig. 1), notamment sur les nageoires, les écailles et les branchies, pour pouvoir se métamorphoser en juvéniles et se disperser (Kat 1984; Nedeau *et al.* 2000; Zardus & Martel 2002). Les caractéristiques biologiques des unionoidés les rendent vulnérables à la modification de leur habitat (Hanson & Locke 2000), ainsi qu'aux perturbations affectant leur(s) poisson(s) hôte(s) (Bogan 1993). Puisque les activités anthropiques menacent à la fois l'intégrité de l'habitat et des communautés ichthyologiques essentielles aux unionoidés, ces dernières sont d'autant plus vulnérables aux perturbations engendrées par l'homme.

Comme le souligne Clarke (1981), la présence ou l'absence d'unionoidés peut s'avérer un indicateur de la qualité de l'eau, de l'habitat et de la santé des communautés ichthyologiques d'un cours d'eau. Les unionoidés sont fortement liés aux communautés aquatiques de par leur dépendance à une population en santé d'espèces de poissons hôtes. Les communautés aquatiques non perturbées sont représentées par une diversité et une abondance importance au sein des espèces d'unionoidés (Metcalfe-Smith *et al.* 1997). C'est dans cette optique que les unionoidés furent évoquées comme étant de bons indicateurs de la santé des écosystèmes aquatiques (Fuller 1974; Williams *et al.* 1993; National Native Mussel Conservation Committee 1997; Vaughn & Taylor 1999). Donc, un déclin au sein des populations de moules d'eau douce, comme celui observé actuellement, envoie un signal qui nous avertit que l'intégrité globale des écosystèmes aquatiques d'eau douce est menacée.

En dépit du fait que les unionoidés paraissent de bons indicateurs de santé des écosystèmes aquatiques, certains aspects de la dynamique des populations de ces mollusques ne sont pas encore bien compris (Bogan 1993; Strayer *et al.* 1994; Haag & Warren 1998; Brim Box & Mossa 1999). L'importance relative de l'impact des perturbations de l'habitat et des communautés de poissons sur l'intégrité des communautés d'unionoidés doit être établie. Les connaissances actuelles sur les relations unionoidés-poissons sont malheureusement incomplètes (Hoggart 1992; Nedeau *et al.* 2000; Hanson & Locke 2001), ce qui rend encore plus difficile l'évaluation des facteurs écologiques limitant les populations d'unionoidés. Au Nouveau-Brunswick, les ouvrages de références sur la diversité, l'abondance, la distribution et voire même, la biologie des espèces d'unionoidés sont très rares (Hanson & Locke 2001).

Les facteurs écologiques qui influencent la diversité, l'abondance et la distribution des populations de moules d'eau douce (Superfamille : Unionoidés) se retrouvent au sein des variables environnementales (macro- et microhabitat) et de la dynamique des populations de poissons hôtes. Plusieurs auteurs soulignent simplement l'importance du rôle des poissons hôtes dans la dynamique (Bogan 1993; Watters 1992) sans toutefois tenter d'étudier ou de quantifier ce rôle. Le rôle des poissons hôtes est souvent abordé en relation aux impacts néfastes qu'ont les barrages sur la migration de certaines espèces de poissons essentielles aux unionoidés (Bogan 1993; Watters 1996; Vaughn & Taylor 1999; Hanson & Locke 2001). Bien que l'on reconnaisse la relation poissons-unionoidés, les études antérieures sur la dynamique des populations d'unionoidés ont principalement porté sur l'influence du macro et du microhabitat (Salmon & Green 1983; Strayer 1983; Neves & Widlak 1987; Holland-Bartels 1990; Strayer 1993; Strayer & Ralley 1993; Layzer & Madison 1995; Di Maio & Corkum 1995; Brim Box & Mossa 1999). L'étude de Haag & Warren (1998) tenta de déterminer l'importance relative des communautés ichthyologiques et des variables environnementales de l'habitat pour les unionoidés. En terme de gestion et de conservation, il est important de connaître les facteurs écologiques et leur influence sur les populations d'unionoidés, afin de pouvoir concentrer efficacement les efforts et le peu de ressources disponibles à la conservation des communautés ciblées (Morris & Corkum 1996; Neves 1997).

Combler ce manque d'information au niveau des populations de moules d'eau douce, tant au niveau des facteurs écologiques régissant leur dynamique, qu'au niveau de la reproduction et du recrutement (poisson-hôtes et leur importance), a été la motivation première de cette présente recherche.

[Note : Dans le texte, je prend en considération la taxonomie telle que mentionnée par McMahon & Bogan (2001) pour les moules d'eau douce d'Amérique du Nord. Ainsi, j'utilise « Uninoidés » comme terme français décrivant les moules d'eau douce de la superfamille des Unionoidea (laquelle comprend les familles Margaritiferidés et Unionoidés). Dans le texte anglais, j'utilise le terme « Unionoids » ou bien « freshwater mussels » pour décrire les moules d'eau douce appartenant à cette superfamille.]

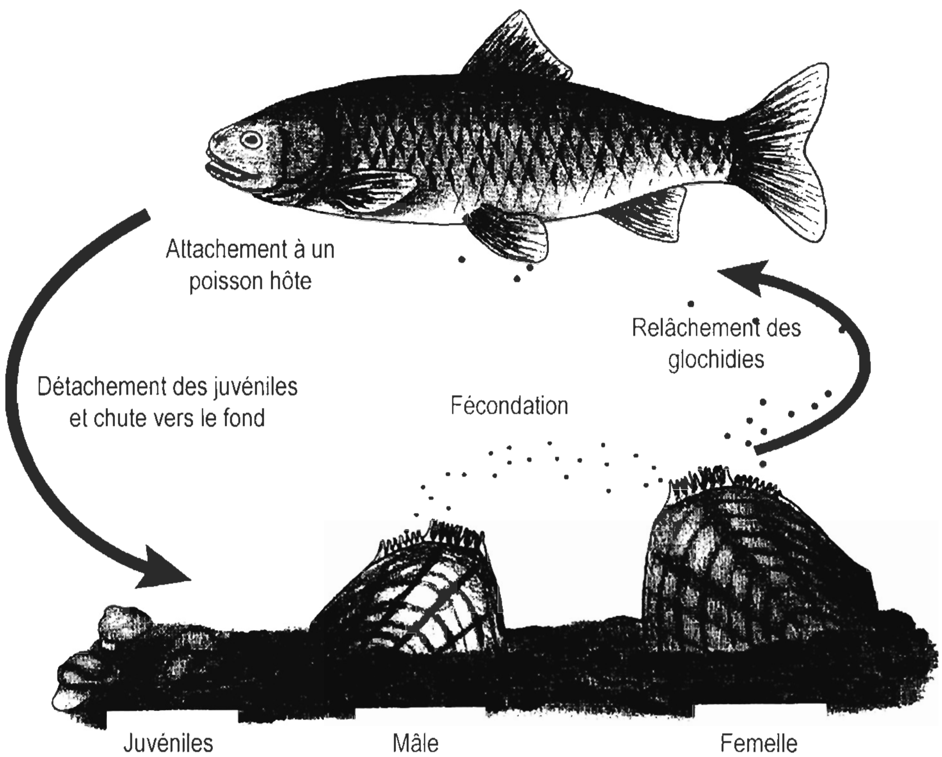

FIGURE 1 : Cycle de reproduction d'une moule d'eau douce et implication d'un poisson hôte (Nedeau *et al.* 2000)

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Chapter I: Factors involved in the diversity, distribution and abundance of freshwater mussels (Unionoidea) in two coastal rivers of New Brunswick

ABSTRACT

Freshwater mussels, members of the Unionoidea superfamily, represent a major component of the benthic biomass of rivers and lakes but are facing severe decline owing to anthropogenic stressors and introduction of invasive species. Several studies were conducted on freshwater mussels and the ecological factors that might influence their population dynamics. We analyse habitat parameters and fish communities using a hierarchical, quantitative sampling design in order to find which and how ecological factors are responsible for structuring mussel communities in two New Brunswick rivers in eastern Canada. A total of 255 mussels belonging to three species were found in the Kouchibouguacis River, whereas a total of 795 mussels belonging to only one species were found in the Kouchibouguac River. The freshwater mussel species were *Margaritifera margaritifera*, the eastern pearlshell, *Elliptio complanata*, the eastern elliptio, and *Alasmidonta varicosa*, the brook floater. Fish populations and habitat (micro- and macro-) variables were studied as factors. There was a highly significant difference in fish assemblage between the two watersheds: 12 species occurred in the Kouchibouguacis River (dominance of cyprinids), whereas only eight were present in the Kouchibouguac (dominance of salmonids). The study results show that none of the habitat variables were explaining freshwater mussel dynamic; thus this could be because 1) of the inability of the sampling design to capture high enough mussel densities to detect relationships, 2) some critical habitat variables were not measured, or 3) because habitat variables do not primarily influence freshwater mussel populations in the two studied rivers. The major difference in fish assemblage between the Kouchibouguac River and the Kouchibouguacis River is suspected to be the playing a role in the freshwater mussel diversity and abundance discrepancy. However, absence of baseline data and inventories do not allow to draw solid conclusion about the relationships between fish assemblage and freshwater mussel communities.

Key words: Freshwater mussels, Unionoidea, Unionoidae, Margaritiferidae, *Margaritifera margaritifera*, *Alasmidonta varicosa*, *Elliptio complanata*, population dynamic, ecological factor, spatial patterns, host fish, mussel-host relationship, distribution, abundance, habitats

INTRODUCTION

Freshwater ecosystems are fragile and react to various types of anthropogenic disturbances. Organisms living in freshwater ecosystems are facing severe and widespread decline owing to alteration and pollution of their habitat and introduction of invasive species (i.e. zebra mussel, *Dresseina polymorpha*) (Fuller 1974; Bogan 1993; Watters 1996; Vaughn & Taylor 1999). In particular, freshwater mussels (Unionoidae; aqlso called "unionoids" in the text) have been severely impacted during the last century when the degradation of freshwater ecosystem intensified (Metcalfe-Smith *et al.* 1998). Unionoids represent a major component of the benthic biomass of rivers (Sephton *et al.* 1980; Strayer *et al.* 1994) and play various and important roles in the maintenance of biological integrity of aquatic ecosystems (Strayer *et al.* 1994, 1999). Unionoids are suspension feeders, and therefore filter a great amount of plankton, organic material, inorganic material, nutrients, and contaminants from the water column. This regulates plankton densities, reduces turbidity, recycles nutrients, and improves water quality (Strayer *et al.* 1994; National Native Mussel Conservation Committee 1998; Welker & Walz 1998; Strayer *et al.* 1999; Nedeau *et al.* 2000). Unionoids also provide food for wildlife such as birds, fishes and mammals (Zahner-Meike & Hanson 2001). In addition, when occurring at high densities they stabilise the substrate and create habitat for other organisms (Strayer *et al.* 1994).

New Brunswick's freshwater mussel assemblages have rarely been studied, even though these mussel communities are facing the same threats as other populations in North America. Although 12 species of freshwater mussels are listed for the Canadian Maritime

Provinces (Williams *et al.* 1993; Metcalfe-Smith & Cudmore-Vokey 2004), little information is available on this group. Few published studies or reports have been published on New Brunswick unionoid (Athearn 1961; Athearn & Clarke 1961; Sephton *et al.* 1980; Hanson & Locke 2000, 2001). An assessment of the diversity, general biology and conservation status of the freshwater mussels of the Maritimes ecozone is currently being prepared by Environment Canada (Martel *et al.* in prep.). Thus, so far the scarcity of published information on New Brunswick freshwater mussels hampers conservation efforts for this group of invertebrates (Hanson & Locke 2001).

Freshwater mussel biological features (i.e. high longevity, low dispersal rate, filter-feeding, particular reproductive system) make them valuable indicators of environmental changes and aquatic ecosystem integrity (Fuller 1974; Clarke 1981a; Carell *et al.* 1987; Green *et al.* 1989; Williams *et al.* 1993; National Native Mussel Conservation Committee 1998; Vaughn & Taylor 1999). However, a high proportion (approximately 72%) of North American unionoids are considered endangered, threatened, or of special concern (Williams *et al.* 1993), which explains why they are gaining attention. This popularity is relatively new and many aspects of unionoid populations are still not well understood. Basic data such as distribution, diversity, reproduction, fish-host relationships and habitat selection are lacking for many species. The basic knowledge of diversity, distribution, and abundance of unionoids in New Brunswick rivers is necessary for assessing conservation status and planning effective management and restoration actions for protecting these freshwater mussel populations. Hastie *et al.* (2000) mentioned that knowing the physical habitat requirements of the mussels would help to identify the underlying processes of their decline, and would enable impact assessments of future river management plans. Since many aquatic ecosystems containing significant freshwater mussel communities are constantly altered by humans (Bogan 1993; Allan & Flecker 1993; Miller & Payne 1998; Vaughn & Taylor 1999; Carignan & Steedman 2000), detailed quantitative descriptions of their habitat, as well as of the fish communities they depend on for reproduction, are required.

Several authors have tried to correlate parameters of freshwater mussel dynamics with ecological factors in order to better understand these complex relationships. The two main ecological categories that have been investigated are the mussel habitat and the associated fish assemblage. Authors have studied microhabitat variables, such as current velocity, sediment size, depth of water (Strayer 1981; Salmon & Green 1983; Neves & Widlack 1987; Strayer 1993; Haag & Warren 1998; Hastie *et al.* 2000), as well as macrohabitat variables, such as hydrological variability (Vannote & Minshall 1982; Layzer & Madison 1995; Di Maio & Corkum 1995), characteristics of riparian cover (Morris & Corkum 1996), stream size and river gradient (Strayer 1983; Strayer 1993), in an attempt to relate the habitat variables to the structure of unionoid populations. Even though mixed results were obtained, microhabitat variables, such as substrate composition, current velocity, and water depth were successful at predicting freshwater mussel distribution (Harman 1972; Salmon & Green 1983; Johnson & Brown 2000). Several authors have been sceptical about how effective traditional microhabitat variables are in explaining unionoid biological patterns and suggested that they should be replaced by macrohabitat variables (Holland-Bartels 1990; Strayer 1993; Strayer *et al.* 1994; Di Maio & Corkum 1995; Morris & Corkum 1996; Strayer 1999; Brim Box & Mossa 1999). However, as mentioned by Johnson & Brown (2000), in small aquatic systems, macrohabitat variation can be minimal, thus microhabitat variables represent the only measure available for predicting unionoid populations. Haag & Warren (1998) are among the few investigators who have considered unionoid habitat and fish variables in an integrated study in order to identify the most influential variables for mussel population dynamics. In the present study, freshwater mussel habitat parameters and fish community were analysed in order to find which and how ecological factors, either habitat characteristics or fish host populations, are influencing the distribution, abundance and diversity of freshwater mussel populations. I also tried to integrate new macrohabitat variables as potential ecological factors involved in mussel community structure.

The specific steps towards achieving the purpose of the study were (1) to investigate the spatial distribution of the freshwater mussel in two river systems, (2) to quantify the abundance of each freshwater mussel species encountered, (3) to quantify river habitat variables, (4) to characterize the fish populations close to mussel populations, and finally (5) to examine the relationships among mussel abundance, habitat and fish community structure.

Materials and methods

Study area

The Kouchibouguac River and the Kouchibouguacis River basins are located south of the Bay of Miramichi along the east coast of New Brunswick, eastern Canada (Fig. 2). The downstream sections of the two drainages pass through the Kouchibouguac National Park. The Kouchibouguac and the Kouchibouguacis rivers are located in the Maritime Lowlands Ecoregion. This region is characterised by flat to gently sloping areas of sedimentary rocks (mainly sandstone and mudstone) (Poole 1976). The glacial and marine processes left a layer of sediments (less than 1.5 m) in most areas of the lowlands (Rampton *et al.* 1984). The watershed areas are 370 and 393 km^2 for the Kouchibouguacis River and Kouchibouguac River, respectively. The Kouchibouguac River is a fifth order stream, whereas the Kouchibouguacis River is a fourth order stream. An old breached dam is located near the mouth of the Kouchibouguac River. The dam was built in the summer of 1917 for electricity production and a breach was created in the late 1930's following concerns and complaints about declining population of anadromous fish. Both watersheds are mainly forested, and activities like forestry, farming, and human habitation are being practised. In more details, 49.0% of the Kouchibouguac watershed is forested, compare to 51.3% for the Kouchibouguacis watershed. Forested activities, such as cutting, thinning and planting, account for 31.4% and 30.2% of the Kouchibouguac and Kouchibouguacis watershed, respectively. Agriculture (i.e. vegetables, christmas trees, blueberries) and human development (i.e. houses, cottages, gravel pits, bridges) represent a fairly small percentage of both watersheds, i.e. 5.6% of the Kouchibouguac watershed and 6.8% of the Kouchibouguacis watershed, and occur mainly in the downstream portion of the two watersheds. Historically, both rivers were affected by the ship building industries and were the scene of wood transportation, wood mills and dams operating along both riverbanks. None of these structures are operating today, but remnants of this period can still be seen (DeGrâce 1984; Beach 1988).

Mussel assemblage

A hierarchical sampling design was used to sample mussel communities. Benthic organisms are often aggregated following spatial heterogeneity of the environment (Morrisey *et al.* 1992). This natural aggregated distribution is encountered in freshwater mussel populations (Hastie *et al.* 2000) and makes sampling difficult when the objective is to study population dynamics (Downing & Downing 1992). In order to take spatial heterogeneity into account, a hierarchical sampling design, as proposed by Morrisey *et al.* (1992) was selected. Stratification of the rivers would have provided valuable information in order to take into account heterogeneity of both rivers; unfortunately this could not be done due to lack of time and resources.

Both rivers were separated into six equal segments of approximately 8.8 km. The six segments were then divided into eight sections of approximately 1.1 km. In turn, each section was divided into five stations of approximately 200 m. The first step was to randomly select three sections. In each of the sections, two stations were selected at random. Within each station, a 100 m^2 site was selected in which three different habitats were encountered (i.e., pool, riffle, and run; as described by Neves & Widlak 1987; Fig. 3). Finally, 10% of the site were sampled (a minimum percentage recommended; Downing & Downing 1992) by using ten 1 m^2 quadrats distributed randomly. A total of 65 sites (650 quadrats) were thus sampled for freshwater mussels from 27 May to 17 August 2002. The Kouchibouguac River and the Kouchibouguacis River were sampled at 32 and 33 sites, respectively. All freshwater mussels on the riverbed (i.e. unburied) were counted, identified at the species level, and returned to the bed. No substrate excavation was done at the sampling site in order to capture buried individuals, although special attention was given to smaller individuals (those with 30-50 mm shell length) by carefully looking under rocks (Strayer & Ralley 1993) and digging in finer sediments behind boulders. Some sites could not be sampled owing to inaccessibility and unsuitability for sampling (depth and turbidity). Sampling occurred only in the main channel; tributaries were not sampled because of lack of time and resources. Mussel species were identified using Clarke (1981a) and Nedeau *et al.* (2000).

Mussel community patterns were described within and between the rivers using cluster analysis with average linkage (Primer v.5). Similarity matrix was computed using Bray-Curtis similarity index. Sites in which mussels were absent were withdrawn from the cluster analysis. Species abundance data for all similarity matrices were fourth-root transformed. Analysis of variance and Two-sample t-tests were used to detect significant difference between density pattern of the most common mussel (*Margaritifera margaritifera*) within and between the rivers. One-way analysis of variance and Kruskal-Wallis (SYSTAT v.9) were used to determine whether *M. margaritifera* abundance differed significantly between rivers and among gradient zones (i.e., upstream, mid reach, and downstream) of each river. Abundance data of *M. margaritifera* used in parametric analysis of variance were log transformed to avoid departure from normality and heterogeneity of variances. A Dispersal index was calculated in order to determine the spatial aggregation of the mussel populations (see Elliot 1977 for details; Hastie *et al.* 2000) at two different levels (quadrats and sampling stations). Departure from random distribution was then analysed using a Chi-square test (Elliot 1977). A Chi-squared test for goodness-of-fit was performed to compare the observed data from those of the Poisson distribution (Elliot 1977). The goodness-of-fit test could not be performed for all unionoids because of lack of density classes in some species.

Habitat characteristics

Macro- and microhabitat variables were measured at each sampling site. The microhabitat variables were: water depth, percentage of different substrate types, percentage of macrophytes, percentage of large woody debris, and distance from shore. Depth was taken in the middle of the quadrat. Five classes, adapted from Simonson *et al.* (1994), were selected for substrate composition: clay (0.004-0.062 mm), sand (0.062-2 mm), gravel (2-64 mm), cobble (64-256 mm), and rocks (>256 mm). Each substrate class was estimated, to the closest 5%, by the field crewmember for each quadrat (adapted from Haag & Warren 1998). The same evaluation, to the closest 5%, was done for the macrophyte and large woody debris cover variables.

Link magnitude, stream order, river width, current velocity, conductivity, pH, width of the riparian zone, and surrounding land uses were macrohabitat variables taken at the sampling site scale. Link magnitude is a measure of the total order 1-streams occurring upstream of the site. This measure accounts for small changes in stream width and velocities; thus, it is considered more sensitive to hydrologic variations (Haag & Warren 1998). The link magnitude and the stream order (Strahler 1957) have been obtained from 1:50 000 topographic maps. Current velocity was measured with a floating device (punctured plastic golf ball). Because of lack of expertise and resources, only two water chemistry variables were measured at the site level.. Conductivity and pH were taken respectively with Hanna model HI8733 and HI9024 kit.

Width of riparian zone and land-use were obtained using Geographic Information System (GIS, ArcView 3.2) layers. Aerial photographies (1:12 500, 1998) of the two watersheds were digitalised and georeferenced in the GIS. In the GIS, the "sampling site" information layer was superposed to the digitalised photographies, allowing the measurement of the length of riparian zones (m) for each mussel locations. To obtain the land-use variable, land use zones of 250 and 500 m in diameter were created around each site. The information in the GIS "forestry" layer (provided by the New Brunswick Service of Natural Resource

1995) was used to calculate the proportion of each land-use class contained in the land use zones. Five land use classes were determined: forested, agricultural, wetland, disturbed forest, and human development.

Simpson diversity index of substrate diversity was computed in order to estimate the substrate diversity of each river using the percentage of each substrate class found in quadrats (Primer v.5). A Kruskal-Wallis was computed with SYSTAT (v.9) to compare substrate diversity between the two rivers. Analysis of variance and Spearman rank correlation were used to test for difference between habitat variables of the rivers, and to examine correlation among variables within river. A critical value table for correlation coefficients was used to test a significance of a correlation (Sokal & Rohlf 1981b).

Fish assemblage

Electrofishing during periods of low flow is considered an appropriate method for sampling fish in shallow, relatively fast-flowing rivers with coarse substrate similar to those found in the Kouchibouguac River and the Kouchibouguacis River (Hartley 1980; Reynolds 1996; Schneider & Merna 2000). A total of 64 100-m^2-site were sampled with an electrofishing backpack unit (Smith-root 12V). Since the objective of fish sampling was not to get a precise species density, but rather a relative species density, one open single pass was selected (Mitro & Zale 2000). Captured fishes were counted, measured and returned to the river. Mean fishing time depended on fish abundance, experience of the field crew, and obstacles within the sites (average of 27 minutes, ranged between 16 and 70 minutes). Fish were identified using Scott & Crossman (1973), Bernatchez & Giroux (2000), as well as expert identification with Dr. Brian Coad, Icthyologist at the Canadian Museum of Nature. In the results section, fish species are listed following the systematic order found in Bernatchez & Giroux (2000).

Cyprinid species were grouped into one class for statistical analysis. American eels (*Anguilla rostrata*) and immature sea lampreys (*Petromyzon marinus*) were withdrawn from all analyses because they are not reported as hosts for mussel glochidia.

The statistical methods used to test fish patterns between rivers are similar to the ones used for the mussels. The fish community pattern was first examined by clustering sites using Cluster group average method (Primer v.5). A Kruskal-Wallis test was used to verify differences in fish pattern between the two rivers. Fish abundances were fourth root transformed, when necessary, to fulfill test assumptions. An R x C test of independence using G-test (Sokal & Rohlf 1981a) was performed to test whether the proportion of fish from three major families (i.e., Salmonidae, Catostomidae and Cyprinidae) differs between the two rivers. Shannon-Weiner diversity index was calculated and used in testing the difference in fish diversity between rivers.

Habitat - mussel relationships

Habitat preference curves were used to examine mussel preferences for specific range of a habitat variable. They were used to examine the optimal ranges of water depth, distance from shore, surface substratum types, and percent coverage by macrophytes and large woody debris for the mussels in the two rivers. In order to assess habitat preference, habitat availability was first taken into account (see Hastie *et al.* 2000 for complete methodology). A Suitability Index (SI) was then calculated and plotted for each mussel and each habitat variable. Suitability index is a score ranging from 0 to 1 representing respectively unsuitable and optimal habitat conditions for each mussel species. Discrete quantitative habitat variables (e.g., distance from shore) had to be classified into groups in order to perform this analysis (Hastie *et al.* 2000).

In order to discriminate habitat variables that might explain variability in mussel abundance, a stepwise discriminant analysis was performed with Systat v.9, using a selection of variables at p=0.15 to enter or remove variables. The stepwise discriminant analysis was only performed on *Margaritifera margaritifera* densities because the occurrence of the two other mussel species were too low to be analysed. *M. margaritifera* densities were grouped into four classes: abundant (> 1.0 ind./m^2), common (0.2-1.0 ind./m^2), scarce (0-0.1ind./m^2), and absent (0 ind./m^2). The habitat variables were tested for colinearity (Draftsman plot analysis, Primer v. 5) and departure from normality (Kolmogorov-Smirnov One Sample Test, Systat v.9). Several variables were withdrawn of the analysis because of colinearity. Stepwise discriminant analysis was performed on 11 habitat variables: pH, conductivity, magnitude, depth, velocity, mean percentage of sand, gravel, cobble, macrophyte and large woody debris cover, and percentage of forested land surrounding the sampling sites. Conductivity, macrophyte cover, large woody debris cover, and magnitude were square root transformed.

BIOENV and RELATE tests (Primer v.5) were performed on the similarity matrices of the mussel assemblages and habitat variables for both rivers. The Bray-Curtis similarity index was selected to compute the mussel matrix, and abundance data were fourth-root transformed. Sites containing no mussels were withdrawn from the analysis. A normalized Euclidian distance was selected for the environmental matrix. The BIOENV analysed first the abiotic data, and then the multivariate pattern of these data is compared to the pattern of the species data. The match obtained between the abiotic and species data pattern reflects how strongly the environmental data explained the biotic pattern (Clarke & Warwick 2001). This is done using a Spearman rank correlation coefficient (Clarke & Warwick 2001). RELATE testing is comparable to testing Spearman rank correlation between the distance matrices (Clarke & Warwick 2001). A simple permutation test (20 000 permutations) was then applied to the correlation coefficient in order to test the null hypothesis that there is no relation between the two similarity matrices (Clarke & Warwick 2001). Finally, simple Spearman rank correlation and Pearson correlation were used to test the relation between habitat variables and mussel densities. A critical values table for correlation coefficients was used to test a significance of a correlation (Sokal & Rohlf 1981b). Bonferroni corrections were used for the Pearson correlation.

Fish and mussel relationships

Spearman rank correlation (SYSTAT v.9) was used to test for relations between the fish and mussel abundances. RELATE testing was also used to examine correlation between distance matrices of fish and mussels.

RESULTS

Unionoid assemblage

Kouchibouguacis River

A total of 255 freshwater mussels of three species were found in the Kouchibouguacis River (330 sampling quadrats, Table 1). Mean unionoid densities of all sampling sites are 0.77 ind./m² in this system. The freshwater mussel community of the Kouchibouguacis River was composed of the eastern pearlshell, *Margaritifera margaritifera* (Margaritiferidae), the eastern elliptio, *Elliptio complanata* (Unionoidae: subfamily Ambleminae), and the brook floater, *Alasmidonta varicosa.* Another freshwater mussel species, the eastern floater (*Pyganodon cataracta*; Unionoidae: subfamily Anodontinae). is present and completes the freshwater mussel list for the Kouchibouguacis River (Chapter 2, Beaudet *et al.* 2002). Mean densities for each species for sampling sites were: 0.66 *M.*

margaritifera/m², 0.082 *E. complanata*/m², and 0.027 *A. varicosa*/m². *M. margaritifera* was found in 40% of the Kouchibouguacis quadrats, whereas *E. complanata* and *A. varicosa* were only found in 4% and 2% of the quadrats, respectively. Considering another spatial scale, *M. margaritifera* was found in 79% of the sampling station, whereas *E. complanata* and *A. varicosa* were found in 21% and 12% of the stations, respectively. The densities along three zones of the river gradient for *M. margaritifera* (ANOVA, p=0.023, n=33) and *A. varicosa* (Kruskal-Wallis, p=0.021, n=33) were significantly different. The lowest densities were encountered in the upstream section. Although densities of *E. complanata* were not significantly different along the river gradient (Kruskal-Wallis, p=0.06, n=33), there was however a tendency to observe higher densities in the mid-reach sampling sites. *A. varicosa* was rare and only collected in sampling sites located downstream, where its density reached 0.075 ind./m² (Fig. 4). *E. complanata* was almost entirely restricted to the «mid-reach» sampling sites where its density averaged 0.2 per m². *M. margaritifera* was distributed in all segments of the river.

Three main clusters based primarily on unionoid diversity were recovered in the Kouchibouguacis River (Fig. 5). The first cluster groups sites were dominated exclusively by *Margaritifera margaritifera*. These sites ranged from small headwater to large downstream sites (link magnitude ranging from 11 to 47). The second cluster incorporates sites in which *Alasmidonta varicosa* were encountered. This cluster corresponds to downstream sites with link magnitude ranging from 78 to 88. The third cluster includes sites in which *Elliptio complanata* were present, or even dominant, mainly at midreach sites, and at two downstream sites (link magnitude ranges from 23 to 36). Seven sites, where mussels were absent, were not included in the cluster analysis. Five of these sites were located in narrow headwater reaches.

Kouchibouguac River

Margaritifera margaritifera (Margaritiferidae) was the only freshwater mussel species found in the Kouchibouguac River (Table 1). A total of 795 individuals, encountered in 62.5% of the 320 quadrats, represented a density estimate of 2.48 ind./m^2. *M. margaritifera* was found in 97% of the sampling stations. The highest *M. margaritifera* density (3.5 ind./m^2) was observed in the mid-reach portion of the Kouchibouguac River. However, there was no significant difference in density of *M. margaritifera* among the three gradient zones (Kruskal-Wallis, p=0.068).

Groups obtained from the clustering of the abundance of the single species of the Kouchibouguac River had high similarity, and were only explained by different mussel abundances.

Margaritifera margaritifera abundance differed significantly between the Kouchibouguacis River and the Kouchibouguac River (ANOVA, p=0.000) (Fig. 6). *M. margaritifera* abundance differed significantly at all of the three gradient zones between the two rivers (*t* test; upstream, p=0.026; mid-reach, p=0.000; downstream, p=0.008).

Unionoids distributions in both rivers were highly aggregated in regard to the Dispersal Index analysis. Table 2 summarises results from the Dispersal Index analysis and the testing of the hypothesis of randomness. The dispersal indexes and results of the Chi-square test suggest that the freshwater mussels of the Kouchibouguacis River and Kouchibouguac River are not randomly distributed at the quadrat level.

Habitat characteristics

Results concerning the habitat variables are summarized in Table 3. The overall habitat variables did not differ significantly in the two rivers, with few exceptions. The Kouchibouguac River basin is a larger system than that of the Kouchibouguacis River and order and link magnitude at each site differed significantly from those of the Kouchibouguacis River (Kruskal-Wallis, p=0.000 and p=0.026, respectively). The Kouchibouguacis River system has a higher mean percentage of macrophyte cover (Kruskal-Wallis, p=0.037), and a higher percentage of land around the sites was represented by wetlands (Kruskal-Wallis, p=0.001). Moreover, a small but significant difference in percentage of human development was observed between the two rivers. The Kouchibouguacis River has a higher percentage of human development (Kruskal-Wallis, p=0.046).

Based on the dominant substrate found in the quadrats, the Kouchibouguacis River system is dominated by cobble, while the Kouchibouguac River system is dominated by gravel.

There were significant differences in percentages of gravel and cobble between the two systems (Kruskal-Wallis, p=0.000 for both). A significant difference between the substrate diversity indexes of the two rivers was found: the Kouchibouguac River system having a higher quadrat substrate diversity index (Kruskal-Wallis, p=0.007, n=33). The two rivers did not differ significantly in any other measured habitat variables.

Fish assemblage

There was a highly significant difference in the fish assemblage between the Kouchibouguacis River and Kouchibouguac River (RxC test, χ^2= 5.990, G=556.36, p=0.001). Twelve fish species were collected in the Kouchibouguacis River, compared to eight species for the Kouchibouguac River (Table 4). Cyprinids were more abundant in the Kouchibouguacis River, whereas salmonids dominated the fish assemblage of the Kouchibouguac River (Fig. 7). Four species of cyprinids were found in the Kouchibouguacis River, whereas only one was found in the Kouchibouguac River. The blacknose dace (*Rhinichthys atratulus*) was the most abundant cyprinid in the two rivers. The abundance of cyprinids and salmonids differed significantly between the two rivers (Kruskal-Wallis, p=0.002 and p=0.000, respectively). The white sucker (*Catostomus commersoni*) was more abundant in the Kouchibouguacis River (Kruskal-Wallis, p=0.007). The slimy sculpin (*Cottus cognatus*) was rare and only found in the Kouchibouguac River. The ninespine stickleback (*Pungitius pungitius*) and the alewife (*Alosa pseudoharengus*) were only found in the Kouchibouguacis River.

The cluster analysis for all sites of the Kouchibouguacis River did not reveal any groupings that could be clearly explained by the fish assemblages or any other environmental factor. However, clusters obtained from the analysis of the sites of the Kouchibouguac River are associated with the dominant fish species and river gradient (Fig. 8). In that river, salmonids dominated the downstream and some of the midreach sites, whereas the only cyprinid species, the blacknose dace, dominated the upstream sites and the other midreach sites. Clusters obtained from the analysis of the fish species and abundance of all the sites (Kouchibouguac River and Kouchibouguacis River) demonstrate clear groups associated with different fish assemblages (Fig. 9).

The Shannon-Weiner index for fish was significantly higher in the Kouchibouguac River (*t* test, p=0.020). However, when salmonids were withdrawn from the Shannon-Weiner index calculation, the Kouchibouguacis River had a significant higher fish diversity (Kruskal-Wallis, p=0.000).

Relationships between freshwater mussels and habitat

No significant correlation was found between freshwater mussel abundances and the habitat variables, with the exception of a negative correlation found between *Margaritifera margaritifera* abundance and length of riparian zone for the Kouchibouguac River (r_s=-0.381, p=0.035, n=31) and the Kouchibouguacis River (r_s=-0.442, p=0.01, n=33). However, when the small upstream sites of the stream were removed from the analysis, relations were weaker and not significant. Correlation between mussel densities and Simpson index of substrate diversity are weak and non-significant. Neither BIOENV, nor RELATE gave conclusive results.

Although a significant correlation could not be found between species abundances and habitat variables, patterns could be derived from the preference curves for the substratum type and distance from shore in the Kouchibouguacis River. Substrate and distance preference curves for the Kouchibouguacis system differ for the three species of mussel (Fig. 10, 11). *Alasmidonta varicosa* were mostly encountered in quadrats at an intermediate distance (between midstream and offshore), mainly on gravel bed. *Elliptio complanata* was mostly found in quadrats located near the shore on finer sediment. Distance from shore did not explain *M. margaritifera* abundance and distribution, but quadrats with substrate composed mostly of gravel and cobble seemed to show higher *M. margaritifera* densities. *Margaritifera margaritifera* density and distribution were not explained by habitat variables of the Kouchibouguac River.

Stepwise discriminant analysis for the Kouchibouguacis River revealed that five habitat variables (i.e., river magnitude, depth, velocity and mean percentages of cobble and large woody debris) predict best the four-*Margaritifera margaritifera* density groups. Results of the standardized coefficient are shown in Table 5 for each variable. The first discriminant function explained 72.4% of the total variation, and showed positive relationships between

M. margaritifera density and the five habitat variables; the second and third discriminant functions explained the remaining 27.6% of the variation. The Jackknifed classification matrix correctly reclassified 70% of the mussel density. Discriminant functions were not computed for the Kouchibouguac River because *M. margaritifera* abundance was similar through all sampling sites, thus no habitat variables could explain differences of density. However, no significant relations were obtained between *M. margaritifera* and these five variables when submitted to statistical analysis.

Relationships between freshwater mussels and fish assemblage

No significant correlation was found between mussel and fish abundance for the Kouchibouguac River. In contrast, significant correlations were detected between mussels and fish in the Kouchibouguacis River. In that river *Margaritifera margaritifera* was negatively correlated with brook trout (*Salvelinus fontinalis*) (r_s=-0.510, p=0.01) and ninespine stickleback (r_s=-0.350, p=0.05). *Elliptio complanata* was negatively correlated with the threespine stickleback (*Gasterosteus aculeatus*) (r_s=-0.337, p=0.05) and salmonid abundance (r_s=-0.398, p=0.01), while *Alasmidonta varicosa* was positively correlated with the threespine stickleback (r_s=0.328, p=0.05) and the alewife (r_s=0.458, p=0.01).

DISCUSSION

Mussel assemblage

The Kouchibouguac and Kouchibouguacis Rivers are similar in terms of drainage area, physical bed material and land use, yet they support different freshwater mussel communities. The scarcity of freshwater mussel studies in New Brunswick rivers does not allow comparisons with the freshwater mussel assemblage found in the Kouchibouguac region. Few studies have been conducted in the Atlantic Slope region and most of them are unpublished reports (Hanson & Locke 2001). Nevertheless, the freshwater mussel assemblage found in the Kouchibouguac River (393 km^2) and Kouchibouguacis River (370 km^2) appears to be representative of the region. Two rivers south of the present study area, the Chockpish River (126 km^2) and Cocagne River (387 km^2), were surveyed in 2003 and two mussel species were found: *Margaritifera margaritifera* and *Elliptio complanata* (Julien & Caissie, unpublished report). These two species were also found in the Kouchibouguacis River. Hanson & Locke (2001) extensively surveyed the Petitcodiac River (1360 km^2) and found five unionoid species. *Alasmidonta undulata*, the least abundant species in the Petitcodiac River, was the only species not found in the Kouchibouguacis River (Beaudet *et al.* 2002). In Morice Lake (New Brunswick), Septhon *et al.* (1980) reported the presence of three unionoid species (*Pyganodon cataracta*, *E. complanata*, and *Leptodea ochracea*) usually associated with lacustrine conditions. Studies conducted in the northern Atlantic Slope reported diversity ranging from five to 13 species (Wiles 1975; Smith 1982; Strayer 1993; Strayer *et al.* 1994; Nedeau *et al.* 2000). These southern drainage areas were much greater, and closer to the center of biodiversity for this faunal group (Nedeau *et al.* 2000), than those of the Kouchibouguac and Kouchibouguacis rivers and this may explain the higher diversity (Watters 1992). Nedeau *et al.* (2000) cited 16 species of freshwater mussels in the Northern Atlantic Slope, from Virginia to Newfoundland, but only 12 are usually found in New Brunswick (Metcalfe-Smith & Cudmore-Vokey 2004; Martel *et al.* in prep). Of these 12 species, *Alasmidonta heterodon* is known to be extirpated, *Lampsilis cariosa* and *Leptodea ochracea* seem to be constrained to interior land, and *Pyganodon fragilis* is thought to occur only in restricted parts of New Brunswick (Clarke 1981a; Nedeau *et al.* 2000; Martel *et al.* in prep.). We are not aware of any published report demonstrating the presence of *P. fragilis* in New Brunswick. Thus, seven species are likely to be found in coastal systems similar to the Kouchibouguac River and the Kouchibouguacis River. The low diversity of freshwater mussel in New Brunswick is representative of the low diversity encountered throughout New England and surrounding aquatic systems (Nedeau *et al.* 2000).

The absence of the alewife floater, *Anodonta implicata*, in the Kouchibouguacis River is unusual because this river is known to be a good spawning ground for its fish host, the alewife (*Alosa Pseudoharengus*) (Tremblay, pers. comm.). Moreover, the River systems sampled in this study seem to offer a suitable habitat for this mussel species, preferring low gradient coastal rivers (Strayer & Ralley 1991). *A. implicata* is widespread in nearly all the coastal watersheds of Maine (Nedeau *et al.* 2000) and in many parts of New Brunswick (Martel *et al.* in prep.) and Nova Scotia (Athearn 1961; Athearn & Clarke 1961). Only two alewife were collected during this study, because of inappropriate timing of sample, weather conditions and sampling gear.

In general, for both rivers, mussel densities and percentages of presence were low, with the exception of *Margaritifera margaritifera*. Because of a lack of historical data, it is impossible to know if the distribution and abundance of these three mussels in the two rivers are representative of such small systems for the areas, or if their range and abundance has declined. In the study of Nedeau *et al.* (2000) in Maine's watersheds, shells or live *A. varicosa* were found in approximately 6.3% of all the sites sampled, whereas *E. complanata* occurred at almost 58% of the sites. The percentage of presence of *M. margaritifera* was higher in our systems (79% of the sites) compared to the results of Nedeau *et al.* (2000) (only 14% of the sites). In the Petitcodiac River, NB, Hanson & Locke (2001) found *M. margaritifera* in 62.1% of their sites and obtained values of 34.8% and 21.2% for *E. complanata* and *A. varicosa*, respectively.

Mussels along the river gradient (upstream-downstream gradient) were characterized by distinctive assemblages in the Kouchibouguacis River. One must be careful when interpreting these patterns since the densities of most of the mussel species were low. Results of a preliminary survey (Beaudet *et al.* 2002) also indicated low *Elliptio complanata* and *Alasmidonta varicosa* densities, although the sampling design used (semi-quantitative timed search) allowed for more frequent encounters of mussel individuals across the Kouchibouguacis drainage. The effectiveness of sampling design and the power of the subsequent analysis were often discussed in freshwater mussel studies (Downing & Downing 1992; Miller & Payne 1993; Hornbach & Deneka 1996; Obermeyer 1997; Strayer *et al.* 1997; Vaungh *et al.* 1997; Strayer 1999; Metcalfe-Smith *et al.* 2000). Since freshwater mussels usually follow contagious distributions like other benthic invertebrates (see Downing & Downing 1992), special attention must be given to the choice of the sampling design (Downing & Downing 1992). Zale & Neves (1982) pointed out that their quadrat sampling was not as efficient as their qualitative sampling since several individuals of a rare species were found in qualitative surveys and none were found in the quadrat sampling. In addition, the distributions of unionoid species of the Kouchibouguac River and Kouchibouguacis River were contagious (Table 2), thus the sampling design might have been less successful at detecting low mussel abundances. However, with the objective of this study, a quantitative sampling design was necessary. In order to achieve higher mussel abundance, a combination of semi-quantitative and quantitative sampling methods, as well as greater sampling effort and site size, might have been more efficient.

Juveniles (individuals< 50 mm) of the *Margaritifera margaritifera*, *Alasmidonta varicosa* and *Elliptio complanata* were found in the Kouchibouguacis River, meaning that recruitment is taking place in this river system. Juveniles of *M. margaritifera* were commonly observed within the Kouchibouguac River, indicating recruitment in this river as well.

Mussel relationships with habitat variables

Except for *Margaritifera margaritifera*, freshwater mussels in the Kouchibouguacis River occurred at low densities. Low encounter rates and low abundance reduce the power to detect relationships between unionoid and habitat variables. However, even when higher diversities and densities were found in other rivers, only few investigators were successful in finding significant relationships among mussel distribution, mussel abundance and habitat variables (Holland-Bartels 1990; Strayer *et al.* 1994; Balfour & Smock 1995; Strayer 1999; Brim Box & Mossa 1999).

Alasmidonta varicosa seems to prefer intermediate distance from the shore as well as gravel substrate in the Kouchibouguacis River. This substrate preference corroborates with observations from previous studies (Ortmann 1919 *in* Clarke 1981b; Athearn & Clarke 1961; Clayton *et al.* 2001). The low density of *A. varicosa* in the Kouchibouguacis River is difficult to explain because of the availability of suitable habitat, including flowing water and gravely substrate. Strayer & Ralley (1993) could not find a consistent substrate preference for *A. varicosa*, although it is thought to prefer coarse sandy and gravely bottoms. This type of habitat is available both in the Kouchibouguac River and the Kouchibouguacis River. One has to be careful, however, when interpreting or generalizing on substrate preference of freshwater mussel. Identification and definition of substrate classes may vary among studies. Since *A. varicosa* was only found in the downstream area of the Kouchibouguacis River, this may suggest that some unmeasured habitat variables (representative of downstream reaches) may be responsible for the distribution of this unionoid. The distribution of *A. varicosa* might also be explained by an unsuspected fish host (explanation below). Any information explaining the presence of *A. varicosa* downstream of the Kouchibouguacis River would be valuable since reaches downstream tend to be more impacted by cumulative anthropogenic stressors. Indeed, downstream reaches are the most populated and utilised areas of these two rivers. Along the Kouchibouguacis River is a suit of cottages (most have no riparian zone and no proper sewage systems), agricultural fields, All-terrain-vehicle crossing in the river, and continuous development of infrastructure. This could be serious threat to a scarce, aggregated freshwater mussel population.

Elliptio complanata is known to use a wide variety of habitat, ranging from clay to cobble bottoms of different river widths (Matteson 1948; Harman 1972; Nedeau *et al.* 2000). In river systems, it is found usually in slow waters on fine particle substrate (Matteson 1948), which was the case in this study even if this type of habitat was not frequently encountered. *E. complanata* was associated with finer sediments, especially silty substrate close to shore in this study.

No macrohabitat variable successfully explained the presence and abundance of *Margaritifera margaritifera*. Nevertheless, *M. margaritifera* seemed to prefer gravel and cobble substrate as observed by Nedeau *et al.* (2000).

As for previous studies, usual microhabitat variables were not useful in predicting distribution and abundance of freshwater mussels (Tevesz & McCall 1979; Strayer 1981; Holland-Bartels 1990; Strayer & Ralley 1993, Strayer *et al.* 1994, Brim Box *et al.* 2002). In previous studies, even when significant relations were found between unionoids and habitat, the reliability of these relations was criticized because of low predictive power (Neves & Widlack 1987; Balfour & Smock 1995; Layzer & Madison 1995; Strayer 1999; Arbuckle & Downing 2002). Macrohabitat variables were viewed as promising variables in the quest of explaining freshwater mussel distribution, abundance and diversity (Strayer & Ralley 1993; Strayer *et al.* 1994; Morris & Corkum 1996; Strayer 1999). However, these macrohabitat variables appear more important when considering freshwater mussel abundance and diversity in large rivers (Holland-Bartels 1990; Strayer 1993; Strayer *et al.* 1994; Di Maio & Corkum 1995; Morris & Corkum 1996). Several investigators who were successful in predicting distribution and abundance of freshwater mussel with macro- and microhabitat variables did not always report the power of their analysis (Salmon & Green 1983; Di Maio & Corkum 1995; Hastie *et al.* 2000, Brim Box *et al.* 2002). Johnson & Brown (2000) mentioned that small systems do not show enough heterogeneity to measure efficient macrohabitat predictors, and this may be one of the reasons why we were not able to obtain correlations between freshwater mussel populations and macrohabitat variables. Our results also corroborate those of Strayer (1993) who concluded that unmeasured habitat variables might be more efficient in explaining distribution patterns of freshwater mussels. In our case, it would be interesting to monitor, in the future, substrate stability, sedimentation, and several water chemistry variables, notably water temperatures, nitrate levels, total phosphorus, alkalinity, Calcium content, turbidity, dissolve oxygen that are known to be important determinants of benthic community structure (Matteson 1948; Harman 1972; Strayer 1993). A stratification of the two rivers would also provide a valuable tool for future studies.

Fish assemblage

The fish composition is different between the Kouchibouguac and the Kouchibouguacis rivers (Table 4). Even though no historical or actual temperature data are available in either of the river for comparison, it seems that the Kouchibouguac River is dominated by cold water fishes (i.e. atlantic salmon, sea lamprey, threespine stickleback, brook trout, and slimy sculpin) and the Kouchibouguacis is dominated by cool water species (Wehrly *et al.* 1998). Indeed, 73% of the 1564 fishes collected from the Kouchibouguac River were cold water species as compared with only 33% in the Kouchibouguacis River. Lack of published historical fish inventories makes it impossible to know if these assemblages were always different as they are today. But this may very well explain the presence of different freshwater mussel species in the two rivers.

The dominance of cyprinids in the Kouchibouguacis River is interesting. Cyprinids are host to several species of freshwater mussels and since the Kouchibouguacis is also supporting a higher diversity of freshwater mussels, it seems fair to assume that the cyprinids may play a role in the freshwater mussels diversity. We also suspect that the dam, contructed in 1917 in the Kouchibouguac River, is a factor that might have affected the fish composition in the past. Again, lack of historical data does not allow us to obtain information on the fish fauna of the Kouchibouguac River prior to the dam construction. A breach was created in the late 30's allowing migration of the anadromous salmonids. Disturbances, like a dam as small as one meter high, are known to severely impact unionoid fauna as well as populations of their fish hosts (Fuller 1974; Bogan 1993; Williams *et al.* 1993; Di Maio & Corkum 1995; Layzer & Madison 1995; Watters 1996; Richter *et al.* 1997; McMurray et al. 1999; Vaughn & Taylor 1999; Khym & Layzer 2000; Hanson & Locke 2000). However, if the salmonid populations have recovered since the creation of the breach (nearly 65 years ago), one would think that other species would recover as well. This again suggests that baseline data is essential when trying to understand or detect changes in actual aquatic communities.

Mussel and fish assemblages

Several studies have been conducted in order to define fish hosts for one or several mussel species by studying parasitism (Wiles 1975; Neves & Widlack 1987; Yeager & Saylor 1995; Weiss & Layzer 1995; Keller & Ruessler 1997; Haag *et al.* 1999; Araujo *et al.* 2000; see Hoggarth 1992 for review). However, few of them have explored such relationship with fish pattern analysis (Smith 1985; Watters 1992; Layzer & Madison 1995; Haag & Warren 1998). In this study, I did not find any reliable correlations between the abundance of fishes and freshwater mussels collected in the sampling sites.

Numerous authors have shown that salmonids are hosts for *Margaritifera margaritifera* (Smith 1976; Fuller 1974; Futish & Millmann 1978; Cunjak & McGladdery 1991, Hoggarth 1992). Brook trout and Atlantic salmon have been identified as host for *M. Margaritifera* in several cases (Smith 1976; Cunjak & McGladdery 1991). In this study, these two species were considered hosts for *M. Margaritifera*. The Kouchibouguac River had more than 12 times the number of salmonids sampled in the Kouchibouguacis River. Furthermore, the Kouchibouguac River had more than three times the number of *M. margaritifera* sampled in the Kouchibouguacis River. Since *M. margaritifera* abundance was not explained by habitat variable, it is suspected that the abundance of the *M. margaritifera* population in each river is related to the abundance of its fish host population. Unexpectedly, these relationships were not confirmed statistically. The negative correlation obtained between brook trout and *M. margaritifera* in the Kouchibouguacis River cannot be accounted for. Layzer & Madison (1995) also failed at finding relationships between suitable hosts and the 17 freshwater mussel species in the upper Cumberland River drainage in Kentucky. They concluded that caution was advised when comparing contemporary host fish abundances with unionoid abundance that is based on previous recruitment. Moreover, comparing highly motile organisms such as fishes with movement-limited freshwater mussels is difficult. These comparative analyses do not take into account differences in spatial movement and distribution between the two groups. Sampling of fish includes transient individual that seasonally migrate, spawn in selected areas, and abandoned modified reaches, while these aspects are not present when sampling unionoid population (Watters 1992).

Abundance and distribution patterns of *Elliptio complanata* and *Alasmidonta varicosa* could not be properly explained because their numbers were too low. Several potential host-parasite relationships were discovered for *E. complanata* in the Kouchibouguacis River (see Chapter 2). Among the five fish species involved in these relationships, the creek chub (*Semotilus atromaculatus*) and lake chub (*Couesius plumbeus*) were not encountered in the Kouchibouguac River, where *E. complanata* is absent. Even though the fivespine stickleback (*Culaea inconstans*) was not reported during the present study, it appears to be present in previous surveys (Beaudet *et al.* 2002; Leblanc, unpublished data). These fish species might thus play a key role in the reproductive processes of *E. complanata* in the Kouchibouguacis River.

Eventhough several "likely" hosts have been identified for *Alasmidonta varicosa* (Nedeau et al. 2000; Wicklow, pers. comm.), no fish hosts have officially been confirmed for this freshwater mussel. However, one glochidia of *A. varicosa* was found attached to the fin of a ninespine stickleback in the Kouchibouguacis River (Chapter 2). The distribution of the ninespine stickleback also corroborates with that of *A. varicosa* in the downstream sampling sites. Once again, the ninespine stickleback was not found in the Kouchibouguac River. The distribution pattern of this mussel (occurrence downstream in a coastal river) as shown in the present study as well as in previous surveys (Nedeau *et al.* 2000; Hanson & Locke 2001), may suggest the role of another fish host, which is suspected to be anadromous (Hanson & Locke 2001). In the present study, the distribution of the threespine stickleback and the alewife, an anadromous fish, also overlap the distribution of *A. varicosa*. The alewife is known to spawn in the downstream portion of the Kouchibouguacis River at approximately the same period of *A. varicosa* glochidia release. Both the threespine stickleback and the alewife represent potential host for *A. varicosa*.

Our results can't support the hypothesis that fish populations are mostly influencing the diversity, abundance and distribution of the freshwater mussels in the Kouchibouguac River and Kouchibouguacis River. Further research is needed with different habitat variables and with new focus on host fish and mussel relationships in order to understand the recruitment and distribution of freshwater mussel populations in coastal New Brunswick. This type of information is important for the conservation of freshwater mussels including their protection, relocation, restoration or any other management decisions aimed at ensuring the integrity of this declining natural resource.

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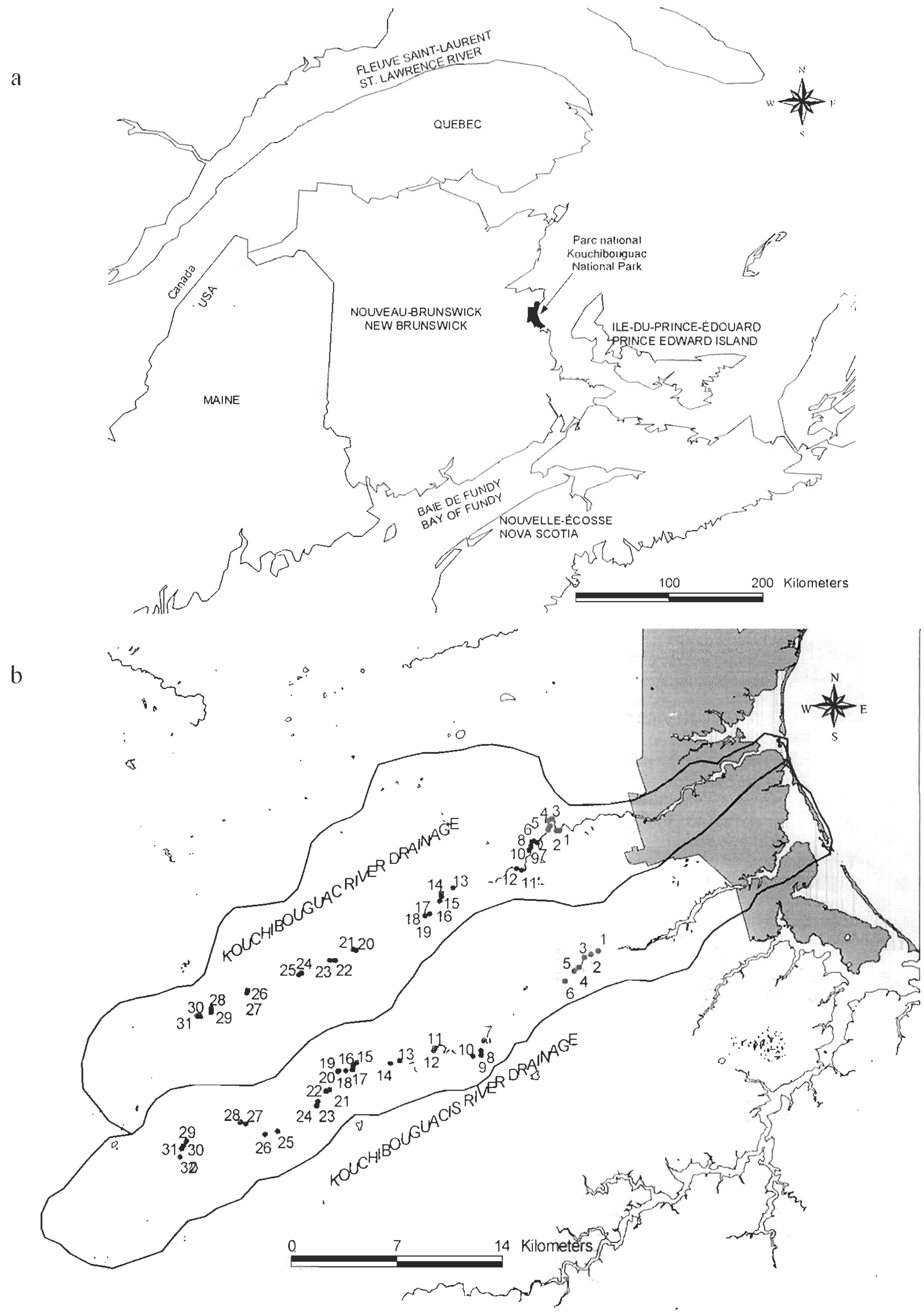

FIGURE 2: Study area. Kouchibouguac National Park (a) and the Kouchibouguac and Kouchibouguacis river watersheds (b), eastern Nouveau-Brunswick, Canada. A total of 65 sites were sampled (black circles): 33 sites in the Kouchibouguacis River and 32 sites in the Kouchibouguac River. Sites were quantitatively surveyed for freshwater mussel and fish population in 2002. Full lines represent the watershed boundaries, and the gray polygon represents Kouchibouguac National Park's boundaries.

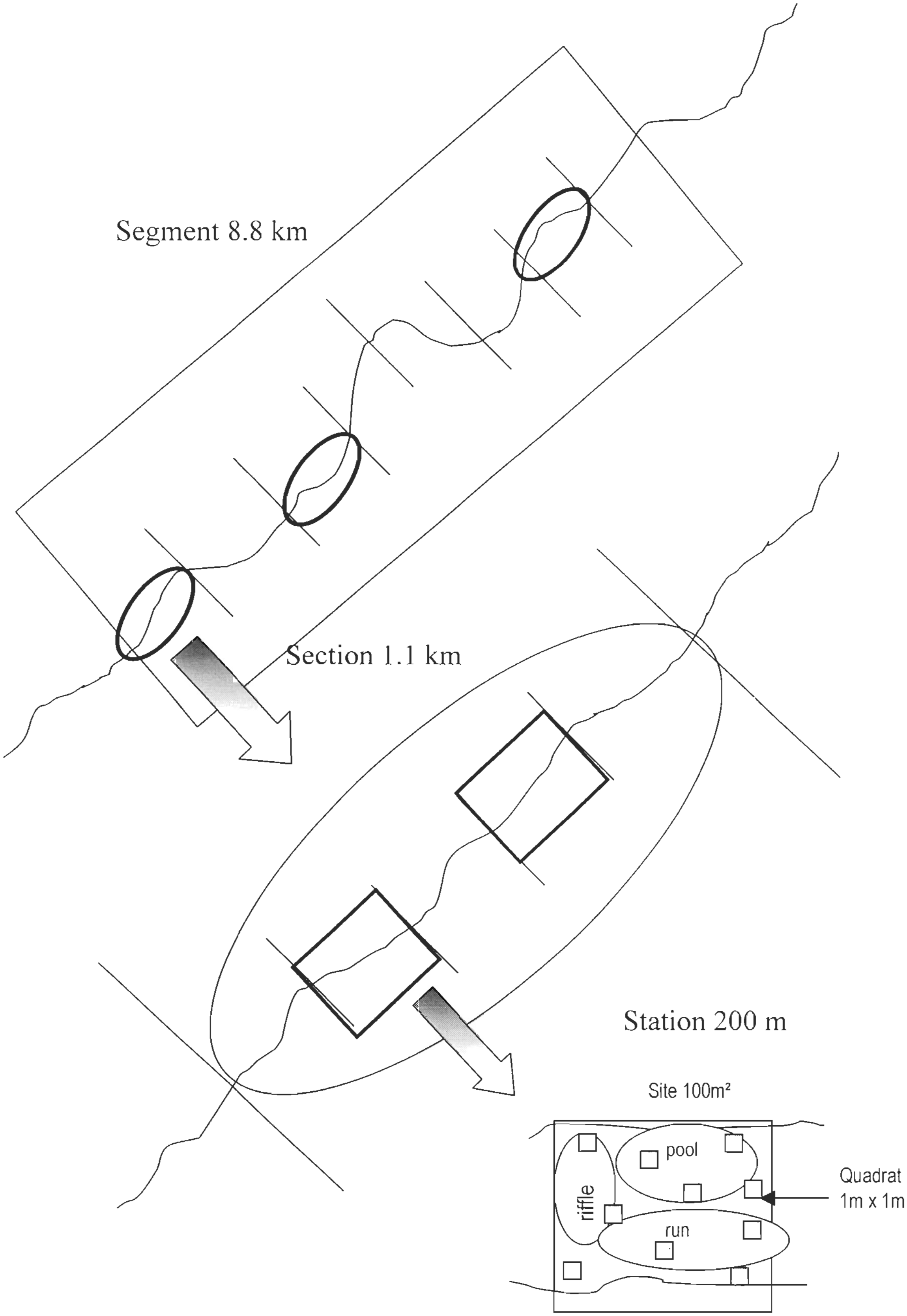

FIGURE 3: Hierarchical/nested sampling design involved random selections of sections, stations, sites, and quadrats within segments of equal length. A total of 32 and 33 sampling sites were randomly selected in the Kouchibouguac and the Kouchibouguacis River, respectively in 2002.

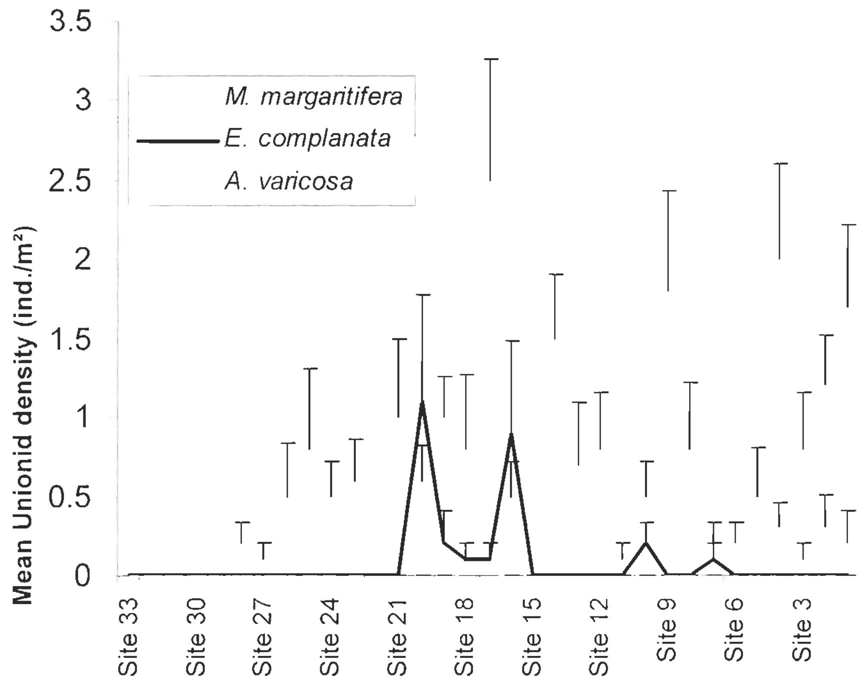

FIGURE 4: Densities (mean ± 1 SE) of the three unionoid species (*Margaritifera margaritifera*, *Elliptio complanata*, and *Alasmidonta varicosa*) encountered in the 33 sites of the Kouchibouguacis River in 2002. Each data point represents the mean freshwater mussel density (10 quadrats) for each species at each site. The x-axis represents the sites (see fig.1) from a right to left, downstream to upstream gradient.

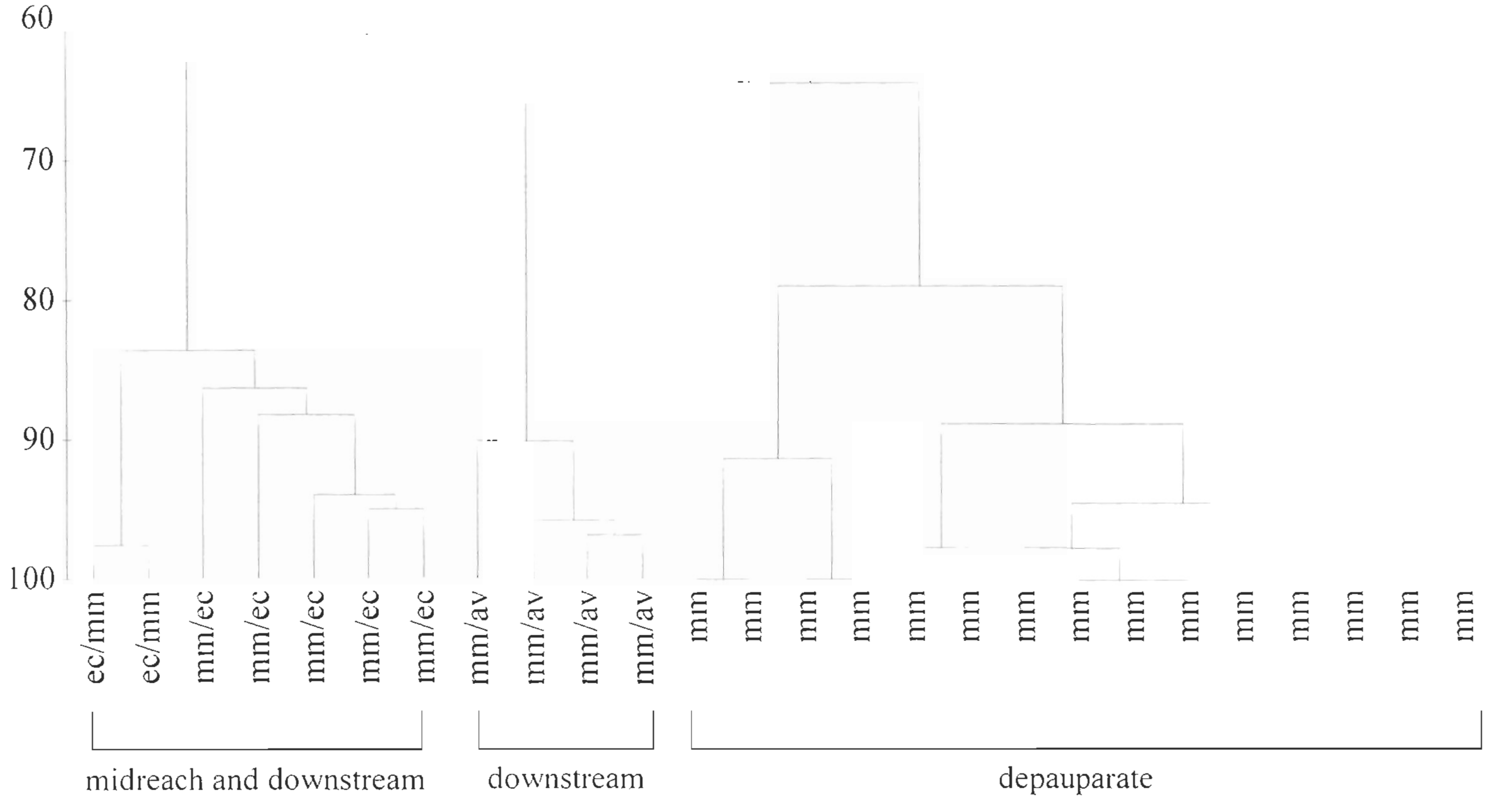

FIGURE 5: Cluster of the unionoid abundance data of the 33 quantitative sites of the Kouchibouguacis River in which at least one individual was found. A **Bray-Curtis similarity index** was used and the cluster mode selected was group average. The assemblages are composed of *Margaritifera margaritifera* [mm], *Elliptio complanata* [ec], and *Alasmidonta varicosa* [av]. The clusters correspond to freshwater mussel assemblages and could be associated with river gradient zones.

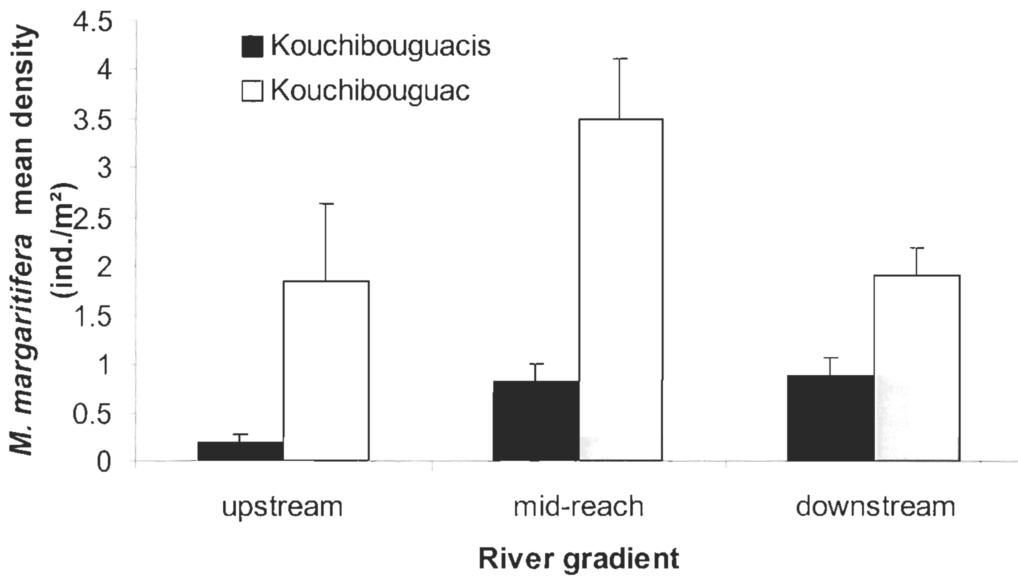

FIGURE 6: Density (mean ± 1 SE) of *Margaritifera margaritifera* (ind./m^2) sampled in the three major gradient zones of the Kouchibouguacis and Kouchibouguac rivers in 2002. Bars represent the mean unionoid density for the three gradient zones calculated from the abundance of *M. margaritifera* found at sites within each gradient zone: the upstream (9 sites), the mid-reach (12 sites), and downstream zone (12 sites).

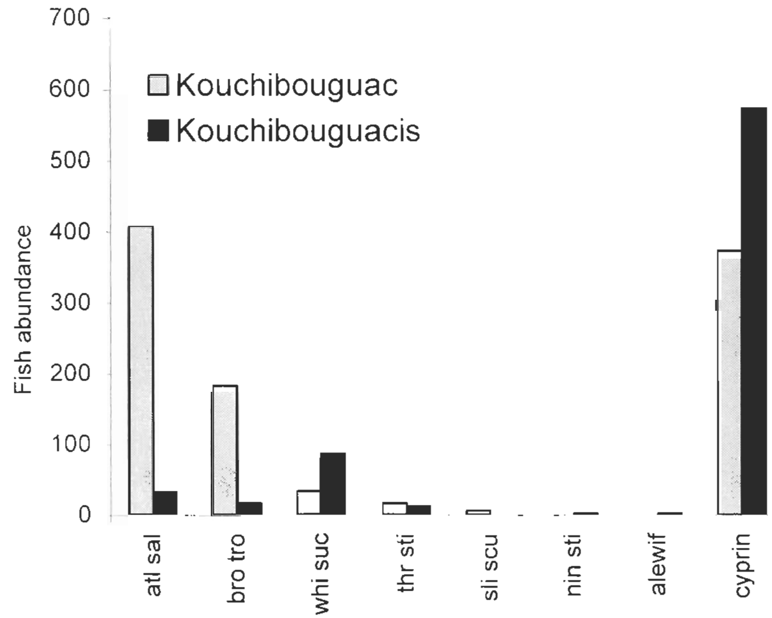

FIGURE 7: Fish abundance in the Kouchibouguac River and Kouchibouguacis River during electrofishing sampling in 2002. The bars represent the fish abundance for eight species found in each river. Each bar represents the total number of fish collected during 64 electrofishing samples ranging from 16 to 70 minutes each and covering 100 m². Fish species are: Atlantic salmon (*Salmo salar*) [atl sal], brook trout (*Salvelinus fontinalis*) [bro tro], white sucker (*Catostomus commersoni*) [whi suc], threespine stickleback (*Gasterosteus aculeatus*) [thr sti], ninespine stickleback (*Pungitius pungitius*) [nin sti], alewife (*Alosa pseudoharengus*) [alewif], and undifferentiated cyprinids (cyprinid) [cyprin]

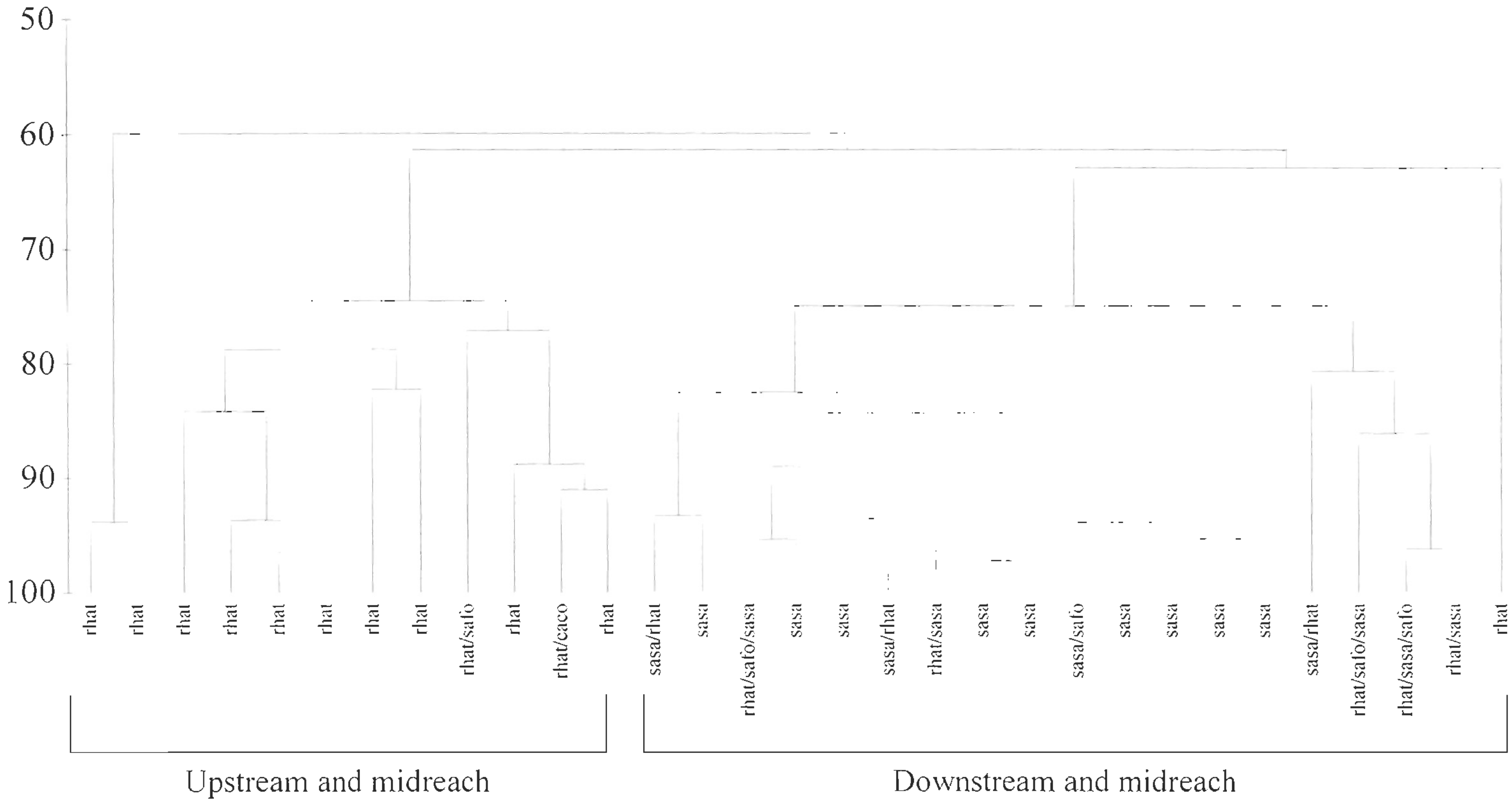

FIGURE 8: Cluster analysis of the fish abundance data of the 31 sites where electrical fishing was performed in the Kouchibouguac River in 2002. Fish abundance data were fourth root transformed. A Bray-Curtis similarity index was used and the cluster mode used was group average. Dominant fish species appearing in the cluster analysis were: Atlantic salmon (*Salmo salar* [sasa]), brook trout (*Salvelinus fontinalis* [safo]), and blacknose dace (*Rhinichtys atratulus* [rhat]). The clusters correspond to the assemblage of dominant fish species

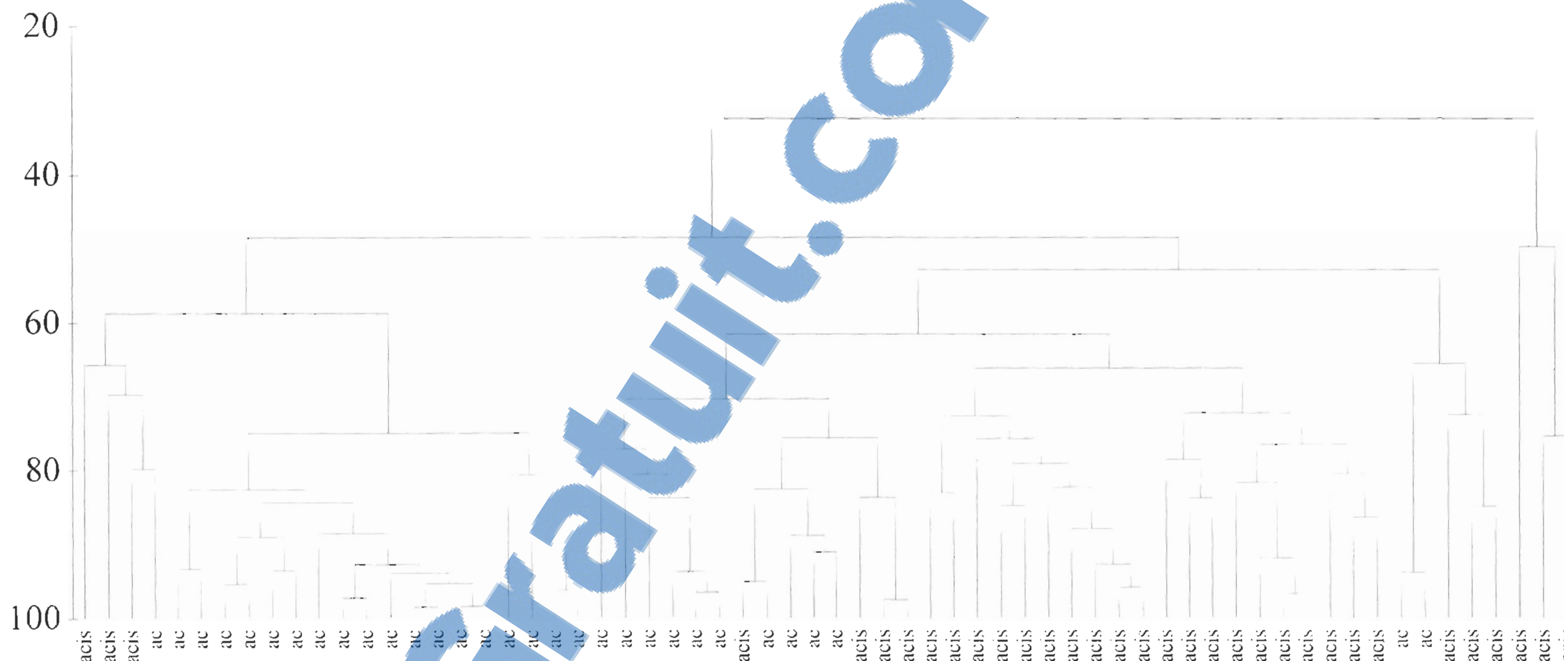

FIGURE 9: Cluster of the fish abundance data of the 64 sites where electrical fishing was performed in the Kouchibouguac [ac] and Kouchibouguacis [acis] rivers in 2002. Fish abundance data were fourth root transformed. **A Bray-Curtis similarity index** was used and the cluster mode used was group average. The clusters correspond to the assemblage of fish species within both rivers.

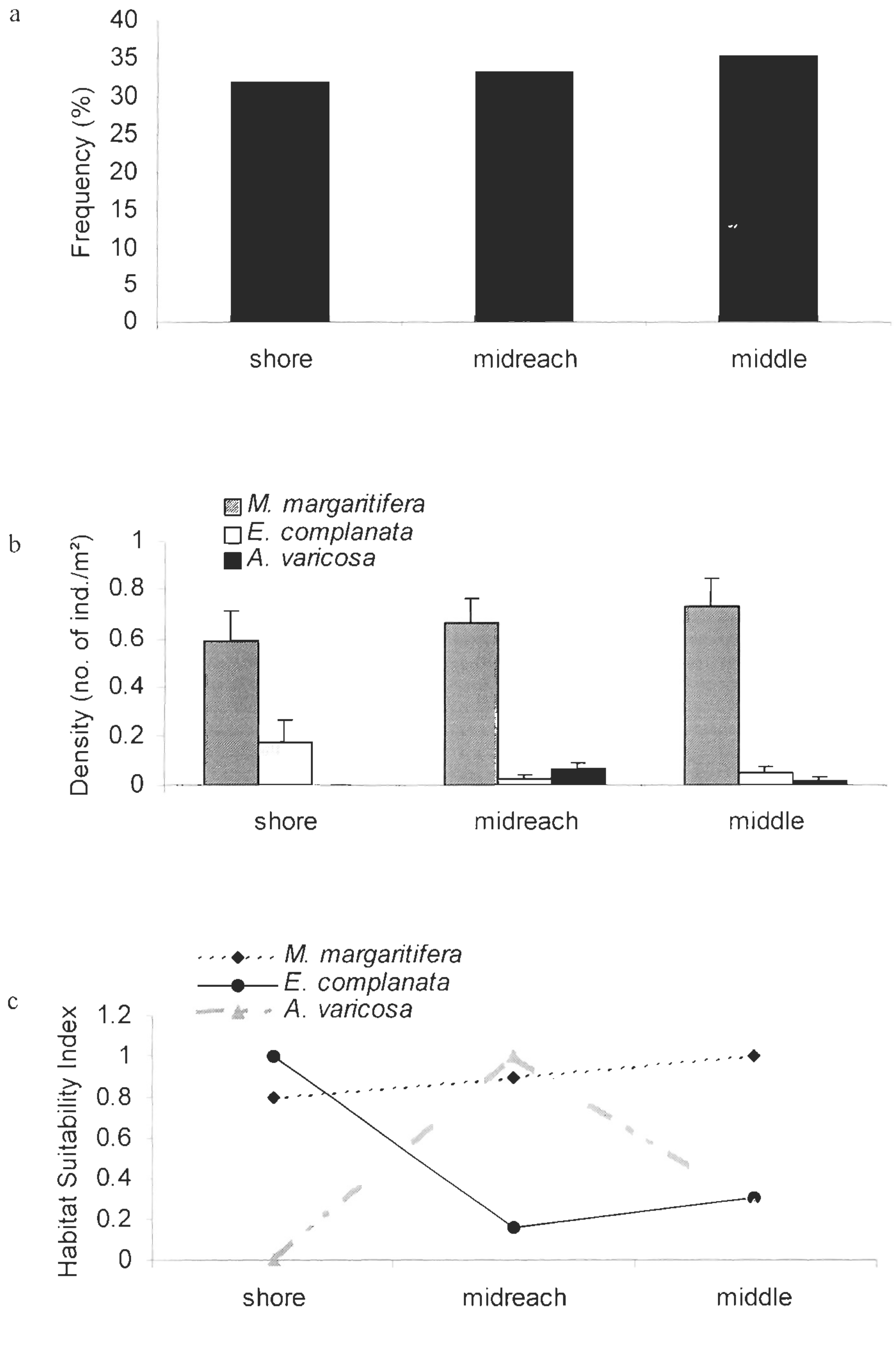

FIGURE 10: Preference analysis: (a) frequency of quadrats found in the three "distance from shore" categories, (b) density (number of unionoid individuals per m²) (mean ± 1 SE) for each unionoid species for the three categories of distance from shore and (c) preference of each unionoid species for the different distances from the shore.

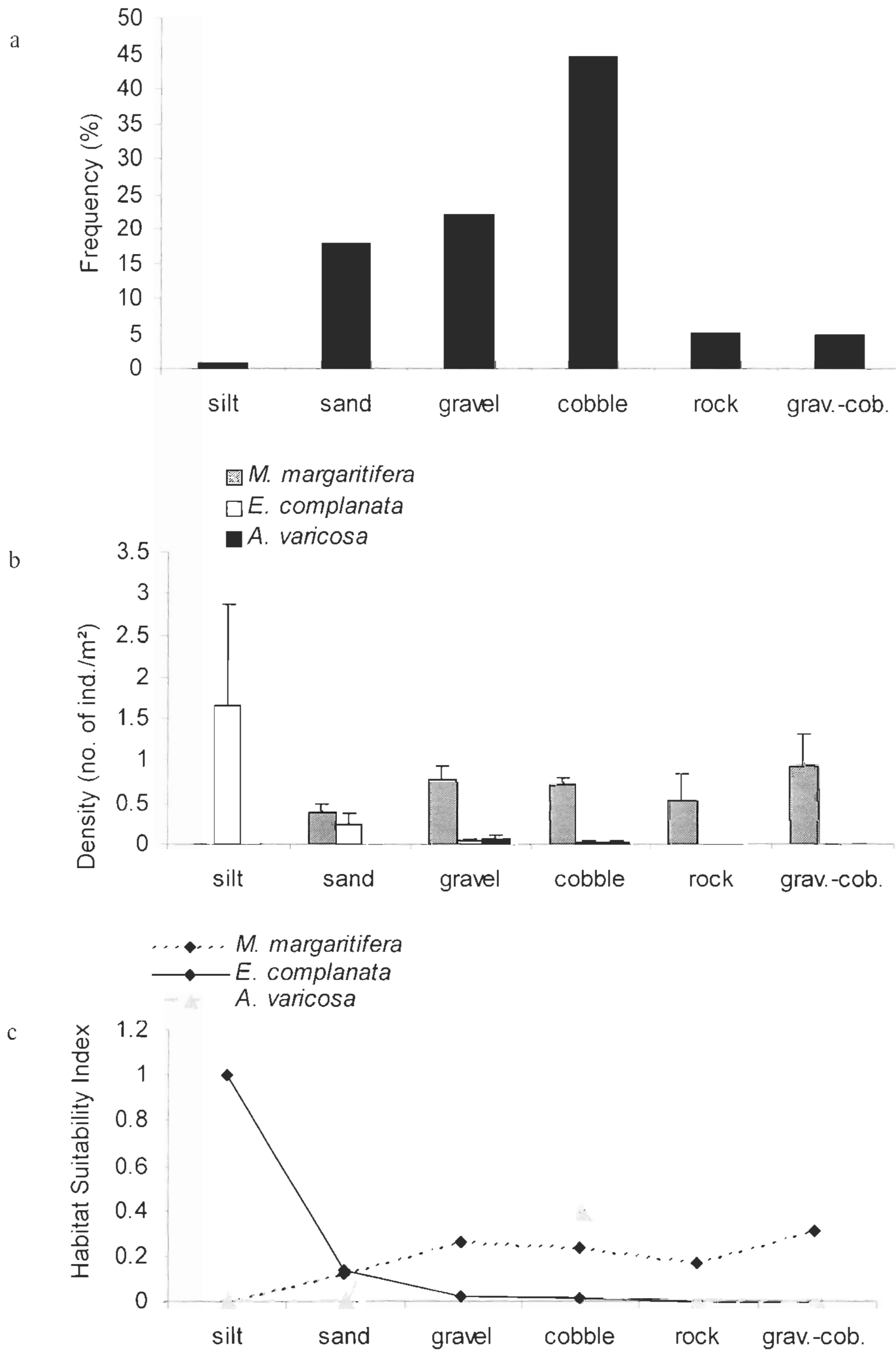

FIGURE 11: Preference analysis: (a) frequency of quadrats found in the six dominant substrate types, (b) density (number of unionoid individuals per m²) (mean ± 1 SE) for each unionoid species for the six dominant substrate types and (c) preference of each unionoid species for the different substrate types observed in the Kouchibouguacis River, 2002. One of the dominant substrate category represents a mixture of gravel and cobble [grav.-cob.].

TABLE 1: Species and number of freshwater mussels encountered in the Kouchibouguac and Kouchibouguacis River during the 2002 sampling season.

Kouchibouguac River		Kouchibouguacis River	
2002 quadrat sampling, n=320		2002 quadrat sampling, n=330	
species	no. of ind.	species	no. of ind.
M. margaritifera	795	*M. margaritifera*	219
		E. complanata	27
		A. varicosa	9
		P. cataracta	0

TABLE 2: Results of the statistical tests performed on the dispersal of the three freshwater mussel species in the Kouchibouguac River and the Kouchibouguacis River (*Margaritifera margaritifera* [mm], *Elliptio complanata* [ec], *Alasmidonta varicosa* [av]). Tests were performed at two levels: the stations and the quadrats. A χ^2 test was used to test agreement with a Poisson (random distribution) series, and verified with a χ^2 goodness-of-fit test.

River	Spatial scale	sp.	n	Density *	σ^2	Dispersal index (I)[a]	χ^2	D[b]	χ^2 goodness-of-fit (ν)	χ^2 goodness-of-fit test
Kouchibouguac	station	mm	32	24.84	368.20	14.82	459.44	22.50	189.40 (7)	p<0.001
	quadrat	mm	320	2.48	12.01	4.84	1542.41	30.30	1063.81 (7)	p<0.001
Kouchibouguacis	station	mm	33	6.64	41.68	6.280	200.96	12.11	104.92 (4)	p<0.001
	station	av	33	0.27	0.64	2.35	75.33	4.337	-[c]	-
	station	ec	33	0.82	5.96	7.29	233.33	13.66	15.81 (1)	p<0.001
	station	all	33	7.73	52.52	6.80	217.48	12.92	92.42 (6)	p<0.001
	quadrat	mm	330	0.66	1.44	2.17	713.74	12.54	187.73 (6)	p<0.001
	quadrat	av	330	0.027	0.04	1.42	467.67	4.95	-[c]	-
	quadrat	ec	330	0.082	0.30	3.67	1207.44	23.51	19.41 (1)	p<0.001
	quadrat	all	330	0.77	1.77	2.30	755.71	13.24	150.89 (3)	p<0.001

[a] variance/mean (Elliot, 1977)
[b] normal variable used when large sample (n>31; Elliot 1977)
[c] goodness-of-fit test not performed because insufficient density classes
* number of individual per station or quadrat

TABLE 3: Micro- and macrohabitat conditions encountered in sampling quadrat in the Kouchibouguac and Kouchibouguacis rivers.

	Variables	Mean (range) Kouchibouguac	Kouchibouguacis
microhabitat	depth (cm)	33 (5-90)	32.5 (20-46)
	% silt (0.004-0.062 mm)	0.2 (0-50)	1.1 (0-95)
	% sand (0.062-2 mm)	19.9 (0-100)	22.8 (0-100)
	% gravel (2-64 mm)	38.2 (0-100)	26.2 (0-100)
	% cobble (64-256 mm)	33.9 (0-100)	42.6 (0-100)
	% rock (>256 mm)	7.5 (0-95)	7.1 (0-100)
	% macrophytes	3.8 (0-90)	11.8 (0-95)
	% large woody debris	5.6 (0-80)	3.8 (0-75)
macrohabitat	pH	7.2 (6.5-8.7)	7.0 (6.0-7.8)
	conductivity (μS)	70.0 (37.7-183)	60.0 (28.3-191.6)
	river width (m)	15.1 (6-24)	16.5 (8-38)
	current speed (m/s)	0.33 (0.98-0.14)	0.23 (1.03-0.05)
	riparian zone (m)	134.1 (0-200+)	134.7 (21-200+)
	% forest	60.1 (14.8-95.1)	52.0 (21.9-88.2)
	% agriculture	4.1 (0-28.7)	5.4 (0-45.9)
	% wetland	5.9 (0.4-23.5)	8.9 (2.9-17.1)
	% disturbed forest	27.6 (0-71.8)	27.7 (0-56.4)
	% human	1.2 (0-5.4)	4.8 (0-36.2)

TABLE 4: Species and number of fish collected at each station using electrofishing in the Kouchibouguac River and Kouchibouguacis River, during 2002.

Kouchibouguac River (n=31) (no. of ind. collected)	Kouchibouguacis River (n=33) (no. of ind. collected)
Sea lamprey - *Petromyzon marinus* (535)	Blacknose dace – *Rhinichthys atratulus* (436)
Atlantic salmon - *Salmo salar* (408)	Sea lamprey – *Petromyzon marinus* (259)
Blacknose dace - *Rhinichthys atratulus* (373)	Common shiner – *Luxilus cornutus* (106)
Brook trout - *Salvelinus fontinalis* (182)	White sucker – *Catostomus commersoni* (86)
White sucker - *Catostomus commersoni* (33)	Atlantic salmon - *Salmo salar* (32)
Threespine stickleback – *Gasterosteus aculeatus* (16)	Golden shiner – *Notemigonus crysoleucas* (31)
American eel - *Anguilla rostrata* (11)	Brook trout - *Salvelinus fontinalis* (16)
Slimy sculpin - *Cottus cognatus* (6)	American eel - *Anguilla rostrata* (15)
	Threespine stickleback - *Gasterosteus aculeatus* (12)
	Lake chub - *Couesius plumbeus* (5)
	Ninespine stickleback - *Pungitius pungitius* (2)
	Alewife - *Alosa pseudoharengus* (2)

TABLE 5: Relationship between density classes of *Margaritifera margaritifera* and habitat variables were tested with a Stepwise Discriminant Analysis (p=0.15 to enter or remove variable) for the Kouchibouguacis River.

		Standardized coefficients for the Kouchibouguacis River						
	Proportion of variation	Square canonical correlation	Eigenvalue	River magnitude	Depth	Velocity	Mean % cobble	Mean % large woody debris
DF1	72.4%	0.773	3.403	1.539	1.005	0.338	0.946	0.899
DF2	18.5%	0.465	0.868	0.085	0.377	1.219	0.060	0.158
DF3	9.1%	0.299	0.427	0.092	-0.013	0.288	-0.566	0.779

Wilk's λ= 0.085; F= 5.075 ; p< 0.0001

CHAPTER II: OCCURRENCE OF *ELLIPTIO COMPLANATA*, *PYGANODON CATARACTA* AND *ALASMIDONTA VARICOSA* GLOCHIDIA ON FISHES OF THE KOUCHIBOUGUACIS RIVER, COASTAL NEW BRUNSWICK, CANADA

ABSTRACT

Aspects of the reproductive biology and recruitment of three species of freshwater mussels were studied from May to August 2003 in the Kouchibouguacis River, coastal New Brunswick. Suitable fish hosts were identified for three unionoids: *Elliptio complanata*, the eastern elliptio; *Pyganodon cataracta*, the eastern floater; and *Alasmidonta varicosa*, the brook floater. A total of 209 fish belonging to seven species were examined from a site with high number of *E. complanata*. Larvae of *E. complanata* were found on seven individuals of five species of fish: lake chub (*Couesius plumbeus*), creek chub (*Semotilus atromaculatus*), blacknose dace (*Rhinichtys atratulus*), white sucker (*Catostomus commersoni*) and fivespine stickleback (*Culaea inconstans*). Most of these species are new fish host records. A total of 961 fish belonging to 12 species were also examined for the presence of *P. cataracta* and *A. varicosa* glochidia. One glochidia of *A. varicosa* was found attached to the pectoral fin of a ninespine stickleback. This represents the first mention of a glochidia of *A. varicosa* on a host fish in Canadian waters. Discovering new potential host(s) could be valuable information since the *A. varicosa* is listed as a candidate species on the Committee on the status of endangered wildlife in Canada (COSEWIC) list. The ninespine stickleback seems to also play an important role in the reproduction and recruitment of *P. cataracta* by showing the highest proportion of attached glochidia. One glochidia of *P. cataracta,* still attached to the fin of a ninespine stickleback, was fully transformed into a juvenile. This is the first confirmed report that the ninespine stickleback is a host fish for *P. cataracta*. The other *P. cataracta* glochidia were found on blacknose dace (*Rhinichtys atratulus*), creek chub (*Semotilus atromaculatus*), threespine stickleback (*Gasterosteus aculeatus*) and common shiner (*Luxilus cornutus*). Both fish gills and fins were parasitized by the glochidia of the three unionoid species. The percentage of the population infested and the intensity of glochidia attachment were both relatively low in the Kouchibouguacis River.

Key words: Freshwater mussels, Unionoidae, *Elliptio complanata*, *Alasmidonta varicosa*, *Pyganodon cataracta*, reproduction, recruitment, fish host, parasitism, glochidia, mussel-host relationship

INTRODUCTION

The reproductive biology of freshwater mussels is complex and many of its aspects are still not well understood. The larvae of freshwater mussels must attach to fish in order to complete its development and metamorphose into a juvenile mussel. Attachment to fish also allows mussels to disperse within the watershed. The need for fishes as hosts thus must be considered in investigations on reproduction, recruitment, and distribution of these freshwater bivalves. Although fish hosts play an important role in the life cycle of freshwater mussels, only few of these mussel-fish relationships are known (Kat 1984; Hoggarth 1992). Studies aimed at investigating the mussel-fish host relationships are increasingly important as they provide valuable data for the conservation and the management of freshwater mussel species, a major component of benthic communities in lake and river systems. Although recent studies have focused on new records of mussel-fish relationships (Weiss & Layzer 1995; Yeager & Saylor 1995; Keller & Ruessler 1997; Roe *et al.* 1997; Haag *et al.* 1999; McMurray *et al.* 1999; Watters & O'Dee 1999; Khym & Layzer 2000), biologists know only a small percentage of the fish hosts for the North American unionoid species and subspecies (Hoggarth 1992; Watters 1994).

Investigations on the freshwater mussel-fish host relationships can either be conducted in the field or in the laboratory. The information obtained by either method may be incomplete (Hoggarth 1992; Khym & Layzer 2000). Indeed, infested fish captured in the wild may not be final proof of glochidial transformation since the glochidia may be shed off unsuitable hosts after a few days (Tedla & Fernando 1969; Zale & Neves 1982; Kat 1984). On the other hand, laboratory experiments and manipulations, with confirmation of glochidia transformation do not necessarily provide enough evidence that the mussel glochidia and their hosts actually encounter each other naturally in the field (Hoggarth 1992; Khym & Layzer 2000). Hoggarth (1992) mentioned 279 glochidia-host relationships for 63 freshwater mussel species of the 297 North-American species. Of these 279 mussel-fish relationships, only 59 were based on natural infestation and confirmed by laboratory experiments (metamorphosis of glochidia attached to fishes) (Hoggarth 1992).

Basic information on the freshwater mussel-fish relationships is lacking for numerous river drainages of North America. This is particularly true for the Northern Atlantic Slope region. Few published surveys or studies dealing with freshwater mussels have been conducted in the Canadian Atlantic Maritime provinces (Athearn 1961; Athearn & Clarke 1961; Wiles 1975; Sephton *et al.* 1980; Kat & Davis 1984; Metclafe-Smith & Green 1992; Hanson & Locke 2000; Hanson & Locke 2001) and most of them did not focus on the mussel-host relationships. Wiles (1975) mentioned that Northeastern American fish host relationships were known only for 9 out of the 31 species present, and that only one species of freshwater mussel from Nova Scotia had a known fish host. This statement is not far from reality, even nearly 30 years later; in fact, almost no information is available [mostly unpublished old preliminary surveys (Hanson & Locke 2001)] on many aspects of freshwater mussel populations of New Brunswick. We are not aware of any published mussel-fish studies conducted in New Brunswick. The main objective of this study was to investigate the mussel-fish relationship for freshwater mussels of the Kouchibouguacis River, New Brunswick, by collecting and examining fish sampled in the field.

MATERIALS AND METHODS

The study was conducted in the Kouchibouguacis River, a small coastal river in eastern New Brunswick (Fig. 2) durning the spring and summer of 2003. The Kouchibouguacis River is located south of the Bay of Miramichi and has a drainage area of approximately 370 km^2, with its downstream waters flowing through Kouchibouguac National Park. Previous freshwater mussel surveys conducted in the Kouchibouguacis River revealed the presence of three freshwater mussels of the family Unionidae: one of the subfamily Ambleminae, *Elliptio complanata*, and two of the subfamily Anodontinae, *Pyganodon cataracta* and *Alasmidonta varicosa* (Beaudet *et al.* 2002). These three species of freshwater mussels were included in the present study of fish host relationships. *Margaritifera margaritifera* was not included in this study since its host fish, in occurrence the Atlantic salmon (*Salmo Salar*) and the Brook trout (*Salvelinus fontinalis*) are known from literature (Smith 1976; Cunjak & McGladdery 1991). Moreover, these species are of interest in a regional economical context, thus the sacrifice of individuals did not seemed to be worth it"

At the beginning of the sampling period, adult freshwater mussels of each species were collected in order to verify timing of gravidity and obtain precise morphological measurements of respective glochidia for identification. Abundance of *Alasmidonta varicosa* in the Kouchibouguacis River was not high enough to support collection of individuals for examination of gravidity and subsequent glochidia measurements. Thus, gravid adults were collected in a large *A. varicosa* bed found in a tributary of the Petitcodiac River (Little River), in the Moncton area (46°01'N; 65°01'W). The habitat characteristics where the *A. varicosa* were collected in the Petitcodiac River system were similar to those of the Kouchibouguacis River. Eleven *A. varicosa* individuals were collected on 28 May 2003. Nine individuals of *Pyganodon cataracta* were collected in a small pond adjoining the Kouchibouguacis River on 17 May 2003, and eight specimens of *Elliptio complanata* were collected from an area of high density in the Kouchibouguacis River on 1 July 2003.

Fish sampling sites were selected by choosing areas of high mussel density known from previous surveys (Beaudet *et al.* 2002), as well as accessibility. Fish sampling was done by setting eight baited minnow traps (mesh size approximately 3 mm) at different locations along both sides of the river. The traps, which were baited with bread crumbs or cat's food, were located where fish densities were thought to be higher (i.e. slow to moderate current, protected macrophyte area, shaded shallow area). The traps were separated from each other by a minimum of 30 m and set for 24 hours. A hoop net was set on June 13, 2003 but yielded no fish. Electrofishing and seining methods were used when possible in order to obtain greater fish diversities and to avoid the selective sampling bias, which occurs when using only one method of sampling. The method used for electrofishing consisted of a single pass within the area where mussels were found. Minnow traps were nonetheless the most effective method of sampling for the conditions encountered during the sampling period.

Amblemine mussels

Fish sampling was conducted in a zone of high density of *Elliptio complanata* (Fig. 12). The furthest minnow trap was no more than 75 m from a bed with approximately 165 individuals of *E. complanata*. This site was sampled seven times from 7 July to 8 August. Collected specimens for each fish species were preserved in 70% ethanol solution. Collected fishes were brought back to the laboratory where they were dissected for gill examination. Because *E. complanata* glochidia are small in size (<280 μm), hookless, and usually attach to the gill filament (Matteson 1948; Kat 1984), left and right gills were removed and examined under a dissecting microscope (Wild M4A, 10-40x) for glochidial attachment. Gills were sent to the Canadian Museum of Nature where they were re-examined under a stereomicroscope (Olympus SZH, 7.5-60x) in order to confirm the identification of glochidia.

Anodontine mussels

Eleven fish samples were taken from 5 June to 4 July at approximately 58.5 km from the source (upstream) of the Kouchibouguacis River, where *Alasmidonta varicosa* was previously encountered in the 2001 survey (Beaudet *et al.* 2002) (Fig. 12). Two sampling locations were selected. The first site was located directly in the area where there was the presence of *A. varicosa* and the second location was 2.1 km upstream of the first site.

All captured fishes were individually anaesthetised with clove oil, examined for glochidia and put into a recovery basin before returning them to the river. Glochidia of *Alasmidonta varicosa* and *Pyganodon cataracta* are large enough (>320 μm) to be examined with a hand lens (10x). The search for anodontine glochidia was conducted by examining the fins of each fish collected. Fish carrying glochidia were kept in a 70% ethanol solution for verification of the glochidia identification and for comparison with the field counts.

Adult freshwater mussels were identified using Clarke (1981a) and Nedeau *et al.* (2000). Dr. André Martel, a malacologist at the Canadian Museum of Nature identified the glochidia found attached to fishes. Identifications were done by morphological and morphometrical comparison between glochidia found on the fish, and glochidia collected from gravid, live specimen or glochidia from the reference collection kept at the museum. Fish were identified using Scott & Crossman (1973), Bernatchez & Giroux (2000), as well as expert identification with Dr. Brian Coad, Ichthyologist at the Canadian Museum of Nature. In the results section, fish species are listed following the systematic order found in Bernatchez & Giroux (2000).

RESULTS AND DISCUSSION

Amblemine mussels

The gills of 209 fishes were examined for the possible occurrence of *Elliptio complanata* glochidia. Seven fish belonging to five different species carried *E. complanata* glochidia: white sucker (*Catostomus commersoni*), lake chub (*Couesius plumbeus*), creek chub (*Semotilus atromaculatus*), blacknose dace (*Rhinichthys atratulus*) and brook stickleback (*Culaea inconstans*) (Table 6). Individual white suckers, creek chubs, and blacknose dace carried single glochidia in their gills or fins and the lake chubs carried two glochidia. A single specimen of brook stickleback carried three *E. complanata* glochidia, which is higher than any of the other fish species. Although found in small numbers, the presence of *E. complanata* glochidia on the creek chub and especially the lake chub indicates that Cyprinidae (minnows, daces, chubs) may play an important role in the recruitment and reproduction of *E. complanata* in the Kouchibouguacis River. It is the first time that *E. complanata* glochidia are found on these five species of fish. Moreover, the blacknose dace, creek chub and brook stickleback had *E. complanata* glochidia attached to their fins, which is unusual for the "hookless" type of glochidia that *E. complanata* uses to attach to gills (Kat 1984). The fish hosts of *E. complanata* reported from literature are yellow perch (*Perca flavesens*), banded killifish (*Fundulus diaphanus*), and the largemouth bass (*Micropterus salmoides*) (Matteson 1948; Wiles 1975; Watters 1994). Since *E. complanata* occupies a variety of habitats and has a widespread distribution across eastern North

America, it likely uses other type of fish hosts (Nedeau *et al.* 2000), such as cyprinids, as demonstrated in this study.

Nine female *Elliptio complanata* were collected on 1 July and none were gravid. This suggested that glochidia had already been released. *E. complanata* is a short-term breeder, or tachytictic, where fertilization occurs in spring and glochidia are released later in the summer (Clarke 1981a; Nedeau *et al.* 2000). The bed where *E. complanata* were sampled was found in a site of shallow water, with no overhanging vegetation, of the Kouchibouguacis River where water temperatures tend to be higher (sometimes peaking at 30°C in July and August), thus it is possible that these *E. complanata* released their glochidia sooner than expected and that the parasitic stage is shorter because of higher temperatures. Many authors have reported a relationship between timing of glochidial release, the duration of glochidial attachment to a fish host and water temperature (Matteson 1948; Tedla & Fernando 1969; Zale & Neves 1982; Yeager & Saylor 1995). In the present study, the infested fish hosts were encountered in the beginning of the sampling period (7 and 16 July), and not afterward. It is worth mentioning that even though none of the collected adult of *E. Elliptio* were gravid, we were able to identify the glochidia found on infested fishes with morphological comparison with glochidia of *E. complanata* kept at the Canadian Museum of Nature reference collections.

Anodontine mussels

A total of 961 fish belonging to 12 species were examined for the presence of *Alasmidonta varicosa* and *Pyganodon cataracta* glochidia (Table 7). Previous morphological measurements of the glochidia of both *A. varicosa* and *P. cataracta* confirmed the identification of the glochidia found attached to the fins of the collected fish. *P. cataracta* glochidia were found on five fish species, and one glochidia of *A. varicosa* was found on one ninespine stickleback (*Pungitius pungitius*). The ninespine stickleback was the most important fish host of *P. cataracta* in the Kouchibouguacis River. We can confirm that the ninespine stickleback is a host for *P. cataracta* glochidia, since a fully transformed, metamorphosed glochidia (showing anterior and posterior adductor muscles) could be observed, still attached, to the fin of this host. As much as 30% (6 out of 20) of the ninespine sticklebacks carried glochidia mainly of *P. cataracta*, but one of them carried a glochidia of *A. varicosa*. Single individuals of the threespine stickleback, common shiner, blacknose dace and creek chub also carried *P. cataracta* glochidia on their fins, suggesting that these fishes may be less important hosts in the life cycle of *P. cataracta* than the ninespine stickleback in the Kouchibouguacis River. Glochidia of *P. cataracta* were encysted only on 1.04% (10 fish out of 961) of all fish examined.

Published studies on *Pyganodon cataracta* have, so far, revealed the existence of six fish hosts: rock bass (*Ambloplites rupestris*), white sucker (*Catostomus commersoni*), pumpkinseed sunfish (*Lepomis gibbosus*), threespine stickleback (*Gasterosteus aculeatus*), carp (*Cyprinus carpio*), and bluegill (*Lepomis macrochirus*) (Wiles 1975; Threlfall 1986; Hoggarth 1992; Gray *et al.* 1999). Two of them, the white sucker and the threespine stickleback, occur in the Kouchibouguacis River. No glochidia of *P. cataracta* were found on the white sucker and only two were found on the threespine stickleback. In the present study, the low frequency of glochidia occurrence on fishes (1.04%) as well as the small number of glochidia attached to fish (usually 1 to 18 glochidia per fish) may reflect the low density of *P. cataracta* near the sampling site and in the Kouchibouguacis River in general. Some authors also found relatively low frequency and density of glochidia attachment for mussels living in riverine environment. Araujo *et al.* (1988), who studied occurrence of glochidia on the *Margaritifera auricularia*, obtained a higher frequency of infestation (12%) but a similar degree of infestation, averaging 4 glochidia per fish. Trdan (1981) witnessed slightly higher frequency (8%) and degree (average of 13 glochidia per fish) of infestation while studying *Lampsilis radiate siliquoidea.* Neves & Widlack (1988) also obtained a higher frequency of infestation (14%) by glochidia of a river in Virginia, but the degree of infestation (1 to 10 glochidia per fish) was similar to the one of this study. Roe *et al.* (1997) and McMurray *et al.* (1999) results both showed low frequencies of infestation of 1.4% and 1.5%, respectively. These observations support the low reproductive success among the freshwater mussels living in river systems.

We examined gravid females in order to obtain glochidia for further identification and comparison. Because the population of *Pyganodon cataracta* in the river was too small, representative individuals of this species were collected in a pond adjacent to the Kouchibouguacis River (Chapter 3). *P. cataracta* was the only species present in the pond. On 17 May, nine adult *P. cataracta* were collected in the pond and four were gravid females with glochidia. Glochidia of *P. cataracta* had already been found attached to fish collected in the pond, indicating that mussels were in the process of releasing glochidia and that this was a favorable time to sample fish in the river. The glochidia of *P. cataracta* were found on fish collected between 5 June and 14 June 2003 in the Kouchibouguacis River. *P. cataracta* is a long-term brooder, or brathytictic, meaning that fertilisation of eggs takes place in summer, and the gravid period is reported to last from August to May and glochidial release takes place in April or May depending on water temperature (Nedeau *et al.* 2000). Our results from the Kouchibouguacis River concur with those of Nedeau *et al.* (2000) since the period of release and attachment occurs in May and early June. It is possible that glochidial release starts in April, but this needs confirmation.

The occurrence of one *Alasmidonta varicosa* glochidia on the ninespine stickleback is of interest. To our knowledge, this is the first mention of a fish host in Canada for *A. varicosa*. Laboratory work should be conducted to verify the metamorphosis of the glochidia on this potential host in order to confirm this relationship. The identification of the encysted glochidia (on pectoral fin) was confirmed by laboratory examination and comparison with voucher glochidia sampled from the marsupial gills of mature females. The ninespine stickleback, as mentioned before, is suspected to play a role in the reproduction and recruitment of the small population of *A. varicosa* found in the Kouchibouguacis River.

Despite the fact that the ninespine stickleback represented only 2.1% of all the fish collected in the Kouchibouguacis River, its role in unionoid dispersal might be of greater importance than any other fish species and should be investigated in future fish host studies.

The low rate of attachment to fish by *Alasmidonta varicosa* (0.1%) was expected since there is only a small number of adult *A. varicosa* living in the Kouchibouguacis River. Other factors might explain why only one glochidia of *A. varicosa* was found on only one fish out of the 961 examined. First, it is possible that the sampling effort was insufficient to detect a fair number of *A. varicosa* glochidia due to the very low abundance of spawning adult individuals in the river. The degree of infection may be naturally low suggesting that a greater sampling effort may be required in order to find additional host fish. Secondly, because of limited resources and high water level, the choice of sampling gear was limited throughout the sampling period. High water velocity and high water level in the spring made the use of nets, electrofishing, and seine difficult. Moreover, using nets in the river was risky because of large woody debris carried down by the current. Minnow traps seemed to be the most useful gear to use. However, focusing primarily on one sampling gear also restricted the amount, size and diversity of the fish collected. Finally, another factor that could have influenced our hability to detect glochidia is the possibility that some glochidia of *A. varicosa* occurred on gills of hosts. Thus, there remains the possibility that some glochidia were overlooked since the fish were examined while alive, and released without looking at the gills. Members of the Anodontinae subfamily (including *A. varicosa*) have large, hooked glochidia that usually attached to exterior or exposed parts of their host (Fuller 1974; Kat 1984). However, results of several studies do not concur with this description since a significant percentage of glochidia of *Anodonta* spp. have been found attached to fish gills (Davenport & Warmut 1965; Wiles 1975; Jansen 1991; Weiss & Layzer 1995). Zale & Neves (1982) indicated that all the glochidia of the rare *Alasmidonta minor* were found on the gills of sculpins. Moreover, Threlfall (1986) found that glochidia of *P. cataracta*, another member of the Anodontinae subfamily, were primarily found (71%) on the gill of threespine sticklebacks. Thus examining only fish fins may have led to underestimating the abundance of *A. varicosa* glochidia on fishes of the Kouchibouguacis River.

The time of sampling is also an important factor to consider when studying mussel fish relationships. Of the 11 live adult mussels collected for gravidity verification, six were females among which two of them were partly gravid, with the marsupium nearly half full with glochidia. Two other females showed signs of recent glochidia release. These results suggest that on 28 May 2003 at the time of collecting the process of glochidia release was still taking place. *A. varicosa* is known to be a long-term breeder (Nedeau *et al.* 2000). Glochidial release has been suggested to occur from April through June (Nedeau *et al.* 2000). It was found that the period of gravidity of *A. varicosa* was from 9 August to 3 May in Pennsylvania (Ortmann 1919 *in* Clarke 1981b). Thus, sampling in June and July is an appropriate time of the year to look at glochidia on fish in the Kouchibouguacis River. For future studies, drift nets would have been useful in determining when the glochidia were being released (Zale & Neves 1982; Neves & Widlack 1988; Jirka & Neves 1992). In future studies, the entire period of fish infestation could be covered, which would evaluate the duration of the attachment of *A. varicosa* glochidia on the hosts.

The literature does not mention any hosts for *Alasmidonta varicosa* (i.e. a host fish on which *A. varicosa* glochidia have been shown to complete metamorphosis and become a benthic juvenile). Longnose dace (*Rhinichthys cararactae*), blacknose dace (*Rhinichthys atratulus*), golden shiner (*Notemigonas chrysoleucas*), pumpkinseed sunfish (*Lepomis gibbosus*), slimy sculpin (*Cottus cognatus*), yellow perch (*Perca flavescens*) and margined madtom (*Schilbeodes marginatus marginatus*) have been reported to serve as "potential" hosts (Wicklow & Richards 1995 *in* Nedeau *et al.* 2000). Among the potential fish hosts mentioned (laboratory testing) for *A. varicosa*, three were collected in the Kouchibouguacis system: blacknose dace, golden shiner and slimy sculpin. However, glochidia of *A. varicosa* were not found on these fish. Blacknose dace and golden shiner are widespread in the watershed, even at places where the *A. varicosa* is scarce or absent. This may be an indication that they do not play a crucial role in the reproduction and recruitment of *A. varicosa*. However, the slimy sculpin was only found once in the downstream section, where the highest *A. varicosa* densities were found (Beaudet *et al.* 2002). The slimy sculpin is rare in the watershed in comparison to other species found. Special attention should be given to this fish as a potential host for *A. varicosa* in coastal New Brunswick.

The most abundant fish species in the downstream section of the Kouchibouguacis River was the threespine stickleback and its distribution is similar to that of *Alasmidonta varicosa* (chapter 1). However, despite the threespine stickleback's high abundance and availability for attachment, *A. varicosa* population abundance remains low in the system. The threespine stickleback was not mentioned as a potential host in the literature. The distribution of *A. varicosa* in our study and others (Hanson & Locke 2001; Nedeau *et al.* 2000) suggest that this mussel species may use an anadromous fish as a host. Results of extensive surveys in Maine (Nedeau *et al.* 2000) show that *A. varicosa* was found in approximately 6.2% of the sampling sites and these sites were mainly from coastal streams readily accessible by anadromous fishes. Observations of the distribution of *A. varicosa* by Hanson & Locke (2001) also suggest that the host species of *A. varicosa* is an anadromous fish. Based on known *A. varicosa* distribution, the fish hosts do not seem to ascend into headwaters of rivers (Hanson & Locke 2001; Beaudet *et al.* 2002). Thus, further investigations should consider the potential of the slimy sculpin, the threespine stickleback, the alewife or another anadromous fish as hosts for *A. varicosa* glochidia.

One important question remains unanswered: is the current low abundance of *Alasmidonta varicosa* representative of its historical abundance? Or is it the result of declines that have occurred more recently following reduction of its fish host population, or habitat degradation? Unfortunately, historical data on *A. varicosa* distribution and abundance is lacking and many aspects of its life cycle still need to be investigated. This also applies to other freshwater mussel species involved in this study.

Understanding the relationship between mussels and fishes in the wild represents highly valuable information for species and habitat management. The fish-mussel linkages discovered in this study for *Pyganodon cataracta*, *Alasmidonta varicosa* and *Elliptio complanata* represent important clues for the reproduction of these mussels in coastal New Brunswick. Again, laboratory experiments are recommended in order to verify glochidia metamorphosis and confirm the suitability of these new hosts records for these three freshwater mussels.

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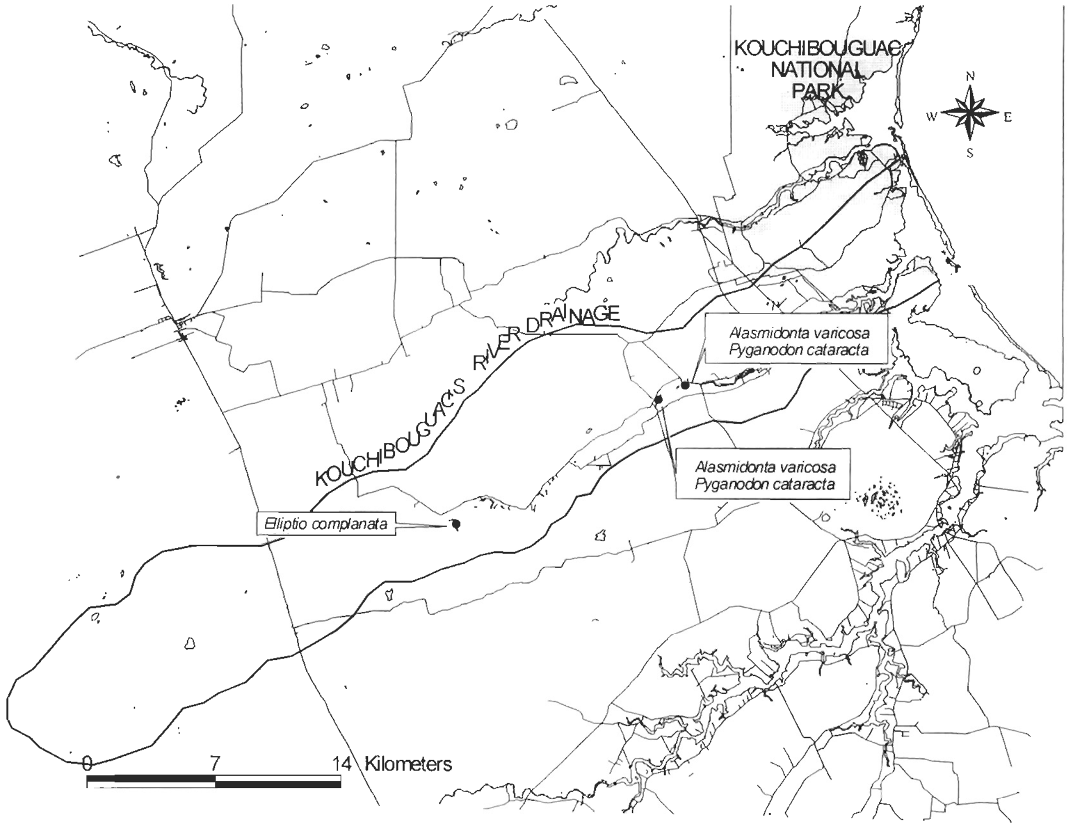

FIGURE 12: Study area in the Kouchibouguacis River drainage, eastern New Brunswick. Location of the fish sampling locations are indicated by black circles. Sampling with minnow traps (n=8 each time) took place from 5 June to 4 July for *Alasmidonta varicosa* and *Pyganodon cataracta* locations, and from 7 July to 8 August 2003 for the *Elliptio complanata* location.

TABLE 6: Attachment and number of *Elliptio complanata* glochidia on fishes of the Kouchibouguacis River, New Brunswick, from 1 July to 8 August 2003.

Species	Number examined	Number infested	Number of glochidia	Date of colletion (2003)
Lake chub (*Couesius plumbeus*)	52	2	3	July 7
golden shiner (*Notemigonus crysoleucas*)	5	0	-	-
common shiner (*Luxilus cornutus*)	57	0	-	-
blacknose dace (*Rhinichthys atratulus*)	15	1	1	July 7
creek chub (*Semotilus atromaculatus*)	55	2	2	July 7 & 16
white sucker (*Catostomus commersoni*)	24	1	1	July 7
brook stickleback (*Culaea inconstans*)	1	1	3	July 7
Total	209	7	10	-

TABLE 7: Attachment and number of *Pyganodon cataracta* and *Alasmidonta varicosa* glochidia of fishes of the Kouchibouguacis River, New Brunswick, from 5 June to 4 July 2003.

Species	Number examine d	Number infested	Total number of glochidia	Identity of glochidia on host fins
brook trout (*Salvelinus fontinalis*)	3	0	0	-
lake chub (*Couesius plumbeus*)	29	0	0	-
golden shiner (*Notemigonus crysoleucas*)	50	0	0	-
common shiner (*Luxilus cornutus*)	175	1	1	*P. cataracta*
blacknose dace (*Rhynichtys atratulus*)	192	1	1	*P. cataracta*
creek chub (*Semotilus atromaculatus*)	4	1	1	*P. cataracta*
white sucker (*Catostomus commersoni*)	11	0	0	-
brook stickleback (*Culaea inconstans*)	23	0	0	-
threespine stickleback (*Gasterosteus aculeatus*)	452	1	2	*P. cataracta*
blackspotted stickleback (*Gasterosteus wheatlandi*)	1	0	0	-
ninespine stickleback (*Pungitius pungitius*)	20	6	27	*P. cataracta*, *A. varicosa*[a]
slimy sculpin (*Cottus cognatus*)	1	0	0	-
Total	961	10	32	

[a] Only one glochidium of *A. varicosa* was found on a ninespine stickleback. Two ninespine sticklebacks carried 18 and 4 *P. cataracta* glochidia respectively, and the remaining ninespine sticklebacks carried only one glochidia of *P. cataracta* each.

CHAPTER III: DIFFERENTIAL USE OF FISH HOSTS BY THE FRESHWATER MUSSEL *PYGANODON CATARACTA* IN A POND ADJACENT TO THE KOUCHIBOUGUACIS RIVER, COASTAL NEW BRUNSWICK

ABSTRACT

Previous studies on freshwater mussel reproductive strategies have, for the most part, focused on fish host identification, glochidial morphology, and recruitment. This study focuses on the differential utilisation of fish species by a known host generalist freshwater mussel: *Pyganodon cataracta*, the eastern floater, in a pond adjacent to the Kouchibouguacis River, eastern New Brunswick, Canada. Glochidia pattern of distribution on fish's body was also study in order to determine how this relates to behaviour. *P. cataracta* was the only freshwater mussel species found in the pond. Eight sites were sampled in the pond using minnow traps at four different periods: 17 May, 1 to 4 June, 2 and 17 July, 2003. A total of 262 fishes belonging to seven species were collected and preserved in a 70% ethanol solution for subsequent examination. Glochidia attached to fishes were observed in the May and June samples. An additionnal 294 fishes were examined only on the field in July 2003 and were released. No glochidia were found on these fishes. The percentage of infestation on the collected fishes was high (90.4%) (May and June samples). Each collected fish was brought back to the laboratory where the fins were photographed, measured and examined for *P. cataracta* glochidia. Fishes were dissected for gill examination. "Available" attachment site areas (fins and head) were then measured, and glochidia density (number of glochidia per cm^2 of fin and head) was calculated for each individual fish. The golden shiner (*Notemigonus crysoleucas*) was the most abundant fish in the pond, but the brook stickleback (*Culaea inconstans*) showed significantly higher *P. cataracta* glochidia abundance and density, with an average of 14.1 glochidia per fish and 5.37 glochidia/cm^2, respectively. Glochidia attached preferentially to some anatomical areas, especially to pectoral fins. Further studies are needed in order to investigate glochidia topographical distribution on the host and the host behavior and distribution.

Key words: Freshwater mussel, Unionoidae, Anodontinae, *Pyganodon cataracta*, reproduction, recruitment, glochidia, fins, gills, parasitic patterns, fish host, mussel-host relationship, pond habitat

Introduction

Freshwater mussels (superfamily: Unionoidae) are commonly a major component of benthic communities in numerous lake and river systems (Strayer *et al.* 1999). Unfortunately, in North America the majority of freshwater mussel taxa have been declining at an alarming rate (Williams *et al.* 1993; Bogan 1996; Neves 1997; Metcalfe-Smith *et al.* 1997). Among the reasons for their decline is their sensitivity to habitat degradation and a low recruitment rate. Freshwater mussels have evolved with different reproductive strategies compared with their salt-water relatives. Larvae of freshwater mussels, called glochidia, are produced by the fertilization of eggs contained in specialized marsupial pouches, or chambers, which are an integral component of the gills of the gravid female. Once released into the water column glochidia only have a short period of time to find a suitable host, usually a fish, on which they stay attached for a period ranging from few days to several months (McMahon 1991). Once attached to an appropriate host, the glochidia encyst in the host tissue, mainly the gills or the fins; otherwise the glochidia are rejected if the host is inappropriate (Davenport & Warmuth 1965; Tedla & Fernando 1969; Zale & Neves 1982; Khym & Layzer 2000). Metamorphosis occurs on the fish and involves the loss of larval structures and the development of juvenile structures, including two adductor muscles and a complete digestive system and a functional foot. Once the metamorphosis is completed, the juvenile mussel detaches from the host and fall to the bottom of the lake or stream to start its benthic life. There are two general reproductive strategies among freshwater mussels with regards to fish host selection: glochidia either select only few and closely related fish species (host-specialist mussels), or select several or many fish of different species or types (host-generalist mussels) (Kat 1984; Jokela & Palokangas 1993; Haag *et al.* 1999; Nedeau *et al.* 2000).

Previous studies on freshwater mussel reproductive strategies and mussel fish interactions have mostly focused on the identification of fish hosts used by glochidia (Matteson 1948; Dudgeon & Morton 1984; Neves *et al.* 1985; Keller & Ruessler 1997; Khym & Layzer 2000), on recruitment (Richardson & Yokley 1996; McMurray *et al.* 1999) as well as on patterns of occurrence or microdistribution of glochidia on the fish host itself (Dudgeons & Morton 1984; Threlfall 1986; Jansen 1991; Jansen & Hanson 1991). Only a few studies have focused on the differential utilisation of host fishes by the glochidia of host-generalist mussel species (Jansen 1991; Martel & Lauzon-Guay submitted).

Only 25% of the 300 freshwater unionoid mussel species of North America have confirmed fish hosts (Hoggarth 1992), but ongoing and future ecological and conservation studies will likely increase this number. Although many unionoids have been shown to involve many fish hosts in their life cycle, few studies have been conducted with the objective of elucidating which host is the preferred or optimally utilised host by a generalist mussel. Some freshwater mussels of the Anodontinae subfamily are known to be host generalist species attaching to a diverse range of fishes. One of those generalist species is *Pyganodon cataracta* (Fuller 1974; Nedeau *et al.* 2000). *P. cataracta* prefers lacustrine environment (Nedeau *et al.* 2000) and is often one of the dominating species in lakes, impounded rivers and pond environments found in coastal New Brunswick (Septhon *et al.* 1980; Hanson &

Locke 2001). Its presence in confined, still water habitats such as ponds, makes *P. cataracta* a valuable species for quantitative studies aimed at determining the intensity of the mussel-fish linkage.

The aspect of reproduction and differential fish linkage in a host-generalist is the type of data needed for effective management and conservation of natural resource. Knowing the list of fishes involved in the life of a mussel is valuable information, but knowing which host species is the major actor in the reproduction and recruitment of a freshwater mussel species provides the additional needed tool for proper conservation of the species. Jansen and Hanson (1991) looked at this differential utilisation in *Anodonta* (*Pyganodon*) *grandis*, but no standardisation (i.e. no division of the number of glochidia per available attachement area, fin or head, in cm^2) was made in order to evaluate the use of each species by glochidia. The study by Martel & Lauzon-Guay (submitted) on *Anodonta kennerlyi* explored the differential use of fishes by glochidia of this unionoid in Vancouver Island Lake. In the present study, although the target species (*Pyganodon cataracta*) is not at risk, information on the differential use of its fish hosts might give some insight into how other "generalist" mussels, including taxa at risk, make "use" of the different fishes involved in their life cycle.

Fish behaviour is another important feature to consider when looking at freshwater mussel - hosts relationships and recruitment. Indeed, fish behaviour may influence the encounter between the fish and freshwater mussel glochidia, and in turn, may influence the position of attachement of the glochidia on the fish. In this study, we also tried to determinate distribution pattern of glochidia on the fish body, in order to relate this to behaviour. Thus this study has two objectives, the first was to determine the fish-hosts of *Pyganodon cataracta* in a small aquatic system adjacent to a coastal river, and obtain a standardised glochidial attachment rate or measure (number of glochidia per available cm^2) on all fish hosts used by this mussel in that system. The second objective was to examine the topographical distribution of glochidia on the fish and determine whether the distribution pattern of glochidia on fishes can be explained by the behaviour of the fish.

MATERIALS AND METHODS

Study area

The sampling was conducted in a small pond of approximately 4500 m^2 (46°36'N; 65°40'W) in the upstream section of the Kouchibouguacis River and adjacent to it, in coastal New Brunswick (Fig. 13). The pond is located immediately beside the actual main river channel, and was most likely created when the railroad and highway were built. The pond is connected to the main river, in the spring by a small outflow, but becomes isolated during the summer when the water level is low. The pond is surrounded by forest except for one side, which is limited by the railroad. The pond is shallow (no more than 2 m deep) and a layer of organic material covers its bottom. *Pyganodon cataracta* is the only freshwater mussel species found and occurs at high densities (approx. 6 ind./m^2), with individuals living partly burried in the thick layer of soft organic material at the bottom of the pond.

Nine *Pyganodon cataracta* were collected in the pond, in order to verify gravidity on 17 May 2003. Four sites were also sampled on 17 May for examination of the glochidia on fish. Four additionnal sites were then added (total of 8 sites) on 1 to 4 June, 2 July and 17 July 2003 (Fig. 13).

Minnow traps were used to sample fishes in the pond (mesh size approximately 3 mm). Within the 24 hours preceding sampling, eight minnow traps (one per site), baited with bread crumbs and cat's food, were placed at approximately equal distance (30 to 50 m from each other) to cover most of the pond perimeter. When the traps were opened the next day, fishes were anaesthetised with clove oil, counted, identified to species and kept in 70% ethanol.

Fishes were brought back to the laboratory and were examined under a dissecting microscope (Leica MZ16A, 7.5-115x) for glochidial counts. The fins were inspected and photographed with a digital camera set on the microscope. Digital pictures were imported in a software (Northern Eclipse 6.0) where the fish fins and head were digitized and the available area (cm^2) for glochidia attachment calculated. Areas of each fin, obtained with the software were multiplied by two to account for both sides of each fin. Other parts of the fish were also carefully examined for the presence of glochidia (i.e. opercula, eyes, mouth, scales, spines, anal region, etc). Standard lengths of fishes were also recorded. Standardised numbers of glochidia per available cm^2 of attachment site were obtained for all individuals of each fish species.

The glochidia lost per fish was estimated in order to see the impact of manipulation and transportation on the samples. Thirty-one jars were inspected after fish manipulation was done. The glochidia that were lost from host tissues were pipetted from the jars and counted. Pipetting ceased when no new glochidia were found in five consecutive pipettings. An estimation of glochidia lost per fish was made with regard to the number of fish contained in each jar. Estimations were made for each species.

In order to measure the head area, the truncated cone equation was used for cyprinids and catostomids, as per Martel & Lauzon-Guay (submitted). The equation estimates the area of the head available for glochidial attachment. Glochidia found on the "head" included those on the gills, eyes, opercula, nasal cavities, chin (under the mouth), and mouth attachment sites. Pictures of the head were taken, and then the equation parameters were measured from these digital pictures (Fig. 14). For the sticklebacks, the head area was simply obtained by multiplying the head area of one side by two, since heads of sticklebacks are narrow and laterally highly compressed, unlike other fish species.

Non-parametric Kruskal-Wallis analyses of variance were used in order to test for difference between infestations rate of each fish species. Non-parametric methods were used as data included numerous "0" values and variances were non-homogeneous, even after transformation. Pearson and Spearman correlation analyses were used to examine relationships between glochidia densities on pectoral fins and on the head area. To verify the significance of the correlations, a Bonferroni correction was used for Pearson correlations, while a table of critical values was used for the Spearman rank correlation (Sokal & Rohlf 1981).

Freshwater mussels were identified using Clarke (1981) and Nedeau *et al.* (2000). Fish were identified using Scott & Crossman (1973), Bernatchez & Giroux (2000), as well as expert identification with Dr. Brian Coad, ichthyologist at the Canadian Museum of Nature. In the results section, fish species are always listed following the systematic order found in Bernatchez & Giroux (2000).

RESULTS

On 17 May 2003, 33 specimens of brook stickleback (*Culeae inconstans*) were collected. In the 1 to 4 June sample, a total of 229 fishes belonging to seven species were collected for a total of 262 fish collected. During the July sampling, none of the 294 fishes examined carried glochidia and the fishes were all returned to the pond. The fish species collected from the pond included the lake chub (*Couesius plumbeus*), golden shiner (*Notemigonus crysoleucas*), common shiner (*Luxilus cornutus),* creek chub (*Semotilus atromaculatus)*, pearl dace (*Margariscus margarita),* white sucker (C*atostomus commersoni)*, and brook stickleback. The golden shiner was the most abundant fish collected in the traps and the pearl dace the least abundant. Although glochidia of *Pyganodon cataracta* were found on all seven fish species collected, their abundance varied greatly among the fish taxa.

The results show a high percentage of fish that were infested by *Pyganodon cataracta* glochidia, ranging from 70.4% to 100%, depending on the species (Table 8). *P. cataracta* glochidia attached in significantly greater numbers to the brook stickleback than to any other fish species in the pond (Kruskal-Wallis, $p<0.001$) (Fig. 15, Table 8). An average of 5.37 glochidia were attached per cm^2 of available tissues of the brook stickleback, compared to 0.96 and 0.95 glochidia per cm^2 on the creek chub and golden shiner respectively, the two other mostly utilised species. The white sucker shows the lowest degree of infection with only 0.22 glochidia per cm^2. An average of 14.1 glochidia per individual brook stickleback whereas the creek chub and the golden shiner carried an average of 12 and 9.5 glochidia per individual respectively. Most of the fish species, except for the pearl dace, creek chub and brook stickleback, had 40% or more of total number of glochidia attached to the head region (Table 8).

The golden shiner and the brook stickleback had significantly higher densities of glochidia attached to their head than any other species (Table 9). The pearl dace is also showing a high density of glochidia on the head, however the result is based only on two individuals. The mean number of glochidia per cm^2 of head was 1.46 and 3.60 glochidia/cm^2 for the golden shiner and the brook stickleback, respectively. Golden shiners had significant higher glochidia attachment on their gills than any other species found in the pond (Kruskal-Wallis, $p<0.001$ for all species) with 41.6% of the glochidia attached to gills.

A significant difference in glochidia density occurred between the different fins of the fish. For nearly all species of fish, higher densities of glochidia occurred on the pectoral and dorsal fins (Table 9). The pectoral fins for the brook stickleback, the white sucker, and the golden shiner showed significantly higher glochidia densities than on other fins (Kruskal-Wallis test for each species, $p<0.001$). An average of 10.2 glochidia/cm^2 was found on the brook stickleback pectoral fins (8.9 and 11.9 glochidia/cm^2 for the right and left pectoral fin, respectively (Table 9). The golden shiner had an average of 1.9 glochidia/cm^2 on the pectoral fins, whereas 0.5 glochidia were attached per cm^2 of the white sucker pectoral fins (Table 9).

Glochidia density results may be underestimated because an estimated 0.64 glochidia were lost per fish in manipulation and transportation. The species that seemed to have lost the higher number of glochidia per fish is the brook stickleback, with an average of 1.6 glochidia lost per fish. However, this slight loss of glochidia does not change the general patterns of the results nor the interpretation derived from them.

DISCUSSION

A total of 90.4% of the 262 fish collected (belonging to seven species) carried glochidia of *Pyganodon cataracta* either on their fins or head. In the pond of the Kouchibouguacis River all fish species collected were infected by *P. cataracta* glochidia, and this concords with the statement that *P. cataracta* is a generalist unionoid using a number of fish hosts (Nedeau *et al.* 2000). Although *P. cataracta* is known as a host generalist, results of this study also indicate that the degree of glochidia attachment shows major differences among the fishes collected in the Kouchibouguacis River pond. If the density of attached glochidia per area is considered a potential indicator of host preference, then the decreasing order of importance of the seven fish species as potential host of *P. cataracta* glochidia would be: (1) brook stickleback, (2) creek chub, (3) golden shiner, (4) lake chub, (5) pearl dace, (6) common shiner and (7) white sucker.

The white sucker is the only fish present in the Kouchibouguacis River pond that had been already mentioned in the literature as a potential host for *Pyganodon cataracta* glochidia (Wiles 1975; Hoggarth 1992; Gray *et al.* 1999). Unexpectedly, the white sucker is the species that presented the lowest rate of glochidia attachment per cm^2 of tissue. The brook stickleback seems to be the most important fish involved in the reproduction and recruitment of *P. cataracta*, with a frequency of infestation (number of infected fishes) of 100% and a mean glochidia density (number of glochidia per cm^2 of fin and head) of 5.37 glochidia/cm^2. The brook stickleback had the lowest available attachment fish area (area of fins and head) of 2.66 cm^2 compared to the other fish species and for which the attachementr area varied between 7.12 and 15.80 cm^2. The creek chub is the second most utilized fish with 100% attachment rate and 0.96 glochidia/cm^2. The golden shiner shows a similar density of glochidia of 0.95 glochidia/cm^2 but with a much higher sample size of 99 individuals compare to only 12 individuals for the creek chub. Since these sample sizes are not equal, one should be careful when drawing conclusions about the importance of these two species in the reproduction of *P. cataracta*. Additional laboratory experiments are needed in order to evaluate their importance in term of metamosphosis of the glochidia on these species.

The degree of infection (number of glochidia per fish) in the study area ranged from 3.6 to 14.1 depending on the species and percentage of fish infested ranged from 70 to 100%. Other studies reported much lower frequency of infestation (percentage of fish parasitized) and degrees of infestation (Neves & Widlack 1988, Araujo *et al.* 2000). Weiss & Layzer (1995) found glochidia of amblemine, anodontine and lampsiline mussel on only 4.1% of the 43 species of examined fish and each fish carried between 1-5 glochidia only. Roe *et al.* (1997) and McMurray *et al.* (1999) showed even lower glochidia attachment rates. Roe *et al.* (1997) found 1.4% of their fish infested by *Potamilus inflatus* glochidia, while McMurray *et al.* (1999) found that only 1.5% of the fish collected in their study hosted glochidia. All these previous studies were conducted in rivers. Some investigators have found higher frequency and degree of infestation. Martel & Lauzon-Guay (submitted) obtained high infection rates for the prickly sculpin (*Cottus asper*) by *Anodonta kennerlyi* in lakes on the west coast of Vancouver Island, in British Columbia. They obtained numbers of glochidia on the prickly sculpin ranging from 5.58 to 84.03 per fish, depending on the sampling period. Tedla & Fernando (1969), in their study conducted in the Bay of Quentin (lake Ontario) on *Lampsilis radiata*, obtained similar frequency of infestation

(86.6% of yellow perch infested) to our study, but higher number of glochidia per fish reaching 60. Trdan (1981) found only 3.2% of the fish in their study infested by *Lampsilis radiata siliquoidea* glochidia, but the intensity of glochidia attachment was similar to the one obtained in this study (ranging from 1 to 58 glochidia per fish, averaging 4 to 17.6). Trdan (1981) also conducted his study in a lake environment.

Trdan & Hoeh (1981) suggested that some high rates and degree of glochidia attachment resulted from high levels of host specificity. On the other hand, Cunjak & McGladdery (1991) hypothesized that intensity and incidence of glochidia attachment were a function of localized adult mussel abundance. Results from the present study support the latter hypothesis because *P. cataracta* is a fish host generalist and glochidial attachment rates in the main river bed were lower (see Chapter 2). One has to be cautious when comparing glochidia attachment rates and degree of attachment between studies. Indeed, most previous studies have been conducted in rivers, where mussel density tends to be much lower compared to that in a pond or lake environment, such as the one examined during the present study. A pond environment creates a semi-closed habitat with standing water where fish movements are limited and where unionoids such as *Pyganodon cataracta* can occur at high densities. This type of environment is likely to favor high glochidia attachment rates since the chance that glochidia will encounter a suitable host is greater than in rivers.

High numbers of glochidia of *Pyganodon cataracta* attached to fish gills were obtained. A total of 29.5% (ranging from 14.6% to 41.6%) of all attached glochidia were found on the gills of the fish hosts. The golden shiner had the higher mean number of 4 glochidia attached to the gills. These results were not expected because freshwater mussels of the Anodontine subfamily have typically large "hooked" larvae and are known to attach to tougher tissues of fish hosts like fins and scales (Fuller 1974; Kat 1984). On the other hand, other studies reported percentage of gill utilization by glochidia as high as 92% for four Anodontinae species (Weiss & Layzer 1995). Zale & Neves (1982) studied the rare *Alasmidonta minor*, another mussel of the anodontine subfamily, and found that glochidia were attached primarily to gill lamellae and epithelial tissue, and none were found on the fins. Wiles (1975) also stated that *Anondonta* sp. glochidia only occurred on gills in fish collected in May, June, and July. Threlfall (1986) studied glochidia of *Pyganodon cataracta* and found that as many as 71% attached to the gills of the fish host, a value much higher than that obtained in this study. Jansen (1991) obtained results similar to those obtained in the present study, with 13 to 20% of the *Anodonta grandis simpsoniana* glochidia infesting the gills of yellow perch, depending on the moment of the collection.

Jansen (1991) hypothesized that the gills of fishes might be infested as a result of inaccessibility of other attachment sites to new glochidia because of crowding. Gills would be alternative locations. Results from this study do not corroborate this hypothesis since the golden shiner, the species carrying the highest densities of glochidia on the gills, did not show any degree of overcrowding on available fin attachment sites. Pearson and Spearman correlations were used to examine possible relationships of glochidia densities between pectoral fins and the head. In a situation of overcrowding of the pectoral fins, there should be more glochidia attaching to the gills, as per Jansen's suggestion. There was, however, no significant correlation between head and pectoral fin glochidia densities for all the host species. Thus, these results do not support Jansen's (1991) overcrowding hypothesis.

In addition to the obvious "preference" of one fish species by *Pyganodon cataracta* glochidia, different fins also showed different glochidia densities. Most glochidia of *P. cataracta* were located on the pectoral fins. This was especially noticeable for the brook stickleback where a highly significant difference was found between the density of glochidia per cm^2 of pectoral fins with that of other fins. Jansen (1991) also observed a preferential use of the pectoral fins of yellow perch by *Anodonta grandis simpsoniana* glochidia in a central Alberta lake. The pectoral fins of the other species of the pond were also more utilized, but the difference in glochidia density between the pectoral and the other fins was less pronounced than that observed in the brook stickleback.

Kat (1984) suggested that examination of host diet and behavior could explain why certain fish and certain fish anatomical parts are more heavily parasitized than others. In the present study, the preference for the brook stickleback as host, as well as the preference for pectoral fins as an attachment site, may be explained by the behavior adopted by this fish while the *Pyganodon cataracta* glochidia are being released. Most of the infested sticklebacks were captured in May. On this sampling date, other fish species were rarely captured, with the exception of two white suckers. A majority of the sticklebacks caught in May displayed a black coloration, indicating that the individuals were mostly spawning males (Bernatchez & Giroux 2000). The nest of the brook stickleback is usually built near the river bottom or on the bottom and the males are known to agitate their pectoral fins in the nest entrance through the egg developmental stage (Reisman & Cade 1967; Scott & Crossman 1973). The spawning time and the unique fanning of the pectoral fins behavior of the brook stickleback could favor its encounter with glochidia of *P. cataracta*. However, fish behavior might not be the only explanation for this difference since some of the other species found in the pond have similar diet and demonstrate territory or nest defensive behavior (i.e. creek chub and pearl dace), and yet showed lower glochidia attachment rates. The timing of the nesting or diet behavior may be an important factor to consider as well.

In their study of the western floater, *Anodonta kennerlyi*, Martel & Lauzon-Guay (submitted) found that the fish that were categorized as the most "benthic" and nearest to the mussel populations, such as sculpins, showed the highest density of glochidia per cm^2 of fins. They also observed that the pectoral and pelvic fins of the preferred fish (sculpins) had a much higher concentration of glochidia. Martel & Lauzon-Guay (submitted) hypothesized that this was due to the more frequent contact between the mussel and the fish as well as more frequent contact of these fins with the bottom, where numerous live glochidia can be resting after glochidia release. A similar conclusion cannot be drawn from the results of this study since there is no obvious patterns of decreasing "connectedness" or "association"of each fish collected in the Kouchibouguacis River pond, as in the lakes studied by Martel & Lauzon-Guay (submitted). Further studies are thus needed in order to evaluate the "preferences" of fish hosts by *Pyganodon cataracta* glochidia.

Understanding the relationship between mussels and fishes in the wild constitutes highly valuable information for species management. The fish-mussel linkage discovered in this study between *Pyganodon cataracta* and the brook stickleback represents important clues for the reproduction of freshwater mussel in a pond environment in New Brunswick. Although the other fish species found in the pond likely play a role in the reproduction and recruitement of *P. cataracta*, the brook stickleback might be a key species that plays a disproportionnally important role in the local recruitment of this freshwater mussel within the pond. This interaction between *P. cataracta* and the brook stickleback should be examined under laboratory conditions in order to be able to confirm the brook stickleback as a host by witnessing the transformation of *P. cataracta* glochidia.

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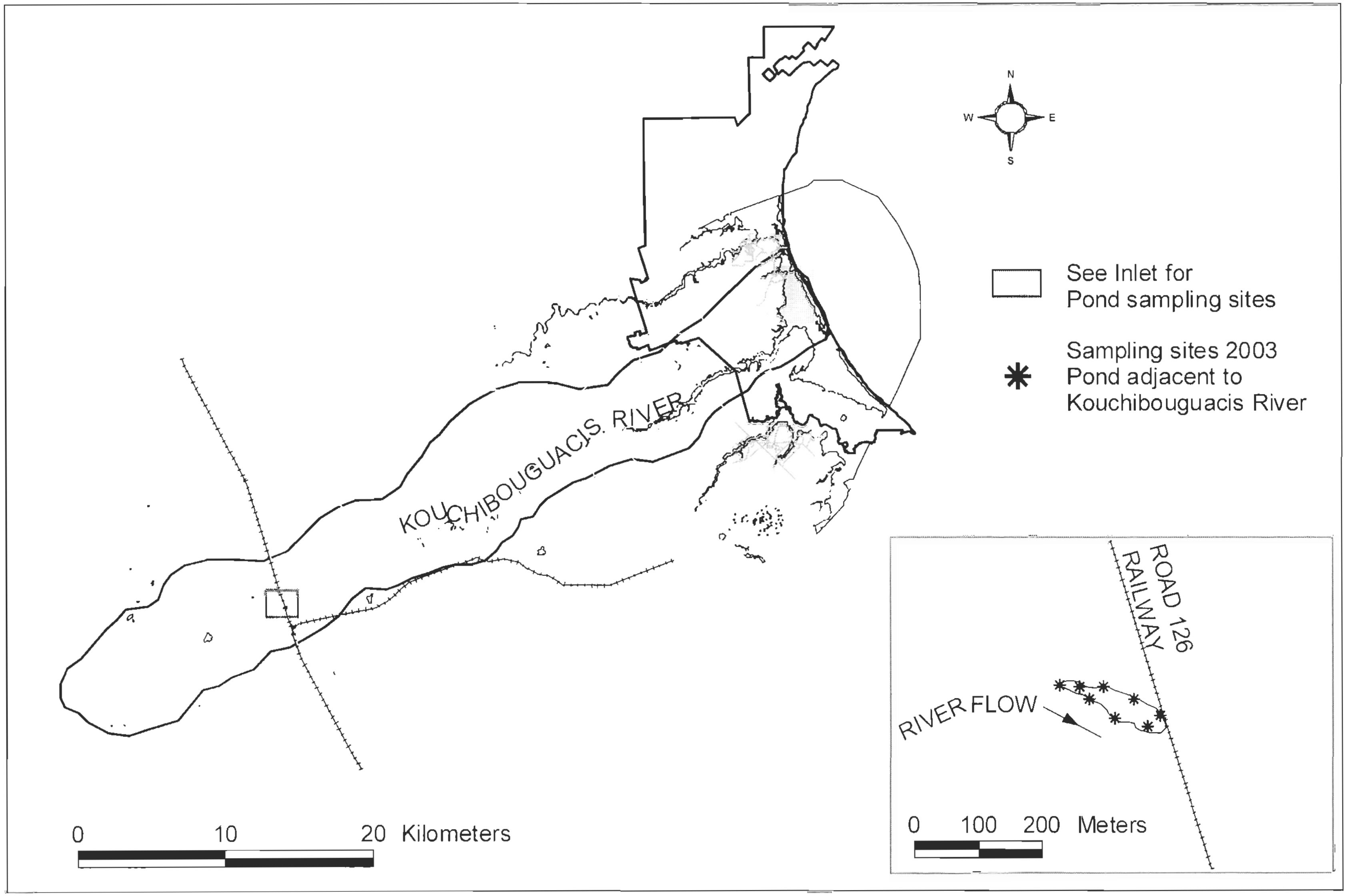


FIGURE 13: Study area in the Kouchibouguacis River drainage, eastern New Brunswick. Location of the pond (inlet) adjacent to the Kouchibouguacis River, where *Pyganodon cataracta* glochidia were examined on fishes in 2003. Minnow traps were set at eight sampling sites in the pond (represented by stars in the map).

$$S = \pi\left(\frac{a}{2} + \frac{b}{2}\right)s$$

$$Total\,Head\,Area = S + \left[\pi\left(\frac{a}{2}\right)^2\right]$$

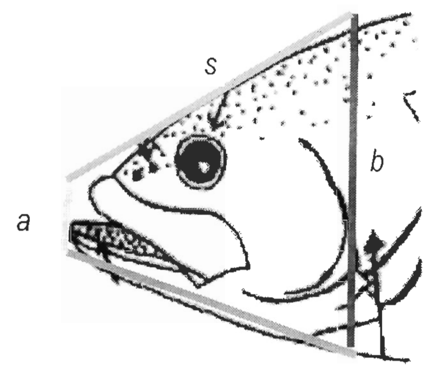
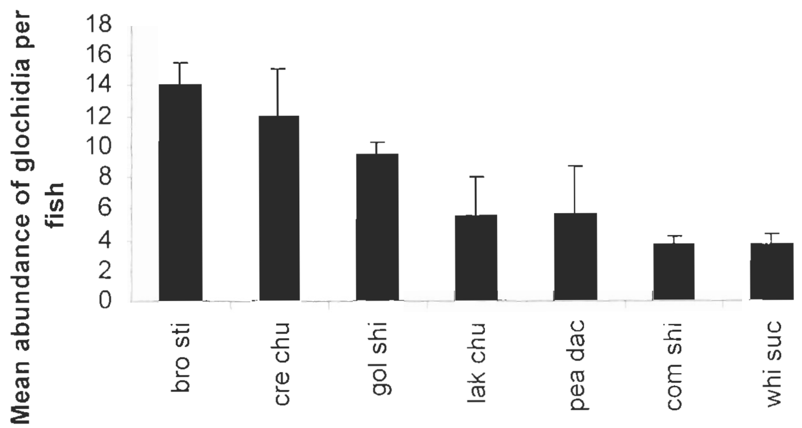


FIGURE 14: Parameters measured (a, b, and s) for the calculation of the head area using the truncated cone equation for individuals of the Cyprinidae. S represents the surface on one side of the head using a as the vertical length at the mouth, b, the height of the head at the operculum, and s, the length of the head from the operculum line to the mouth.

a)



b)

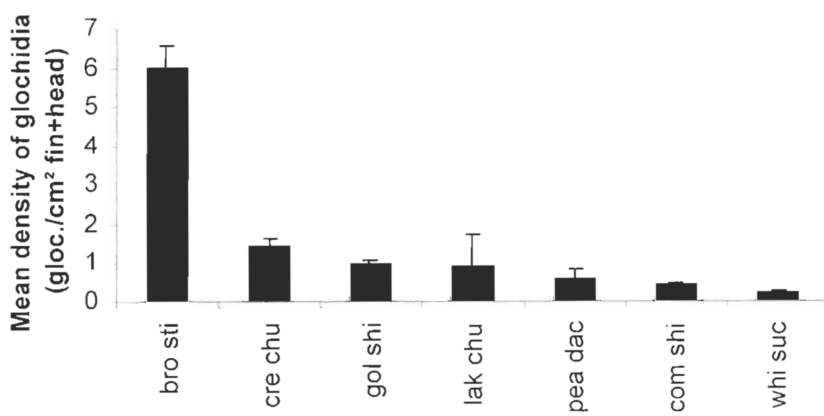


FIGURE 15: a) Graph showing the mean abundance (mean ± 1 SE) of glochidia (no. of glochidia per fish) and b) graph showing mean density (mean ± 1 SE) of glochidia (no. of glochidia per cm^2 of fin and head) for the seven host fish collected in a pond adjacent of the Kouchibouguacis River on 17 May and 1 June 2003. Fish species abbreviations used on the x-axis: lake chub [lak chu], golden shiner [gol shi], common shiner [com shi], creek chub [cre chu], pearl dace [pea dac], white sucker [whi suc], and brook stickleback [bro sti].

TABLE 8: Distribution and abundance of *Pyganodon cataracta* glochidia on each fish species collected in the pond adjacent to the Kouchibouguacis River. Sampling occurred in 2003. Head includes: gills, operculum, nasal cavities, eyes, chin and mouth.

Fish species	n	% fish carrying at least one gloc.	mean abundance of gloc. per fish (SD)	mean density of gloc. per cm² of fish tissue (SD)	% gloc. on gills only	% gloc. on fins only	% gloc. on head
lake chub (*Couesius plumbeus*)	2	100	4.5 (4.9)	0.7 (0.9)	18.2	45.4	54.5
golden shiner (*Notemigonus crysoleucas*)	99	96.0	9.5 (7.4)	0.9 (0.7)	41.6	50.8	47.2
common shiner (*Luxilus cornutus*)	51	86.3	3.6 (3.8)	0.4 (0.4)	28.6	57.8	41.1
creek chub (*Semotilus atromaculatus*)	12	100	12 (11.0)	0.9 (0.5)	14.6	76.4	22.2
pearl dace (*Margariscus margarita*)	5	80	4.6 (5.0)	0.4 (0.4)	17.9	82.1	17.9
white sucker (*Catostomus commersoni*)	44	70.4	3.6 (4.4)	0.2 (0.3)	28.9	59.7	40.3
brook stickleback (*Culaea inconstans*)	49	100	14.1 (10.1)	5.4 (3.3)	17.1	75.0	23.7

TABLE 9: Topographical distribution and densities of *Pyganodon cataracta* glochidia found attached to fish collected in the pond of the Kouchibouguacis River in 2003. Mean number of glochidia per cm² of each anatomical parts infested by glochidia. Standard deviation (SD) is represented in parenthesis.

Location	head	pectoral (R) fin	pectoral (L) fin	pelvic (R) fin	pelvic (L) fin	dorsal fin	anal fin	caudal fin
lake chub (*Couesius plumbeus*)	1.3 (0.2)	2.0 (2.8)	1.2 (1.6)	0	2.0 (2.8)	1.0 (1.4)	0	0
golden shiner (*Notemigonus crysoleucas*)	1.5 (1.7)	1.8 (3.4)	2.0 (2.3)	0.7 (1.1)	0.5 (1.0)	0.6 (1.3)	0.2 (0.5)	0.2 (0.5)
common shiner (*Luxilus cornutus*)	0.5 (0.7)	0.5 (1.2)	0.5 (0.9)	0.4 (1.0)	0.5 (1.2)	0.3 (0.6)	0.2 (0.5)	0.1 (0.3)
creek chub (*Semotilus atromaculatus*)	0.6 (0.7)	1.8 (2.0)	3.0 (3.2)	1.7 (2.0)	1.0 (1.1)	1.1 (0.9)	1.8 (2.0)	0.2 (0.4)
pearl dace (*Margariscus margarita*)	0.2 (0.5)	1.3 (2.9)	1.1 (1.8)	0	0.5 (1.2)	0.7 (0.9)	0.2 (0.5)	0.2 (0.2)
white sucker (*Catostomus commersoni*)	0.2 (0.3)	0.5 (0.7)	0.6 (1.6)	0.2 (0.6)	0.03 (0.2)	0.2 (0.4)	0.1 (0.3)	0.1 (0.3)
brook stickleback (*Culaea inconstans*)	3.6 (3.0)	8.9 (8.9)	11.9 (10.6)	3.8 (13.3)	3.3 (11.4)	8.0 (9.0)	3.7 (4.1)	4.4 (5.4)

CONCLUSION GÉNÉRALE

Les moules d'eau douce (Unionoidés) bénéficient d'un intérêt croissant depuis plusieurs années. Cet intérêt découle de la situation précaire d'un grand nombre d'espèces de moules d'eau douce en Amérique du Nord. Au cours du dernier siècle, les unionoidés ont connu un déclin sévère en terme de diversité et d'abondance (Bogan 1993; Metcalfe-Smith *et al.* 1997). Soixante-douze pour cent (72%) des espèces d'Amérique du Nord sont considérées comme étant en danger de disparition, menacées ou à statut préoccupant à travers leur aire de distribution (Williams *et al.* 1993). Deux familles d'unionoidés sont bien représentées dans cette région du globe, soit les Margaritiféridés et les Unionidés, pour un total de 297 espèces et sous-espèces connues (Williams *et al.* 1993). De ces 297 espèces, 55 sont présentes au Canada (Metcalfe-Smith & Cudmore-Vokey 2004) et 12 au Nouveau-Brunswick (Metcalfe-Smith & Cudmore-Vokey 2004). Seulement 35% des moules d'eau douce sont estimées « en sécurité » à l'échelle nationale (Metcalfe-Smith & Cudmore-Vokey 2004). Des 12 espèces retrouvées au Nouveau-Brunswick, seulement cinq ont un statut de conservation « stable » à l'échelle du continent selon l' « American Fisheries Society Endangered Species Committee » (Williams *et al.* 1993). Un comité homologue canadien, soit le Comité sur la Situation des Espèces en Péril au Canada (COSEPAC) possède un statut officiel pour seulement deux des 12 espèces présentes au Nouveau-Brunswick. Selon le COSEPAC, une troisième espèce, *Alasmidonta varicosa*, figure maintenant comme une espèce prioritaire dont le statut nécessite une évaluation immédiate. Il est cependant difficile d'évaluer le statut de ces mollusques étant donné le manque de données historiques sur leur abondance, diversité et distribution au sein des lacs et rivières. Règle générale, au Canada tout comme aux États-Unis, les études relatant des données historiques indiquent un déclin inquiétant en terme d'abondance et de diversité au sein des populations de moules d'eau douce (Napela *et al.* 1991; Metclafe-Smith *et al.* 1997, 1998, 2000; Brim Box & Mossa 1999; Strayer & Fetterman 1999; Vaugh & Taylor 1999; Hanson & Locke 2000; Pip 2000; Martel *et al.* 2001).

Le mode de vie et la stratégie de reproduction des moules d'eau douce les rendent vulnérables à deux types de perturbations, soit celles affectant directement leur habitat et celles affectant les populations de poissons. Ces derniers sont indispensables à la métamorphose et la dispersion de leurs larves. Au Canada, très peu de données sont disponibles sur la relation existant entre les glochidies des moules d'eau douce et leur(s) poisson(s) hôte(s). Voilà pourquoi il est très important d'étudier conjointement l'habitat et les populations de poissons, deux facteurs écologiques susceptibles d'influencer l'abondance, la distribution et la diversité des moules d'eau douce. La diversité de moules d'eau douce dans la rivière Kouchibouguac est différente de celle retrouvée dans la rivière Kouchibouguacis et cette différence semble trouver une explication dans la composition de la faune ichthyologique entre les deux cours d'eau. En effet, la composition des poissons entre les deux rivières est significativement différente, tandis qu'aucune relation significative n'a été obtenue entre l'abondance et la distribution des moules d'eau douce et les variables environnementales mesurées. La rivière Kouchibouguac est nettement dominée par la présence des salmonidés, tandis que les cyprinidés dominent la rivière Kouchibouguacis en terme de diversité et abondance. Cette importante différence au niveau de la composition des populations de poissons ne peut être expliquée à première vue par les caractéristiques physiques des deux bassins versants. Toutefois, la présence d'un ancien barrage à l'embouchure de la rivière Kouchibouguac a permis d'émettre l'hypothèse de l'impact potentiel que ce barrage aurait eu sur la composition de poissons, notamment un impact négatif sur les cyprinidés. Des études ultérieures, visant principalement l'étude des cyprins de la rivière Kouchibouguac, seraient souhaitables afin de déterminer l'état des populations de cette famille de poissons.

Ces résultats, soupçonnant l'influence des poissons hôtes en tant que facteurs écologiques, sont souvent instigateurs d'études complémentaires se penchant sur les relations entre les glochidies des espèces de moules et les poissons hôtes parasités. Des recherches intensives ont été menées sur les poissons hôtes de trois espèces de moules d'eau douce de la rivière Kouchibouguacis (*Elliptio complanata*, *Alasmidonta varicosa*, *Pyganodon cataracta*). Ces recherches ont permis d'établir de nouvelles relations, auparavant inconnues, entre ces espèces de moules et certains poissons hôtes. Une des découvertes intéressantes est la relation possible entre *A. varicosa* et l'épinoche à neuf épines (*Pungitius pungitius*). Cette relation n'a jamais été mentionnée dans la littérature et constitue une contribution importante au niveau de la conservation de cette espèce qui figure sur la liste d'espèce « candidate » à la liste du COSEPAC. Donc, l'étude de cette espèce de poisson pourrait peut-être expliquer les patrons de distribution et d'abondance de *A. varicosa* au sein de la rivière Kouchibouguacis. Il est intéressant de noter que l'épinoche à neuf épines, poisson hôte de *A. varicosa*, ne se retrouve pas dans la rivière Kouchibouguac. Il en est de même pour plusieurs autres espèces de poissons hôtes de *E. complanata* et *P. cataracta*, notamment les cyprinidés, découvertes lors de la présente étude.

En effet, d'autres relations moules-poissons furent découvertes au sein de la rivière Kouchibouguacis, et pour la majorité d'entre elles, il s'agissait d'une première mention pour l'espèce. Tout d'abord, des relations entre *Elliptio complanata* et le méné de lac (*Couesius plumbeus*), le naseux noir (*Rhinichtys atratulus*), le mulet à corne (*Semotilus atromaculatus*), le meunier noir (*Catostumus commersoni*) et l'épinoche à cinq épines (*Culaea inconstans*) ont été établies grâce à la découverte des glochidies de *E. complanata* sur les branchies et les nageoires de ces poissons. D'autres relations furent établies entre *Pyganodon cataracta* et l'épinoche à trois épines (*Gasterosteus aculeatus*), l'épinoche à cinq épines, l'épinoche à neuf épines, le méné à nageoires rouges (*Luxilus cornutus*), le naseux noir, le mulet à corne, le méné jaune (*Notemigonus crysoleucas*), le méné de lac (*Couesius plumbeus*), le meunier noir, et le mulet perlé (*Margariscus margarita*). À la lumière des résultats, il est intéressant de noter l'importance des épinoches (principalement à cinq et à neuf épines) en tant qu'hôtes pour les espèces de moules présentes dans l'aire d'étude. Certains auteurs suggérèrent ou démontrèrent que l'étude comportementale des poissons s'avérerait utile pour mieux comprendre l'utilisation plus intensive de certaines espèces de poissons par rapport à d'autres (Kat 1984; Martel & Lauzon-Guay submitted).

L'étude comportementale des poissons peut également servir à expliquer la distribution topographique des glochidies sur les individus hôtes. Une étude complémentaire, réalisée dans un étang d'eau douce adjacent à la rivière Kouchibouguacis, portait sur les glochidies de *Pyganodon cataracta* et leurs poissons hôtes. L'étude portait sur l'utilisation préférentielle d'une espèce de poisson, en occurrence l'épinoche à cinq épines, ainsi que sur les parties anatomiques affichant les plus hautes densités de glochidies par cm^2. Bien que l'épinoche à cinq épines soit le poisson offrant la plus petite superficie disponible pour l'attachement des glochidies, ce dernier était néanmoins le poisson le plus densément parasité. Les nageoires pectorales des épinoches à cinq épines affichaient une densité moyenne de 10.4 glochidies/cm^2. L'utilisation d'un même habitat, de même que le comportement de l'épinoche à cinq épines en période de nidification, pourrait expliquer ce haut degré de parasitisme entre cet épinoche et *P. cataracta*. Donc, une moule d'eau douce dite « généraliste » peut afficher tout de même une préférence pour une espèce de poisson, et afficher également une « préférence » pour certaines parties de l'anatomie des individus hôtes.

Ces informations contribuent de façon significative à nos connaissances sur la dynamique de reproduction et de recrutement des moules d'eau douce dans les secteurs côtiers du Nouveau-Brunswick. De même, les résultats obtenus peuvent s'aviser très utiles pour la conservation des moules d'eau douce dans la région étudiée.

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