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Comparing responses in the performance of sentinel populations of stoneflies (Plecoptera) and slimy sculpin (*Cottus cognatus*) exposed to enriching effluents

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ABSTRACT

Programs in Canada that assess the effects of wastewater discharges on organisms, such as Environmental Effects Monitoring (EEM), primarily focus on fish populations and benthic invertebrate communities. Although these methods are widely accepted, there are many situations where fish monitoring is difficult and benthic community data is difficult to interpret; in these instances alternative approaches should be used. There are, however, few alternative methods available. One potential alternative is to use invertebrate population endpoints to determine effects in the receiving environment. In this study we examined effects of sewage and pulp mill effluents in the Saint John River, New Brunswick, on two stonefly genera (Plecoptera, Perlidae, *Acroneuria* spp. and *Paragnetina* spp.) and compared the responses to those of a small-bodied fish, the slimy sculpin (*Cottus cognatus*). Stonefly measurements included condition, developmental stage, gonad weight, and size upstream and downstream of sewage and a pulp mill discharge. Condition, developmental stage, and absolute gonad weight were greater in *Paragnetina* spp. downstream of the sewage discharge. *Acroneuria* spp. showed persistence of the late developmental stage downstream of the sewage inputs. Slimy sculpin exposed to sewage effluents also showed increased condition, but the impacts downstream of the pulp mill effluent were inconsistent in both sculpin and *Paragnetina* spp. Our findings suggest that stonefly populations and slimy sculpin respond to effluents in similar ways and that the responses of large long-lived invertebrate populations, such as stoneflies, may be a viable alternative to fish population monitoring in environmental assessments of point source discharges.

1. Introduction

Canadian Environmental Effects Monitoring (EEM) programs apply to pulp and paper mills and metal mines and are being developed for municipal discharges. The EEM program requires that regulated dischargers periodically evaluate whether effluents in compliance with national discharge guidelines have residual biological effects on 'fish', 'fish habitat', and 'the use of fish resources' (Glozier et al., 2002; Ribey et al., 2002; Walker et al., 2002). In exposed and reference areas, the 'fish' survey measures changes in the population performance of a sentinel species and alterations to 'fish habitat' are estimated using the structure of the benthic invertebrate community (Environment Canada, 2005). In EEM programs effects are used to direct the focus of future studies to determine the potential cause of the previously documented effects; facilities with confirmation of no impacts can reduce the intensity of their monitoring.

There are many situations where the design options and interpretation of adult fish surveys are limited due to the local distribution of a species, confounding factors, sampling restrictions, other concerns about threatened or endangered species, and difficult or dangerous sampling conditions (Courtenay et al., 2002; Munkittrick et al., 2002). In instances where an appropriate fish sentinel cannot be identified or where spatial or ecological factors limit the study design, an investigator can use either a modified or an alternative method that has been approved by Environment Canada, such as mesocosms, caged bivalves, marine bivalves, or a non-lethal fish study (Environment Canada, 2005). In some circumstances, no alternative or modified methods are available. For instance, effluent from some metal mines is discharged to small receiving streams that have no resident fish species. Studies in these environments have relied on information from the benthic invertebrate community (Lowell et al., 2007). While both sets of information are useful, fish population and benthic invertebrate community endpoints respond differently

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(Lowell et al., 2003) and it is not clear if the subtle differences in the responses are because the studies use different organisms (fish vs. invertebrates) or that the analyses focus on different levels of biological organization (population vs. community). Developing more viable alternatives for EEM studies will enhance the effectiveness of the program and the detection of environmental effects of effluents. We suggest that it is possible to expand the monitoring options in freshwaters by measuring the performance of lotic invertebrate populations.

Aquatic invertebrate populations have many attributes that are useful for a monitoring program: they are sedentary, abundant, and locally and regionally widespread. These populations meet the criteria used to select fish sentinels: they should be consistently exposed to the stressor(s) of interest (low mobility), abundant in the receiving waters (meaningful study designs can be developed), and their collection should yield interpretable data (important life history characteristics can be measured; Munkittrick, 1992; Munkittrick et al., 2000). They respond to environmental factors, such as temperature and food availability in measurable ways including changes in growth, diapause, voltinism, or size-at-maturity (Anderson and Cummins, 1979; Rempel and Carter, 1987; Beer-Stiller and Zwick, 1995; Taylor et al., 1998; Lowell and Culp, 1999; Nesterovitch and Zwick, 2003). Changes in the rearing conditions of the larvae can have direct consequences on the adult stage and the reproductive performance of the population. For instance, decreased food for larvae can reduce the weight of adults (Anderson and Cummins, 1979; Beer-Stiller and Zwick, 1995) and lighter and smaller females may have lower fecundity (Peckarsky and Cowan, 1991). This effect on adults is exacerbated in species that do not feed following emergence, such as the stoneflies in the genus *Acroneuria* (Peckarsky, 1979).

Other characteristics of invertebrates, in particular of stoneflies, are useful in developing meaningful studies. The sex of stonefly larvae can be externally identified (Beer-Stiller and Zwick, 1995) and their growth and developmental stage are easily measured. When stoneflies of some genera emerge, females possess fully developed ovaries that can be used to estimate reproductive performance (Peckarsky, 1979; Lillehammer et al., 1989). Stoneflies are found in many receiving environments across North America where identifying subtle impacts on fish can be difficult and they have relatively short life-spans that will reflect recent environmental conditions. These characteristics suggest that the performance of stonefly populations can be used to identify the anthropogenic disturbance of ecosystems. Although several studies have examined the effects of stressors on invertebrate life history characteristics, including growth, survival, and reproduction (Kreutzweiser et al., 1992; Johnson et al., 1993; Kiffney and Clements, 1996; Alexander et al., 2008), few have used this approach to examine sublethal responses to complex municipal or industrial effluents in field settings (Lowell et al., 1995).

The Saint John River at Edmundston, New Brunswick, Canada, receives multiple discharges from municipalities and industries. Previous monitoring for the Canadian pulp and paper EEM program has shown that condition and relative abundance of slimy sculpin is increased downstream of the sewage discharges (Galloway et al., 2003; Arciszewski et al., 2007). This species was chosen over other potential sentinels (Galloway et al., 2003) because it is abundant, has a home range of less than 10 m (Gray et al., 2004; Cunjak et al., 2005) and high reproductive effort in both sexes (Van Vliet, 1964), and responds to nutrient sources in measurable ways (Gray et al., 2002; Galloway et al., 2003). Increases in invertebrate abundance have also been found

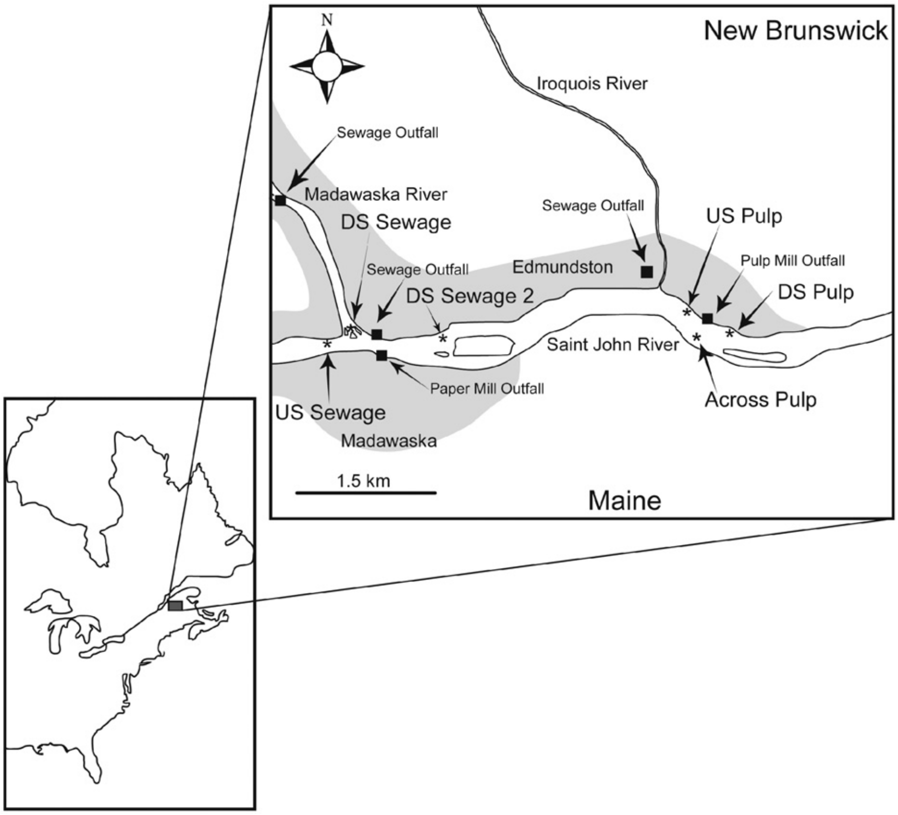

Fig. 1. Position of study area in northwest New Brunswick; inset shows the Edmundston reach of the Saint John River with the sites sampled for stoneflies and slimy sculpin (*) and the point sources of contamination (■); two additional sewage outfalls are found on the Madawaska River beyond the boundaries of the map; shading shows the approximate municipal area on the north and south shores of the Saint John River for Edmundston and Madawaska, respectively.

downstream of pulp mill effluent releases (Arciszewski et al., 2007). Given the considerable information that exists in this reach on how nutrients are affecting fish populations and invertebrate communities, it was an appropriate site to compare the responses of fish and invertebrate populations to municipal and industrial effluents.

The main objective of this study was to evaluate growth (size and condition), survival (size distribution and developmental stage), and reproduction (gonad size) in stoneflies at a well-characterized EEM site and to assess whether invertebrate populations may be a suitable substitute for sentinel fish population assessments in EEM programs. Stoneflies in the Perlidae family were collected upstream and downstream of sewage and pulp mill outfalls in the Saint John River. Changes in growth, survival, and reproduction of the stoneflies were compared to previous and concurrent collections of slimy sculpin. The purpose of this study is not to determine the mechanisms and specific causes (i.e., nutrients or temperature) of the responses, but to understand how the performance of fish and invertebrate population-level endpoints compare in their response to complex municipal and industrial effluents.

2. Methods

2.1. Study site

The Saint John River flows almost 700 km from the north woods of Maine (USA) through New Brunswick (Canada) to the Bay of Fundy (Fig. 1). The first substantial developments along the river are at the municipalities of Edmundston, New Brunswick (20,000 people), and Madawaska, Maine (population $<10,000$), that straddle the Saint John about 200 km from the headwaters; upstream of Edmundston the catchment is heavily forested with little human activity (Curry and Munkittrick, 2005). The study reach is approximately 20 km long, with an average width of less than 75 m, a mid-channel/mid-summer depth of less than 2 m, a predominant cobble/gravel substrate (B.A.R. Environmental and Gore and Storrie Ltd., 1994), and an average discharge in the summer that varies from ~20 to 200 m^3 per second (USGS Station 01014000).

The Edmundston reach has multiple municipal wastewater outfalls (Fig. 1). There are three separate sewage discharges into the upstream Madawaska River, some being derived from combined sewer/stormwater outfalls. Approximately 10,000 m^3 of treated sewage is discharged into the Madawaska tributary or the Saint John River proper by the City of Edmundston. Studies have been done in the area since the early 1990s and water quality tests in 2001 and 2005 showed that levels of coliforms and *Escherichia coli* were high in the Madawaska River close to its confluence with the Saint John River (Table 1). Sewage is also being discharged on the American side upstream of the paper mill outfall at a rate of 2200 m^3 per day.

Industrial effluents are discharged into the Saint John River by a pulp mill in Edmundston, NB, and its related paper mill across the Saint John in Madawaska, ME (Fig. 1). The Edmundston pulp mill produces 850 air-dried metric tons (ADMT) of pulp per day on separate sulfite and groundwood production lines. The effluent is treated with a primary clarifier and a three-celled aerated stabilization basin and discharges via a multi-port diffuser located 4 km downstream of the Madawaska River at a rate of approximately 70,000 m^3 per day. The pulp mill effluent (PME) concentration in the river is typically between 1 and 5% of the flow of the Saint John River in the summer (B.A.R. Environmental and Gore and Storrie Ltd., 1994; Arciszewski et al., 2007). The paper mill in Madawaska primarily processes pulp from the Edmundston facility, and discharges 30,000 m^3 of effluent per day from a secondary treatment facility located on the American side of the Saint John River, opposite the confluence of Madawaska River.

Water quality data have been collected for other studies over several years at sites that overlap spatially and temporally with this study. In 2005, total nitrogen (TN) at St. Hilaire (approximately 10 km upstream of Edmundston on the Saint John River) was 0.5 mg/L and total phosphorus (TP) was 0.008 mg/L (Culp et al. 2006). In 2006, TN was <0.5 and 0.70 mg/L and TP was 0.33 and 0.40 mg/L upstream and downstream, respectively, of the pulp mill discharge (Arciszewski et al., 2007). Because no nutrient measurements were done immediately upstream and downstream of the sewage outfalls, their influence on TP or TN is unknown but likely high because of the elevated coliforms measured in the Madawaska River, the number and volume of the sewage discharges, and the high TP measured upstream of the pulp mill outfall, and downstream of the sewage inputs, in 2006.

2.2. Slimy sculpin collection and endpoints

Slimy sculpin were collected in both 2005 and 2006. Sculpin were collected at 4 sites in July (5–7, 15–17), August (15–19), and September (12–14). Four sites were sampled in 2005 because of the availability of habitat and success of fish capture: US Sewage, DS Sewage, US Pulp, and DS Pulp. In 2006, sampling was conducted in June (6–8), August (14–18), and September (16–18) and focused on in the same four sites as in 2005, an additional site downstream of the sewage (DS Sewage 2), and a site on the American side of the river across from the pulp mill outfall (Across Pulp).

Slimy sculpin were collected using a backpack electrofisher (Smith-Root Model 12B, Vancouver, WA, USA). In 2005 and 2006, a minimum of 10 fish per date were collected at each sample site (2005—four sites; 2006—six sites) and all fish were measured for total length (± 1 mm) and wet weight (± 0.001 g). There are no sex-related differences in the condition of slimy sculpin during the summer months (Brasfield, 2007) and the sexes were pooled during the analyses for this study. Slimy sculpin condition data were expressed by Fulton's condition factor [$k=100{,}000(\text{wet weight}/\text{total length}^3)$]. Fish were lethally sampled for a concurrent study, but no gonad weights were collected for the assessment of among-site differences in GSI; slimy sculpin begin developing their gonads in the fall (Brasfield, 2007) and a previous study found no differences in sculpin GSI at the same sites in October (Galloway et al., 2003).

Instead of GSI estimates (because of low gonad development in the summer), we collected 100 slimy sculpin at all sites (except DS Sewage) in August 2006 to evaluate reproductive success. The proportion of young-of-the-year (YOY) sculpin and catch-per-unit-effort of this year class were used to assess the reproductive success at each sample site. These fish were sampled non-lethally, weighed and measured with the same precision as the adults, and returned to the sites following collection. The YOYs were discriminated from adults during analysis using length frequency diagrams at each site (Arciszewski et al., 2007). Methods for the collection and sampling of slimy sculpin were approved by the Animal Care Committee at the University of New Brunswick.

2.3. Stonefly collections and endpoints

Sampling events were conducted for stoneflies (*Acroneuria* spp. and *Paragnetina* spp.) four times in 2006 (June, July, August, and September). The sites were upstream (US Sewage) and downstream (DS Sewage 2) of the Madawaska River confluence, and upstream and downstream of the pulp mill diffuser (US Pulp and DS Pulp; Table 2, Fig. 1). An additional site was sampled in August on the American side of the Saint John River (Across Pulp; downstream of the paper mill). Stoneflies of both genera and sexes were not collected at all sites. For this reason, some of the intended analyses, therefore, could not be conducted. These included the temporal analyses of *Paragnetina* spp., several of the spatial comparisons of *Acroneuria* spp., and most of the male stonefly analyses. Initial analyses indicated that there is no sex-related difference in development within genus and sexes could be pooled for the analysis of developmental stage. Stonefly data were compared between sites within dates at the level of genus (*Acroneuria* spp., *Paragnetina* spp.).

Table 1
Total coliform and *Escherichia coli* data from the Madawaska River in the Edmundston area collected in 2001 and 2005 by the City of Edmundston; adapted from Société d'aménagement de la rivière Madawaska et du lac Témiscouata inc. (2005); the site downstream of the sewage inputs is in an impoundment.

Location on the Madawaska River	Approximate distance from confluence with the Saint John River (km)	Year	Total coliforms (cells/mL)	*E. coli* (cells/mL)	Water temperature (°C)
Upstream of St. Jacques	15	2001	2590	20	19.6
Downstream of St. Jacques	10	2001	4030	60	20.7
Downstream of sewage inputs	1	2001	80,000	1600	21.8
Upstream of St. Jacques	15	2005	> 5000	< 100	–
Downstream of St. Jacques	10	2005	> 5000	< 100	–
Downstream of sewage inputs	1	2005	> 40,000	> 5000	–

A pooled-sexes group of *Acroneuria* spp. was compared for differences over time (from June to September) between US Sewage and DS Sewage 2 in development. Condition, dry weight, and thorax length of female *Acroneuria* spp. was compared between US Sewage and DS Sewage 2 in June and July 2006. *Paragnetina* spp. females collected in August 2006 were tested for differences in thorax length, dry weight, condition, GSI, and absolute gonad weight between all study sites. A pooled-sexes group of *Paragnetina* spp. was compared in August 2006 for changes in developmental stage across sites. Sample sizes for the *Acroneuria* spp. analyses ranged from 3 to 43 and from 3 to 11 for analyses of *Paragnetina* spp.

The stoneflies were collected incidentally during electrofishing for slimy sculpin, with a kick net, or by turning over submerged or semi-submerged rocks and checking them for larvae; all three sampling methods and similar effort were used at each site. The study sites were along the margins of the river where the water was shallow and the predominant substrate was cobble. Stoneflies were frozen in Whirlpaks® for later identification to genus and further processing in the laboratory.

Endpoints for stoneflies were chosen to be similar to those used in the fish survey component of the EEM program, including body size (thorax length and dry weight), energy storage (condition), development, and reproductive investment (gonad weight). All length measurements were done on thawed individuals using electronic calipers (±0.01 mm) and included head capsule width (Hcw; the width across the midline of the eyes), wing pad length (Wpl; length of the wingpad on the right side from the tip to the posterior attachment point of the wingpad to the thorax), and thorax length (ThL; measured from the anterior limit of the pronotum to the posterior margin of the third thoracic segment along the dorsal midline). Stoneflies were individually dried for at least 48 h at 60 °C and measured for dry weight to an accuracy of ±0.001 g. Condition (k) was calculated using thorax length and dry weight with the formula $k=10{,}000$ (dry weight/thorax length^3). The sex of stoneflies was determined by the presence of precursors of external genitalia on the ventral body surface of the eighth abdominal segment; there is a short medial interruption of the setal fringe in females (Frison, 1935; Beer-Stiller and Zwick, 1995). Developmental stage was calculated using the methods outlined in Beer-Stiller and Zwick (1995) and used the body segment ratio of wingpad length to head capsule width (Wpl:Hcw). Mature females of the genus *Paragnetina* spp. were collected in sufficient numbers to evaluate among-site differences in ovarian mass and gonadosomatic index (GSI). The ovarian tissue was removed from the abdomen and posterior of the thorax into pre-weighed glass vials after the stoneflies were dried. The vials were then re-weighed and the difference taken to give the mass of the gonad tissue. Gonadosomatic index (GSI) was calculated as the percentage of body mass composed of gonad tissue [100(dry gonad weight/total dry weight)]. Few *Acroneuria* spp. females possessed developed eggs so these stoneflies were excluded from the GSI analyses.

2.4. Data analysis

Thorax length and dry weight of stoneflies were analyzed using analysis of variance (ANOVA) and post-hoc Tukey HSD tests when initial differences were

Table 2
Sites of stonefly and sculpin capture in the Edmundston reach of the Saint John River in 2005 and 2006.

Site	Latitude (dd°mm′ss″ N)	Longitude (dd°mm′ss″ E)	Site description	Distance from US Sewage (m)
US Sewage	47°21′38″	68°19′34″	Sewage reference	0
DS Sewage	47°21′38″	68°19′22″	Sewage exposed	500
DS Sewage 2	47°21′31″	68°18′34″	Sewage exposed	750
Across Pulp	47°21′15″	68°16′15″	Pulp mill reference	4000
US Pulp	47°21′20″	68°16′14″	Pulp mill reference	4000
DS Pulp	47°21′18″	68°16′08″	Pulp mill exposed	4500

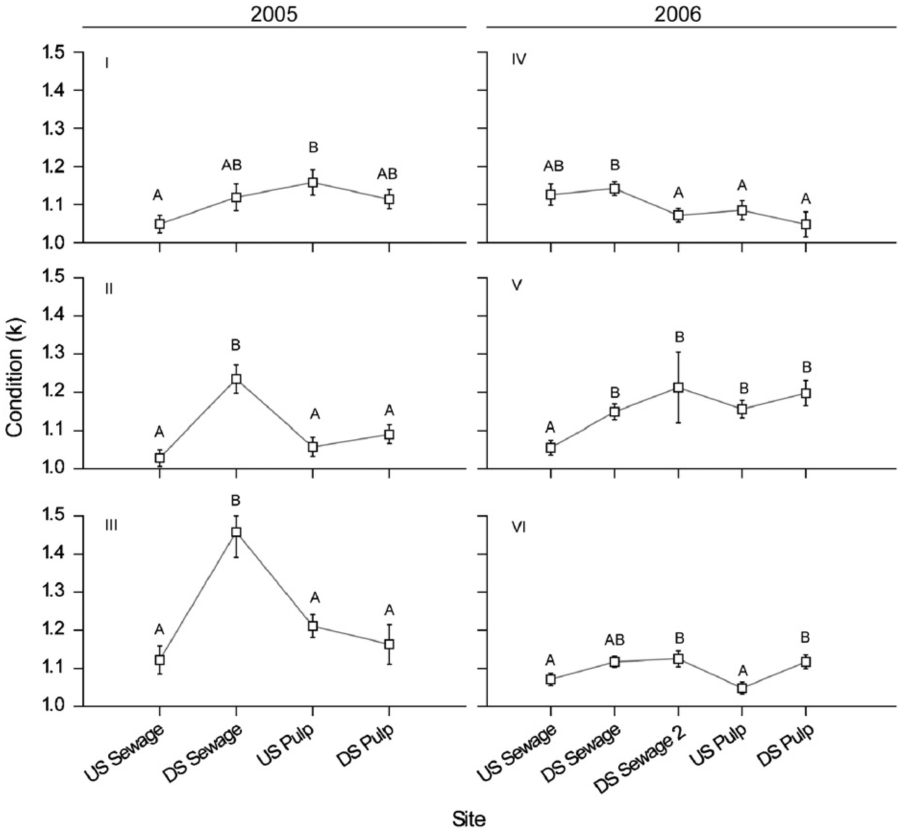

Fig. 2. Mean condition (±1 SE) of slimy sculpin captured in the Edmundston reach of the Saint John River in July (I), August (II), and September (III) of 2005 and June (IV), August (V), and September (VI) of 2006; different letters indicate a significant difference between sites.

significant. Analysis of covariance (ANCOVA) was used to compare changes in condition (dry weight vs. thorax length), developmental stage (wingpad length vs. head capsule width), and GSI (gonad weight vs. dry weight). Absolute gonad weight was analyzed using ANOVA and a Tukey HSD test. Condition of adult and YOY sculpin was analyzed using ANCOVA with length as the covariate to determine whether these data varied between sites within months. Length and weight were analyzed using ANOVA and post-hoc Tukey tests were used to determine the differences when more than two sites were compared. A chi-squared test was used to compare the relative proportions of adult and YOY slimy sculpin in August 2006. Catch-per-unit-effort (CPUE) data of YOY sculpin was not compared statistically. All statistical analyses were conducted on $\log_{10}$ transformed data and evaluated at a significance level of 0.05.

Power analysis was also conducted using the invertebrate data collected in this study to determine the numbers of organisms that may be required in future studies. Sample size estimates were calculated for the condition of *Acroneuria* spp. and *Paragnetina* spp. males and females and GSI of *Paragnetina* spp. females. For the developmental stage endpoint, a pooled-sexes group of *Acroneuria* spp. and a pooled-sexes group of *Paragnetina* spp. were used. The power analyses, used $\alpha=0.05$ and $\beta=0.2$, and estimated the samples required to detect critical effects sizes in the range of 10–50% (see Munkittrick et al., 2009). The variability (SD) was estimated using all of the invertebrates per genus and per sex captured at the US Sewage reference site during all sampling trips in 2006.

3. Results

3.1. Slimy sculpin 2005

In 2005, there were strong responses in the condition of slimy sculpin to the sewage, but fewer differences were found in response to the pulp mill effluent. Sculpin condition was higher downstream of the sewage discharge for some sampling dates. In July 2005, slimy sculpin condition was not significantly different at DS Sewage or DS Pulp when compared to the US Sewage site ($p > 0.086$; Fig. 2), but was significantly higher at US Pulp compared to US Sewage ($p=0.011$; Fig. 2). No among-site differences were noted in length ($p > 0.140$) or weight ($p > 0.350$) of fish exposed to sewage or pulp mill effluent when compared to their respective reference sites (data not shown). In both August and September of 2005, condition of fish at DS Sewage ($p \leq 0.003$), US Pulp ($p \leq 0.025$), and DS Pulp ($p \leq 0.013$) was significantly higher than at US Sewage. There were no other among-site differences on these dates ($p \geq 0.355$).

3.2. Slimy sculpin 2006

In 2006 the among-site differences in condition were smaller and the sewage influence was detected farther downstream when compared to 2005 (Fig. 2). In June 2006, the condition of slimy sculpin was significantly higher at DS Sewage 2, US Pulp, and DS Pulp ($p < 0.035$) compared to US Sewage (Fig. 2), and not different between the US Pulp and DS Pulp sites ($p=0.304$). Condition was higher at all downstream sites in August 2006 compared to US Sewage ($p \leq 0.043$; Fig. 2) and was not significantly different between the US Pulp and DS Pulp sites ($p=0.289$; Fig. 2). In September 2006, the condition of slimy sculpin was significantly higher at DS Sewage 2 compared to US Sewage ($p=0.024$) and US Pulp ($p=0.005$) and there was a significant increase in condition from US Pulp to DS Pulp ($p=0.007$; Fig. 2).

Catch-per-unit-effort of YOY slimy sculpin was highly variable throughout the study reach, but showed spatial patterns related to the discharge of sewage and pulp mill effluent. The CPUE of YOY sculpin was 600% higher at DS Sewage 2 and 570% higher at US Pulp compared to US Sewage (Fig. 3). The CPUE at DS Pulp was 290% higher than at US Sewage.

The mean length of the YOY sculpin also showed large increases downstream of the sewage outfall. The mean length of YOY sculpin was significantly longer at DS Sewage 2 compared to US Sewage ($p < 0.001$) and all other sites ($p < 0.001$; Fig. 3). The mean length of YOY sculpin at DS Sewage 2 and US Sewage was 43.58 and 39.57 mm, respectively. YOY sculpin captured at US Sewage, Across Pulp, US Pulp, and DS Pulp had statistically similar mean lengths ($p \geq 0.051$). Mean weight of YOY sculpin was also significantly higher at DS Sewage 2 compared to all other sites ($p < 0.001$; data not shown). The YOY sculpin captured at all other sites had statistically similar wet weight ($p \geq 0.658$). Condition of YOY sculpin (data not shown) was significantly lower at Across Pulp and US Pulp compared to US Sewage ($p \leq 0.004$) and was significantly lower at US Pulp, Across Pulp, and DS Pulp compared to DS Sewage 2 ($p \leq 0.002$). Condition of YOY sculpin was statistically similar between US Sewage and DS Sewage 2 ($p=0.647$).

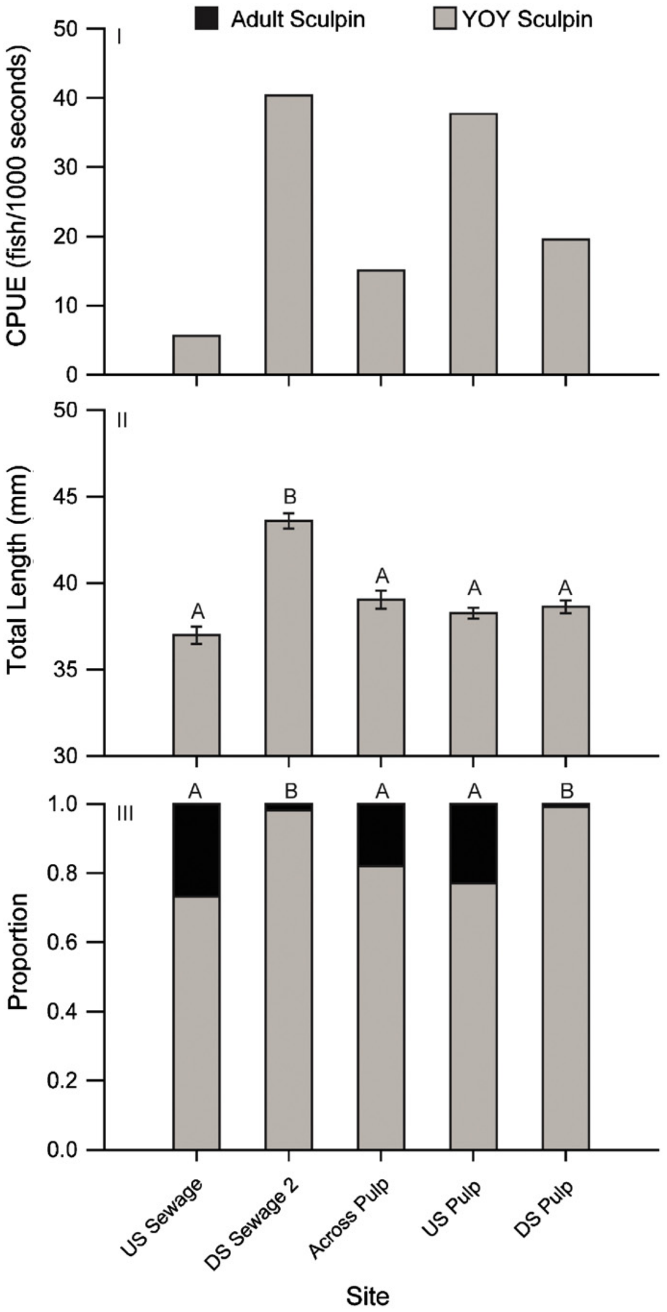

Fig. 3. Catch-per-unit-effort (I), mean length (±SE; II), and proportion of young-of-the-year (YOY) and adult slimy sculpin (III) captured in the Edmundston reach of the Saint John River in August of 2006; different letters indicate significant differences between sites.

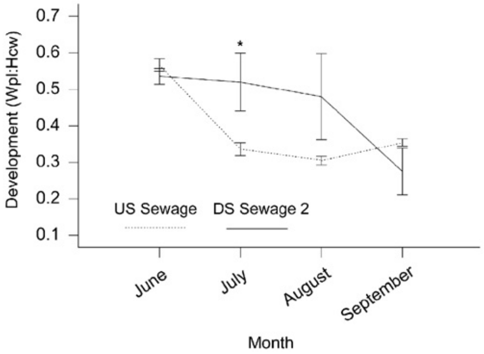

Fig. 4. Mean (±SE) developmental stage of *Acroneuria* spp. at US Sewage (dashed line) and DS Sewage 2 (solid line) in the Edmundston reach of the Saint John River across months in 2006. Asterisk (*) indicates statistical differences ($p<0.05$) between sites within month.

The proportion of YOY sculpin increased downstream of both the municipal and pulp mill discharges. In August of 2006 the relative proportion of YOYs was significantly higher downstream of the sewage inputs (DS Sewage 2, $p<0.001$; Fig. 3) and the pulp mill outfall (DS Pulp; $p<0.001$) compared to US Sewage, Across Pulp, and US Pulp. The proportion of YOYs at DS Sewage 2 and DS Pulp was not significantly different ($p \geq 0.764$).

3.3. *Acroneuria spp.*

Individual *Acroneuria* spp. (sexes pooled) were significantly more developed at DS Sewage 2 compared to US Sewage in July ($p=0.030$), but not in June, August, or September ($p>0.05$; Fig. 4). Condition of female *Acroneuria* spp. also increased downstream of the municipal wastewater discharges. Female *Acroneuria* spp. had higher condition (by 24%) downstream when compared to upstream of the sewage in July ($p<0.001$; data not shown); there was a similar but non-significant increase in their condition of 9% from upstream to downstream of the sewage inputs in June 2006 ($p=0.080$; data not shown). In June 2006, there was no significant difference in the thorax length of female *Acroneuria* spp. ($p=0.980$) or dry weight ($p=0.526$; data not shown). Similarly, in July 2006, there was no significant difference in thorax length ($p=0.158$) or dry weight ($p=0.120$; data not shown).

3.4. *Paragnetina spp.*

In August 2006, the second stonefly genus collected in this study, *Paragnetina* spp., showed a 27% increase in condition in females at US Pulp ($p=0.010$) and an 11% increase at both DS Sewage 2 ($p<0.001$) and DS Pulp ($p<0.001$) when compared to US Sewage (Fig. 5). On this date there were no statistical differences in length ($p>0.090$; data not shown) or dry weight between any site ($p \geq 0.090$; Fig. 5) for female *Paragnetina* spp. Developmental stage of the pooled male and female *Paragnetina* spp. was 24% higher ($p<0.001$) at DS Sewage 2 and 23% higher at US Pulp ($p=0.020$) when compared to US Sewage (Fig. 5). At the DS Pulp site, developmental stage of pooled male and female *Paragnetina* spp. was similar to that of stoneflies collected at US Sewage ($p \geq 0.330$) and US Pulp ($p \geq 0.120$).

Some of the reproductive endpoints assessed for the stoneflies also showed differences between sites upstream and downstream of the wastewater discharges. In August 2006, GSI of female *Paragnetina* spp. did not differ between sites ($p \geq 0.250$; Fig. 6), but absolute gonad weight was greater at DS Sewage 2 compared to US Sewage ($p=0.014$; Fig. 6). Females with eggs were twice as common at DS Sewage 2 compared to US Sewage. Gonad weights

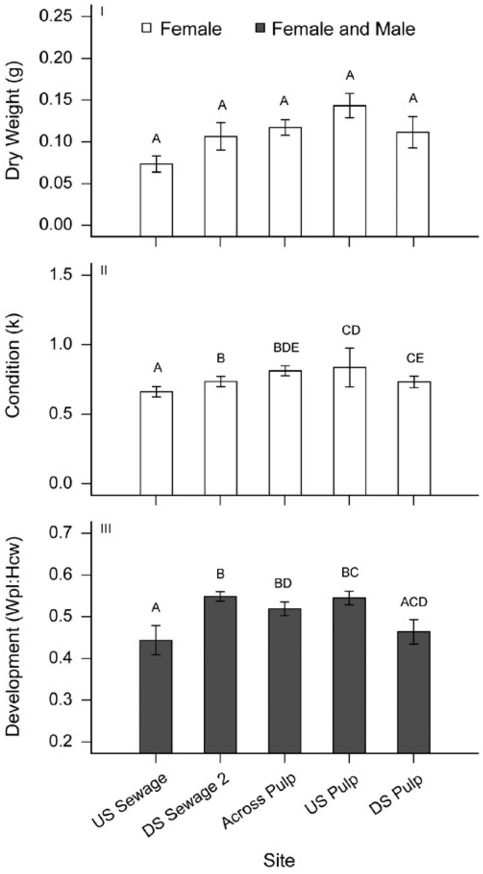

Fig. 5. Mean (±SE) dry weight (I), condition (II), and developmental stage (III) of *Paragnetina* spp. captured in the Edmundston reach of the Saint John River in August of 2006. Different letters denote significant differences ($p<0.05$) in females between sites; developmental stage used a pooled group of males and females; different letters indicate statistical differences of females between sites.

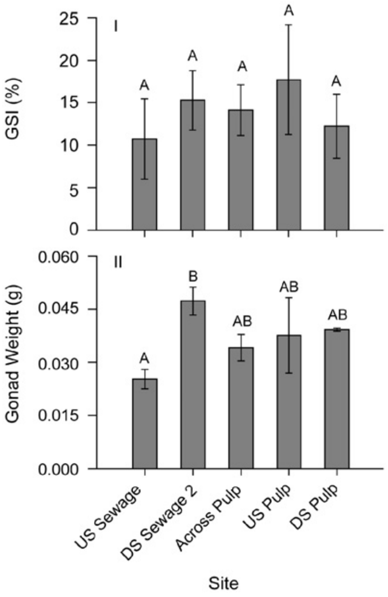

Fig. 6. Mean (±SE) gonadosomatic index (I) and mean (±SE) absolute gonad weight (II) of female *Paragnetina* spp. captured in the Edmundston reach of the Saint John River in August 2006; different letters indicate significant differences between sites.

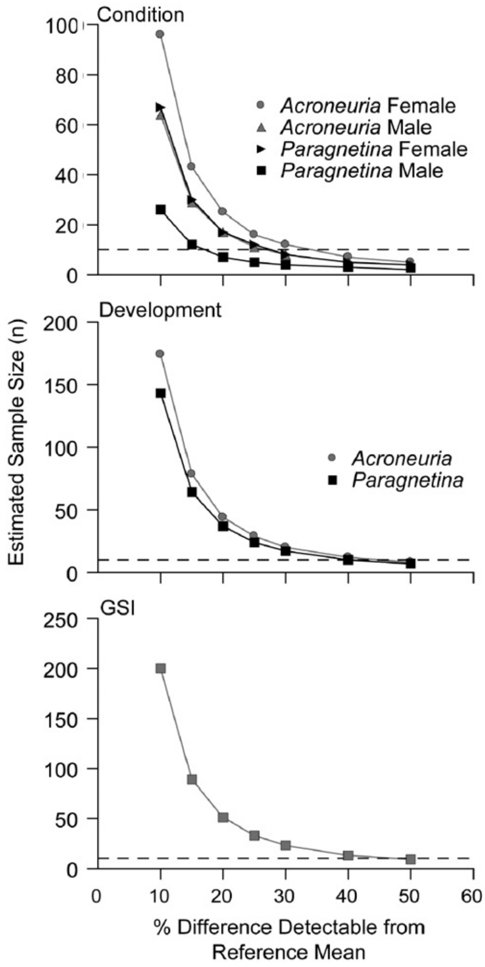

Fig. 7. Sample size requirements to detect differences from the mean (%) for condition (I), developmental stage (II), and GSI (III) in stoneflies; horizontal dashed line indicates where $n=10$; GSI analysis done with *Paragnetina* spp. females only.

of female *Paragentina* spp. at US Pulp, Across Pulp, and DS Pulp were intermediate between US Sewage and DS Sewage 2 ($p \geq 0.108$; Fig. 6).

3.5. Power analysis of invertebrate data

Power analyses conducted for the stoneflies showed that sample sizes needed to detect a 10% difference in all endpoints ranged from 26 to 200 (Fig. 7). Condition measurements were variable between sexes and genera. The *Acroneuria* spp. females had the highest estimated sample size ($n=96$) at a 10% difference. Males from the *Paragentina* genus had the lowest sample size requirements ($n=26$). For condition analyses, all sex and genus combinations converged near an effect size of 25%; all sample sizes at 25% were near 20.

Separate power analyses were conducted for developmental stage for each genera with a pooled-sexes group. Both genera showed similar estimated sample sizes for each critical effect size used. In *Acroneuria*, an estimated sample size of 174 was needed to detect a 10% difference in development; in *Paragnetina* this value was 143. For both groups, a sample size of 10 could detect a 40% difference in development and the estimated sample sizes converged between the two genera at a critical effect size of approximately 20%.

The power analysis for GSI was done for *Paragnetina* spp. females only. In this analysis, a sample size of 10 could detect a critical effect size of 50% in GSI between sites, while detecting a difference of 10% required a sample size of 200 stoneflies.

4. Discussion

In both years of our study, adult slimy sculpin showed increases in condition downstream of the sewage inputs in the Saint John River near Edmundston, NB. Increases in fish condition may be linked to changes in food quality or quantity (Pothoven et al., 2001). YOY sculpin also showed strong responses to the discharge of sewage. In YOY sculpin, the relative abundance and growth (mean length) was higher downstream of the sewage inputs. The responses of YOY sculpin are more complex than the differences in adults between sites, but could also be related to changes in food quality or quantity. The lack of difference in YOY condition downstream of the sewage inputs could be attributed to a density-dependent effect since relative abundance of YOYs was elevated at this site. Little work has been done on the responses of young fish in environmental monitoring programs (Gray et al., 2002), but this life stage may yield important information on the environmental impacts of enriched effluents. Nutrient enrichment is suspected in the Edmundston reach of the Saint John River, and previous studies have shown increased abundance of benthic macroinvertebrates, earlier emergence (Culp et al., 2003), increased size and condition of slimy sculpin (Galloway et al., 2003) and faster growth and increased size of fathead minnow in stream side bioassays (Parrott et al., 2003, 2004). Previous studies during the EEM program suggested that there were several sources of nutrients in the reach, including sewage outfalls, the paper mill, and the pulp mill (B.A.R. Environmental, 1996; ESG International, 2000). Cycle Three EEM studies (2000–2003) also suggested that nutrient enrichment was affecting fish populations in the Edmundston reach (Galloway et al., 2003).

Similar to the adult and YOY sculpins, stonefly populations showed clear responses downstream of the sewage discharge. In the invertebrates, higher condition and increases in development were found in *Paragnetina* spp., and the continued collection of more developed individuals was found in *Acroneuria* spp. Increased development of *Paragnetina* spp. could be related to increased food quality or quantity; other researchers have found accelerated development downstream of nutrient-rich discharges at these sites (Culp et al., 2003). Collecting stoneflies at an advanced developmental stage over time may be due to the accelerated development of multiple cohorts, or increased survival of a single cohort (and their continual emergence), rather than the non-emergence of that cohort (Harper and Pilon, 1970).

In contrast to the strong responses to sewage in both the sculpin and stoneflies, there were inconsistent responses in these organisms to the pulp mill effluent. Adult slimy sculpin responded intermittently to the pulp mill effluent. At the pulp mill sites, compared to US Sewage, YOY sculpin had higher relative abundance and lower condition, suggesting a reach effect of sewage on these endpoints. In *Paragentina* there was a positive response in condition and a negative response in development downstream of the pulp mill outfall. The discrepancy in condition and development in the *Paragnetina* spp. exposed to the pulp mill effluent is further complicated since both of these stonefly endpoints responded positively to the discharge of sewage. The order in which multiple effluents are discharged into rivers is an important factor in attributing effects in a study such as this. Masking of the effects of one effluent by the impacts of another is a problem in this type of study (Scrimgeour and Chambers, 2000; Chambers et al., 2000; Dubé et al., 2006). The sewage plume likely intermittently envelops both the US Pulp and DS Pulp sites, making separation of the influence of these effluents (and mixing) dependent on the recent flow conditions in the river (Jeffries et al., 2008).

The strength of the sewage impacts is likely due to the location of the discharge. The clear changes in condition and development downstream of the sewage outfalls in our study system are likely linked to the lack of confounding factors in the reference area. The nearest point source of anthropogenic contamination upstream of the sewage inputs is a poultry processing facility approximately 40 km upstream (Galloway et al., 2003), and fish condition and liver size returns to ''normal'' (equivalent to sites upstream of any effluent inputs to the river) before 15 km upstream of the Madawaska confluence. The influence of the sewage on the US Pulp site, documented in the condition of adult fish and the relative abundance of YOY, is unusual and occurs only during the lowest flows; in most data collections we have done at US Pulp, the population endpoints are approximately equal to the values at the US Sewage site. We also have some evidence (i.e., sculpin condition in August 2006) that the sewage plume dissipates before the US Pulp site, but that a signature of nutrient enrichment re-emerges downstream of the pulp mill discharge. This suggests that the effluents may be complementary, but, compared to US Sewage, increased condition was found at US Pulp and decreases (or no differences) were also documented at DS Pulp, relative to the US Pulp site. This suggests that there are complex physical, chemical, and biological relationships between the two effluents in the study reach.

Although the order in which the effluents are discharged to the river likely influences the spatial patterns of responses in the Saint John River, differences in the magnitude of responses to the two effluent types may be attributable to innate differences in the effluents. Sewage and pulp mill effluents are both complex (and unique) mixtures of chemical, physical, and biological components. Nutrients, bacteria, and temperature are commonly elevated below outfalls of both effluent types (Chambers et al., 2000), but other components may be specific to one type of discharge. In addition, both common and unique components of the effluents can fluctuate on varying schedules. Both effluents could also contain unique androgenic or estrogenic compounds specific to vertebrates (Thornton et al., 2003) or invertebrates (Kreutzweiser, 1997). Emerging contaminants such as pharmaceuticals and their derivatives have been found in sewage (Lishman et al., 2006) and compounds that disrupt the endocrine system of fish have been found in pulp mill effluent (i.e., Hewitt et al., 2008); it is not known how much overlap there is in the activity or structure of compounds found in industrial and/or municipal effluents. The presence of compounds that affect invertebrates may be more common in pulp mill effluents if they are present in the trees as a defense against consumption or colonization by insects (Kreutzweiser, 1997). It is not known what happens to these natural insecticides during the pulping process, but hormone mimics may also be formed during the high heat/high pressure cooking of liquor and wood chips, or the original compounds may not be destroyed (Hewitt et al., 2008). These compounds could have complex effects on the physiology of invertebrates, but not affect fish, or vice versa.

There were similar responses in fish and invertebrate populations to sewage. The results from this study suggest that longer-lived invertebrates like stoneflies can be used as sentinels in ecotoxicological field studies. An important advantage is that the techniques used here do not require extensive training or expensive analyses. Although this study would be greatly enhanced by the inclusion of biochemical or physiological endpoints, that was not the purpose of this study. We wanted to know if invertebrates could be used in areas where fish are not available as sentinels. This preliminary study on the use of established invertebrate growth and development indicators showed impacts to the performance of populations, but some important lessons were also learned. For instance, condition estimates should be done separately for males and females (because of sexual size dimorphism), but developmental stage is not affected by sex. The analyses for developmental stage combined the sexes into a single estimate and had stronger results than those for other endpoints. For this reason, developmental stage may have an important advantage over other endpoints in that the statistical power can be easily elevated by combining the sexes.

No previous data was available for our study area on the size, condition, and development of stoneflies, effects sizes, or on the sample sizes needed to detect significant changes in response to anthropogenic activity. In our study we tested whether or not the sampling effort used for fishes in this reach ($n=10$) would be adequate to detect differences in invertebrate populations. In many instances, the sample size of 10 per sex was adequate to detect significant differences of 40–50% in GSI and development, but to detect smaller differences of 10–20%, larger sample sizes are required. Condition was less variable than either development or GSI and smaller differences (25–35%) were detectable with a sample size of 10 for this endpoint. It is not known, however, how relevant differences of 10–50% are for these invertebrate endpoints. Effect sizes of 10–25% have been adopted in the analysis of fish data in the Canadian EEM program, but this program also uses ± 2 SD to interpret the relevance of the differences in the invertebrate community endpoints (Environment Canada, 2005). There is, however, considerable debate on the applicability, relevance, and consequences of different effect sizes in ecotoxicological field studies (Munkittrick et al., 2009). Further use of invertebrate growth and development in field studies will require an understanding of effect sizes that matter.

The use of aquatic invertebrate populations to assess the effects of effluents is rare. The more common approach is to use benthic invertebrate communities. Populations respond to subtle disturbances before communities do and, therefore, may show early signs of stress that would not be detected in a community survey (Munkittrick, 1992; Munkittrick and McCarty, 1995); community surveys that use indices of diversity detect differences in the number of individuals among taxonomic group, which first requires alterations to mortality and recruitment or potentially immigration and emigration rates. These factors can be affected by both direct and indirect effects. A community response to an effluent is more complex than a population response since indirect effects will also be included in the assessment (Rohr et al., 2006) and the effects must first impact individual populations (Munkittrick and McCarty, 1995). Community assessments may be best used to examine explicit stresses, like smothering or broad processes like recovery from a catastrophic event (Yount and Niemi, 1990). Population assessments can detect subtle and unexpected effects and can help us identify emerging issues, such as endocrine disrupters and the consequent development of intersex in fish (Jobling et al., 1998) or the potential crash of a population (Kidd et al., 2007). In the future, performance of populations may allow us to look at the present organisms and determine the occurrence of subtle impacts despite compliance of a discharger with an applicable legislation, or if further action is required to achieve globally desirable goals.

The characteristics we measured in the stoneflies were selected to be analogs of the endpoints used in the framework of the population performance of fish (i.e, Gibbons and Munkittrick, 1994; Gibbons et al., 1998): growth, survival, and reproduction. In fish studies these characteristics are rarely measured directly, but instead are estimated using reasonable surrogates. The common endpoints in a fish study in EEM are mean age, growth (length-at-age), liver somatic index (LSI), GSI, and condition (Munkittrick et al., 2002). Applying these surrogates to invertebrates may be difficult if there are important ecological distinctions between organisms. For instance, directly measuring the age of an invertebrate would be difficult.

Developmental stage could be a useful surrogate to assess age or cohort differences between sites. Developmental stage in invertebrates is likely more closely linked with growth and maturity and is not necessarily directly related to the age of the stonefly. The size range at which a fish matures can vary greatly (Twomey et al., 1984) as can the size-at-emergence of invertebrates (Peckarsky et al., 2001). Therefore, faster growth in an invertebrate should elicit maturity (i.e., emergence) at a younger age. Increases in the developmental rate could be affected by an increase in the local food resources (Lowell et al., 1995; Culp et al., 2003), but other studies have found that duration of development was not altered by environmental factors (Sweeney et al., 1986). Data on the potential differences in egg incubation time (Marten, 1991) may also be useful in determining the environmental effects of effluents on the development of invertebrates.

An index of gonad size and reproductive investment is another key endpoint in the study of fish populations for EEM (Gibbons and Munkittrick, 1994) and requires further consideration for stoneflies. Relative gonad size (GSI) was measured in the larvae of female stoneflies and responded positively, albeit not significantly, to known nutrient sources in *Paragnetina* spp. Higher absolute gonad weight of *Paragnetina* spp. was, however, found downstream of the sewage inputs compared to upstream; the higher gonad weight was concomitant with a non-significant increase in female body size between sites. Our findings and those from other researchers (Peckarsky and Cowan, 1991; Taylor et al., 1998; Yoshimura, 2003) show that larger body size is linked to an increase in absolute gonad weight and an increase in population fecundity downstream of the sewage inputs. However, increasing fecundity by increasing the body size of an organism may also increase predation pressure (Ball and Baker, 1996; Brodin and Johansson, 2004) and reduce reproductive success. The type of reproduction used by an organism may affect the usefulness of GSI to estimate the effect of environmental conditions. Semelparous organisms, like stoneflies, may incidentally increase reproductive output by increasing their body size (Peckarsky and Cowan, 1991) while iteroparous organisms, like many fishes, may allocate relatively more energy reserves towards (re)developing gonad tissue (Gibbons and Munkittrick, 1994; Lester et al., 2007). In our study it was not uncommon to measure gonad size of larval stoneflies at 30–40% of the total dry body mass which may be a physiological or anatomical limit. This suggests that population fecundity was also higher downstream of the sewage inputs. We did not estimate density of stoneflies, but mild enrichment was found downstream of the pulp mill discharge in a concurrent study: invertebrates were more abundant at the DS Pulp site compared to US Pulp in August 2006 (Arciszewski et al., 2007).

5. Conclusion

Eutrophication downstream of some wastewater discharges or other anthropogenic activities continues to be an issue in Canadian and global rivers (Chambers et al., 2000; Smith et al., 1999). It is well known that the increased nutrient loads can increase fish biomass (i.e., Askey et al., 2007) and that fish populations often have larger gonads and faster growth in enriched areas (Gibbons and Munkittrick, 1994; Munkittrick et al., 2000), but other effects on fish physiology are also commonly reported in response to sewage effluents (i.e., Jobling et al., 1998). Impacts on the benthic community are also commonly reported (Culp et al., 2003). What is unclear is how municipal and industrial effluents affect invertebrates below the level of the community.

Ecosystems respond to disturbances in complex ways that can be difficult to document and understand, and then predict and mitigate. In many areas, studies to determine ecologically significant and relevant effects of an effluent are limited by many factors, including the difficulties of identifying appropriate sentinels, the presence of confounding factors, and satisfying the varied opinions of environmental biologists. The results of our study suggest that benthic invertebrate populations may be a viable option in monitoring programs and can help us understand the ecological consequences of discharging municipal and industrial wastewater into rivers. Benthic invertebrates are ubiquitous, easily captured, and acknowledged as useful biological indicators in community assessments. We have shown the potential for improving biomonitoring approaches by including population estimates of fecundity and developmental stage of invertebrates. Invertebrate life cycles are complex and their responses to environmental changes can be equally complex (Sweeney and Vannote, 1978; Sweeney et al., 1995). Stonefly populations appear useful in indentifying responses to effluents, but more work will be required to understand the different responses between species (Alexander et al., 2008), how populations are affected by multiple stressors, like temperature and food level (Sweeney et al., 1986), and how invertebrate populations respond to emerging stressors such as endocrine disrupters (Soin and Smagghe, 2007). Although more work is required to fully understand the responses of a benthic population to various stressors and their broader usefulness as indicators, stoneflies offer an opportunity to enhance the efficiency and efficacy of monitoring programs and help increase confidence in management decisions.

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