Effects of thermal effluent on the diversity and distribution of benthic invertebrates in the St. Lawrence River

Kayla Hamelin
Department of Biology
McGill University, Montréal

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ABSTRACT

Temperature is a primary physical constraint on the distribution and abundance of aquatic organisms. Increasingly, human activities modify natural thermal regimes of aquatic systems, thereby altering the composition and structure of the organismal community. For decades, the Gentilly-2 Nuclear Power Plant (G2NPP) in Bécancour, QC, has discharged coolant water into the St. Lawrence River at temperatures more than 10ºC above ambient. My study aimed to quantify how the diversity, composition, and abundance of the St. Lawrence benthic macroinvertebrate community has been altered by G2NPP thermal effluent. Benthic samples were collected at sites along the thermal gradient downstream of G2NPP in May and September 2012. Environmental predictors varying with distance downstream of G2NPP (e.g., temperature, depth, flow rate) were recorded at each station. Macroinvertebrate diversity and abundance were measured for each site and analyzed in relation to local environmental variables. Taxonomic richness and abundance (density and biomass) were elevated close to the power plant, but these patterns were best explained by environmental variables such as depth and sediment type. Taxonomic evenness was reduced in the warmest sites, and this pattern was driven by a few highly tolerant taxa, including thermophilic invasive species like the Asian clam, *Corbicula fluminea,* which was amongst the most dominant species in terms of abundance and biomass. Despite the strong thermal gradient at G2NPP, diversity indices and characteristics of community composition primarily reflected abiotic habitat characteristics other than temperature, whereas temperature was an important predictor only for a minority of taxa, like *C. fluminea*. With the closure of G2NPP in December 2012, and the subsequent eradication of cold-sensitive *C. fluminea*, these results provide a baseline for monitoring the succession of the benthic community in future years.

RÉSUMÉ

La température de l’eau est une composante principale dans la distribution et l’abondance des organismes aquatiques. Aussi, l’essor des activités anthropogeniques modifient les régimes thermiques des écosystèmes aquatiques et changent par le fait même la composition et la structure des communautés d’organismes. Pendant des décennies, la centrale nucléaire Gentilly-2 (CNG-2) à Bécancour, QC, a déchargé ses eaux de refroidissment dans le Fleuve St-Laurent à des températures supérieures à 10ºC la température ambiante du cours d’eau. L’objectif de mon étude a été de quantifier les patrons de diversité, de composition, et d’abondance de la communauté benthique de macroinvertébrés du Fleuve St-Laurent affectée par l’effluent thermique de la CNG-2. Des échantillons benthiques ont été récoltés à différentes stations le long du gradient thermique en aval de la CNG-2 en mai et septembre 2012. Les variables environnementales qui variaient en fonction de la distance par rapport à la source de l’effluent thermique (e.g., température, profondeur, courant) ont été enregistrés à chaque station. La diversité et l’abondance des macroinvertébrés ont été mesurées à chaque station et analysées par rapport aux variables environnementales locales. La richesse taxonomique et l’abondance (densité et biomasse) étaient élévées près de la centrale nucléaire, mais ces observations étaient davantage liées à des variables environnementales comme la profondeur et le type de sédiments. La regularité taxonomique était réduite aux stations les plus chaudes expliquée par la présence de quelques taxa seulement tolérant aux températures élevées, dont des éspèces envahissants thermophiles comme la petite corbeille d’Asie, *Corbicula fluminea*, qui était parmi les plus dominantes en termes d’abondance et de biomasse. Malgré le gradient thermique prononcé à la CNG-2, les indices de diversité et les caractéristiques de la composition de la communauté benthique reflètent essentiellement l’importance de caractéristiques abiotiques de l’habitat autres que la température, une componsante importante pour une minorité d’espèces seulement, telle *C. fluminea*. Avec la fermature de la CNG-2 en décembre 2012, et l’éradication de *C. fluminea*, les resultats présentés seront une référence au cours des prochaines années quant au suivi de la succession de la communauté benthique.

PREFACE

Contributions of Authors

This thesis was prepared according to the "Guidelines for Thesis Preparation" designated by the Faculty of Graduate and Postdoctoral Studies Office. It was completed under the supervision of Dr. Anthony Ricciardi in the Redpath Museum at McGill University, who helped to conceptualize the project, provided guidance during the execution of the research, and provided editorial comments throughout the manuscript. I designed and executed the field research, conducted the laboratory work, performed the statistical analyses, and wrote the manuscript. Temperature logger data used in one of my analyses were collected by Dr. Anouk Simard at the Québec Ministry of Sustainable Development, Environment, Wildlife and Parks (Développement Durable de l'Environnement de la Faune et des Parcs). Data for the analysis of invertebrate community biomass were collected by Honours student Andrea Morden, under my supervision. The biomass data were previously submitted as part of the Honours thesis of A. Morden, and were re-analysed by me for inclusion in this thesis. A. Morden also assisted with the collection of data from the settling plate pilot project under my supervision.

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CHAPTER 1: General Introduction

Modification of aquatic ecosystems

Covering less than 1% of earth's surface (Gleick 1998), freshwater environments host a disproportionately rich diversity of species and habitat types, and provide significant ecosystem services (Collen et al. 2013). However, human activities have altered the fundamental physical characteristics of large freshwater ecosystems on a global scale (Carpenter et al. 2011). Modification of river flow (e.g., Nilsson and Renöfält 2008; Poff et al. 1997), nutrient pollution (e.g., Hilsenhoff 1977), and changes to thermal regimes (Wellborn and Robinson 1996, Caissie 2006) have resulted in substantive consequences for biodiversity (Ricciardi and Rasmussen 1999). Aquatic organisms, primarily poikilotherms, are particularly vulnerable to changes to thermal regimes because of the role of temperature in regulating metabolic processes (Eriksen 1964, Vannote and Sweeney 1980, Parmesan and Yohe 2003). However, the thermal regimes of freshwater systems have been altered by various anthropogenic activities on global (e.g., climate change) to local scales (e.g., riparian deforestation, reduction of river discharge, release of thermal effluent) (Caissie 2006).

Aquatic macroinvertebrates as a study system

The scope of the present study is the response of aquatic macroinvertebrates to environmental change, specifically changes to thermal regime. Macroinvertebrates are commonly used in biomonitoring and environmental assessment and have a number of characteristics which make them particularly useful sentinels for environmental change (Hellawell 1986, Hilsenhoff 1987, Rosenberg and Resh 1993, Chessman et al. 2007). 1) Macroinvertebrates are relatively easy to collect and identify, although they are patchily distributed (Covich et al. 1999), and many groups are also ubiquitous, which allows for comparison between systems (Allan and Castillo 2007, Hauer and Lamberti 2011). 2) Different species or higher taxa have variable known tolerances, permitting the identification of indicator species for particular types of environmental change (Hilsenhoff 1987). Such information allows for the interpretation of both individual and collective community-level responses. 3) Many macroinvertebrates are rapid colonisers which allow us to identify environmental changes with reduced lag time, while others have long life cycles (e.g., mussels) which integrate environmental conditions over time, acting as longer-term monitors of water quality. 4) Macroinvertebrates are important indicators of biological integrity because they serve as a primary food resource for virtually all fish communities (including commercial species) and thus act as important conduits of energy through aquatic food webs (Wallace and Webster 1996, Lakly and McArthur 2000).

Thermal biology and invertebrate communities

There are two primary ways in which environmental temperatures are linked to biodiversity in freshwater: on large continental scales whereby climate envelopes set an outer limit to ranges of species, and on local scales where biotic interactions, seasonal timing, and biomass are affected (Stenseth et al. 2002, Hawkins et al. 2003, Parmesan and Yohe 2003, Burgmer et al. 2007, Hillebrand et al. 2010). On large scales, latitudinal gradients in species diversity have been recognised, with low latitudes supporting higher species richness, perhaps indicating a positive temperature-diversity relationship owing to greater productivity in warmer environments. However, freshwater invertebrates have been identified as a potential exception to this general pattern (Pianka 1966), and numerous other mechanisms could explain this overall latitudinal gradient in organismal communities (e.g., evolutionary processes, habitat complexity, or climatic stability) (Pianka 1966, Ricklefs and Schluter 1993).

On a local scale, we have more insight into mechanisms between temperature and invertebrate community patterns, because of knowledge derived from comparisons over natural thermal gradients, from research pertaining to global warming, and from temperature tolerance experiments. For example, we know that many critical life history variables (e.g., growth, reproduction, emergence) are regulated by temperature (Sweeney and Schnack 1977, Stanford and Ward 1983, Sweeney and Vannote 1984, Hauer and Benke 1991). The consequence is generally faster development, higher rates of reproduction and growth, and shorter generation times in heated waters (Arthur et al. 1982, Arnell 1998, Hughes 2000, Yanygina 2011). The production of biomass is also positively related to temperature (Mulholland et al. 1997). Biomass can be useful in estimating ecosystem productivity as it indicates how the organisms are able to use food resources (Hauer and Lamberti 2011). However, there are trade-offs associated with increased growth and reproductive rates, especially at the individual level; most notably, increases in metabolic and thus feeding rates elevate the demand for dissolved oxygen and high-quality food (Wotton 1994).

There are numerous ecosystem-level changes that could result from elevated temperature (e.g., from discharge of heated effluent) with consequences for the invertebrate community, including increased primary production, enhanced organic matter decomposition and nutrient cycling, longer growing seasons, reduced habitat for cool-water species, and reduced suitable summer habitat (i.e., more extreme summer die-off) (Mulholland et al. 1997). Over time, environmental changes can promote tolerant species and eliminate sensitive species, thereby restructuring the community (Tarzwell 1970). Thermophilic species would be able to move into the warmed areas from which heat-intolerant or stenothermic species are excluded (Gibbons and Sharitz 1981, Parmesan and Yohe 2003). This phenomenon has been hypothesised to occur as a consequence of climate change in alpine streams (Hauer et al. 1997, Durance and Ormerod 2010) and marine intertidal systems (Sagarin et al. 1999), and may occur on a smaller scale in freshwater artificial thermal plumes. Warming habitats could therefore favour invading rather than native organisms, both through the lifting of thermal barriers previously excluding them, and through reduced biotic resistance by the native community (Mandrak 1989, Schindler 2001). However, temperate organisms are often adapted to a broader seasonal variation in temperature, and can generally acclimate to more extreme temperatures than those that may currently define their distribution (Tarzwell 1970). Furthermore, it is essential to note that invertebrate distributions are not necessarily restricted by absolute temperature maxima, but rather by accumulated degree-days - that is, both the magnitude and rate of temperature change may be significant (Sweeney and Vannote 1986, Pritchard et al. 1996, Hawkins et al. 1997).

Thermal effluent in aquatic environments

It is unclear whether predictions based on thermal physiology, climate change, or geographic gradients can give insight into organismal effects of thermal plumes that involve abrupt temperature gradients over small spatial scales. The present study uses the influence of thermal effluent (hot water discharged from power-generating stations and industrial operations; Mason 2002) to test hypotheses related to the effects of temperature on macroinvertebrate abundance and distribution within a river system. Power stations often operate with an overall efficiency <40%, and therefore water is removed from natural systems for use as a coolant to capture this waste heat and is then returned to the environment at an elevated temperature (Mason 2002). Introducing water with an artificially high temperature into a natural system may affect resident biota by determining which areas serve as suitable habitat for organisms with specific temperature tolerances (Caissie 2006).

Research on the effects of thermal effluent was primarily initiated in the late 1960s (Gibbons & Sharitz 1981; Figure 1). This followed the enactment of the Federal Water Pollution Control Act in the United States, which was amended by the Water Quality Act in 1965, and designed to facilitate the establishment of water quality standards for interstate and coastal systems (Becker 1973). Water quality was largely defined by the physical, chemical, and biological characteristics required by aquatic organisms, industry, agriculture, and other human uses (Becker 1973). This time period also corresponds to the advent of nuclear power generation and thus a large proportion of thermal effluent studies focus on nuclear reactor sites. The majority of thermal effluent studies were therefore conducted in the 1970s and 1980s, with some ongoing work and follow-up studies conducted in more recent decades (Figure 1). Research was initiated based on the justifiable concern that waste heat from electrical generating stations could have negative ecological impacts. For the purposes of this review, I will focus on describing the invertebrate community changes commonly observed in these thermal plumes (summary of selected studies in Table 1).

Observed effects of thermal effluent on macroinvertebrates

Changes to community diversity

A primary method for investigating the impact of thermal effluent has been through the assessment of taxonomic diversity within and along thermal plumes from power plants.

Diversity could be expected to decrease in the presence of thermal effluent owing to the removal of intolerant organisms (Gibbons and Sharitz 1981). Considering the mathematics of common indices such as Shannon-Weiner or Simpson's Diversity, measured diversity could decrease both through a reduction in species richness (loss of sensitive organisms) and a reduction in evenness (owing to disproportionate numbers of a few tolerant organisms). This prediction is supported by a substantive body of research on thermal effluent plumes, which tend to show a pattern in which the loss of stenothermic organisms reduces taxonomic diversity, while a few species become dominant (Howell et al. 1974, Gibbons and Sharitz 1981, Wellborn and Robinson 1996, Ponti et al. 2010). For example, at the highest temperatures in the Delaware River, 40 taxa were found in control sites compared to 4 taxa at thermally enhanced sites (Coutant 1962). A review by Langford (1990) noted the rare clarity of these results, which can be attributed to the presence of good control sites that differed primarily in temperature. Another study used three experimental channels to compare thermal effects (Alston et al. 1978): one unheated, one heated but stratified, and one heated thoroughly, with a temperature difference of between 3-10ºC in the unheated compared to heated channels. The highest Shannon-Wiener diversity index was found in the unheated channel, and this was statistically significant during the warmest months of the year (summer, fall). In contrast, while the heated channel had the greatest number of organisms of the three, the fewest number of taxa were found there.

These results lead one to consider that temperature can have effects in both space (among sites) and time (seasonally), as demonstrated in a study by Snoeijs (1989). He evaluated macroinvertebrate communities in an experimental channel at the outflow of the Forsmark nuclear power plant in Sweden, and found significantly reduced diversity during the warmest season (May-November) as well as the lowest diversity at the most strongly heated sites. It was found that only a few species dominated the most strongly heated sites in both seasons, illustrating drastically reduced diversity.

In contrast, some studies demonstrate an increase in macroinvertebrate diversity within thermal plumes. It has been suggested that if temperatures do not reach lethal limits, heat may actually enrich the community (Lessard and Hayes 2003). If one considers thermal enrichment to be a form of disturbance, this pattern could be interpreted as reflecting the intermediate disturbance hypothesis (Connell 1961), whereby diversity increases with temperature to a maximum point (for many fish and invertebrates, this threshold is ~32ºC; Langford 1983) after which it declines.

However, many studies indicate no changes in diversity or changes in diversity that are not well-explained by temperature patterns. Ferguson and Fox (1978) reported low biomass and low generic diversity in Lake Keowee, South Carolina in the vicinity of the Oconee Nuclear Station, which was attributed to thermal stress (temperature increases ~10ºC), but also an absence of organic matter. Similarly, a study found more taxa in unheated lake areas compared to lakes affected by thermal pollution, but this was attributed to increased habitat heterogeneity in the control lake (Langford 1990). Temperature may also act at different levels of taxonomic or community organization. For example, a study by Jacobsen et al (1997) investigating temperature/diversity relationships found that while insect family richness had a positive relationship with temperature, species richness did not. However, community composition at the species level was significantly related to differences in mean summer temperature. This indicates that taxa replacement - not changes in diversity - could be the best indicator of thermal effects.

Changes to community composition

To address the discrepancies associated with differences in diversity results, many studies do not apply a diversity index but rather measure the presence and abundance of specific species. Community composition must be evaluated to take into account effects specific to regional fauna (Čiamporová-Zaťovičová et al. 2010). An increase in temperature may alter the higher taxonomic groups of organisms occurring in a community, without measurable changes in overall species richness (Logan and Maurer 1975). In most thermal effluent studies, there were significant changes in community composition even if there was no measurable change in diversity metrics (Langford 1983).

Differences in macroinvertebrate functional groups have been found between heated and unheated sites of streams, presumably because of an energy shift from allochthonous to autochthonous inputs in heated sites (Lakly and McArthur 2000). Although it is rare to be eliminated by high temperature alone, declines and disappearances of many taxa occur between 33-35ºC (Langford 1983). Taxonomic groups most commonly found in discharge plumes include chironomid larvae (Alston et al. 1978, Langford 1990), tubificid worms (Durrett and Pearson 1975, Alston et al. 1978, Rasmussen 1982, Osborne and Davies 1987), and gastropod snails (Langford 1983, Snoeijs 1989). Taxa most commonly reported to be excluded by effluent include Amphipoda (Wrenn et al. 1979), Coleoptera (Coutant 1962), Ephemeroptera (Coutant 1962), Plecoptera (Arthur et al. 1982) and Trichoptera (Coutant 1962, Arthur et al. 1982). Although taxonomic changes vary by site, these are the dominant replacement trends observed in the literature.

Species composition could be used to evaluate impacts by identifying indicator organisms, which has been done in creating biotic indices for other types of pollution, e.g., organic pollution (Hilsenhoff 1977). Indeed, many organisms resistant to thermal pollution tend to be those resistant to other types of contaminants, while those eliminated in effluent are notoriously sensitive groups. In a review by Langford (1990), it was suggested that since power plants are often located in developed, industrial regions, it is predictable that species found in polluted rivers would be tolerant to other (including thermal) stresses. These changes in taxa can be driven by direct lethal effects or by indirect effects stemming from biotic interactions, including competitive exclusion by more tolerant or efficient taxa (Ferguson and Fox 1978). However, there are sufficient examples of exceptions to this that a biotic index may not be reliably applied to these sites. For example, notoriously intolerant species (e.g., members of the "EPT" group - Ephemeroptera, Plecoptera, Trichoptera) are sometimes equally abundant in thermal effluent sites and natural sites (Langford 1990). Also, it was suggested that isopods are very heat-tolerant organisms (Langford 1983), yet other studies have found isopods eliminated after June in effluent by seasonal increases in temperature (Arthur et al. 1982). Evidently, like diversity patterns, the results of changes in community are context dependent.

There must be a combination of variables allowing some animals to survive in a given temperature and perish in another spot at the same temperature. For example, Cairns (1970) reported that at the Glen Lyn power station in Virginia, caddisflies and mayflies were relatively abundant at all stations and chironomids increased downstream of the effluent, but molluscs declined in the heated water and did not recover more than 3 km downstream, where the temperature increase was no longer significant. This elimination of molluscan taxa was attributed to a confounding factor, but the study did not speculate what this may have been. Composition changes may also only be detectable when one considers seasonality, as Coutant (1962) found that composition and abundance at heated and control sites were similar during winter months, but as temperatures warmed on an annual cycle, mayflies, caddisflies, and beetles were eliminated. The complexity of the systems being studied has hindered the identification of specific mechanisms for changes in invertebrate assemblages in many cases.

Non-native species in thermal plumes

A further complication to identifying shifts in diversity or community composition in thermal plumes is that many aquatic systems have been invaded by non-native species. The disturbance associated with thermal discharge combined with the reduction of thermal barriers in these freshwater systems may facilitate the spread of invasive species into new habitats (Moyle and Light 1996, Schindler 2001). There are very few examples of complete species replacements occurring with the introduction of tolerant nonnative taxa (Langford 1983). However, a number of species, such as *Branchiura sowerbyi*, *Physa acuta*, and *Corbicula fluminea*, do thrive specifically in artificial thermal plumes outside their native range (Scott-Wasilk et al. 1983, Langford 1990, Brancotte and Vincent 2002, Paunović 2007, Simard et al. 2012). Some of these animals, notably *C. fluminea*, yield potentially significant environmental impacts, including changes via bioturbation of the sediments, high rates of filtration, and increased habitat complexity from shell accumulations (Sousa et al. 2008).

In addition to facilitating warm-water invaders, thermal plumes may alter the abundance and distribution of those already established. For example, the zebra mussel, *Dreissena polymorpha,* has had enormous ecological impacts in the Great Lakes-St. Lawrence River system, but the species cannot tolerate prolonged exposure to temperatures greater than 30ºC (McMahon 1996), excluding it from some thermal plume environments (Langford 1990). The response of invaders to the release of thermal effluent is species-specific and context-dependent, so their abundance and distribution in thermally altered systems are often difficult to predict. By offering a warm-water environment, thermal plumes may act as a refuge for source populations of subtropical and tropical invaders which would not otherwise be able to establish in temperate regions. Range expansion by these organisms may be facilitated by anthropogenic climate change (Hellmann et al. 2008) and potentially by adaptation to cold resistance (Barrett et al. 2011).

Other environmental changes

Invertebrate distribution and community structure is related to numerous environmental factors (Cook and Johnson 1974, Pinel-Alloul et al. 1996). While temperature is certainly an important environmental variable, it is evident that the effects of thermal effluent are too complex to be attributed to temperature alone. For example, while temperature might not kill off sensitive organisms, stress from an unnatural thermal regime can make invertebrates vulnerable to parasites, diseases, and other types of pollution (Langford 1983). Temperature itself is dynamic and variable (Coutant and Brook 1970), and even effects that can be attributed directly to temperature depend on the rate of the temperature change, exposure time, and season.

Furthermore, discharge from power plants differs in a number of ways from water in natural systems. Thermal effluent not only has an elevated temperature, but it is also typically low in dissolved oxygen, discharged at a high flow rate, and may contain biocide chemicals like chlorine to eliminate fouling organisms from pipes and machinery (Langford 1983). Given that warm water holds less oxygen, it is not surprising that the organisms described as resistant to high temperatures by thriving in thermal effluent are also species that tend to be resistant to hypoxia (e.g., certain chironomids). Thus, there are some parallels between indicator species of organic pollution and candidate indicators of thermal pollution (Langford 1990). In some cases, effects of thermal effluent may be attributed to low oxygen rather than the exceeding of thermal tolerance limits.

Thermal discharge also often augments flow, altering parameters considered important to the structure of lotic communities such as substrate (Lakly and McArthur 2000). Indeed, a number of studies have found that substrate type or the effect of scour were strong predictors of benthic invertebrate community characteristics (UNECE 1975, Langford 1983, Snoeijs 1989). This is unsurprising, as particle size is an important factor in determining community structure in lotic systems (Minshall 1984). A study found that bivalves *Pisidium sp.* and *Bithynia sp.* disappeared in the thermal plume of the Gentilly-I power plant on the St. Lawrence River, where temperatures reached >30ºC, but these same species are found living in effluents of similar temperature in Britain (UNECE 1975). It was later concluded that scour was the distinguishing factor. Conversely, other studies have suggested that warm water has decreased viscosity, leading to an increase in sedimentation (Langford 1983).

Enhanced primary production is an important indirect effect of temperature as well. For example, the dominance of oligochaetes at some sites in Sheep River, Alberta, was attributed to the prevalence of cyanobacteria; the macrofaunal change was driven by this shift towards heat-tolerant primary producers, rather than a direct effect of temperature on the worms (Osborne and Davies 1987). Macrophyte productivity, too, can be enhanced by increased temperatures from thermal effluent, and the subsequent change in habitat may benefit epiphytic invertebrates (Rasmussen 1982).

In some studies, where spatio-temporal variability cannot be explained by temperature or its correlates, refuges may be a factor (Mason 2002, Caissie 2006). Benthic invertebrates may be shielded from the extreme temperatures generated by thermal effluent if the receiving body of water stratifies, creating a cool refuge in the hypolimnion. For example, the benthos of Lake Erie in the vicinity of the Monroe Power Plant was largely unaffected by the temperature increase likely owing to the creation of a strong thermocline (Cole and Kelly 1978). As previously described, subsequent increases in oligochaetes and chironomid larvae in this region of Lake Erie were associated with blooms of cyanobacteria in the discharge rather than a direct effect of temperature.

Summary of thermal effluent effects

Both direct (lethal) and indirect effects of temperature have been documented, where indirect effects can have delayed lethal effects or produce a non-lethal stress that causes changes to population dynamics (Tarzwell 1970). Studies of stream recovery after exposure to thermal pollution indicate that effects compound over time, making a return to the previous community state increasingly improbable (Lakly and McArthur 2000). However, thermal effluent cannot necessarily be considered thermal pollution - which requires demonstrable negative effects - given that increased temperatures in an aquatic system can also create subsidies or enhancement in some instances (Gibbons and Sharitz 1981). As we have gained insight into the resistance and adaptation possible at all levels of biological organisation, early conceptions about the harmful effects of elevated temperatures have been modified (Gibbons and Sharitz 1981). With the variable directions and magnitudes of change observed in aquatic systems impacted by power plant thermal discharge, it is essential to evaluate their individual influence on their local community on a case-by-case basis (UNECE 1975).

Research Objectives: Effects of thermal effluent on benthic fauna in a large river

The goal of my research was to evaluate how characteristics of the benthic macroinvertebrate community in a large river have been altered by thermal discharge downstream of a nuclear power plant. This study system is a section of the St. Lawrence River downstream of the Gentilly-2 Nuclear Power Plant (G2NPP) at Bécancour, Quebec (Langlois & Vaillancourt 1990). Operating from 1983 to 2012, the heated discharge from G2NPP was released to the environment at temperatures greater than 10ºC above ambient river temperatures. I used this system to test predicted relationships between temperature and invertebrate community diversity, composition, and abundance (described in the following chapter). Special consideration was given to a new dominant invasive species established at this site, the Asian clam (*Corbicula fluminea*), which was able to use the G2NPP plume as a thermal refuge (Simard et al. 2012). A multivariate approach was used to distinguish the effects of the thermal enhancement from the effects of changes to the structural habitat and physico-chemical properties of the river. Given that modification associated with power plants involves more than merely heated discharge, I expected other variables (e.g., flow rate, substrate, dissolved oxygen) to be implicated in observed patterns in invertebrate distribution and abundance.

TABLES

Table 1: Summary of some changes observed in invertebrate communities exposed to thermal effluent. Location indicates the locale of the power plant; Max Temp indicates maximum temperature recorded during study; Temp Diff indicates thermal enhancement relative to ambient; Community Change describes invertebrate community changes in heated sites relative to unheated sites.

Location	Max Temp (ºC)	Temp Diff (ºC)	Community Change	Reference
Martin's Creek, Delaware River, Easton, Pennsylvania	35	~10-12	Lower diversity, lower abundance, lower biomass	Coutant 1962
Severn River, UK	28	13	No relationship with richness	Langford 1972
Trent River, UK	27	10 (max.)	No relationship with richness	Aston 1973
North Lake Reservoir, Dallas County, Texas	42.2	5-15	Lower diversity, higher abundance (Nov-Feb), lower abundance (summer)	Durrett and Pearson 1975
Lake Keowee, South Carolina	31.8	10-13	Lower biomass, lower generic diversity	Ferguson and Fox 1978
Indian River, Delaware	36.5	10.5 (max)	Lower abundance and richness	Logan and Maurer 1975
EPA Field Station, Monticello, Minnesota *experimental	40.8	8-10	Higher density (winter, spring), lower density (summer), few organisms alive during peak temperature	Arthur et al. 1982
Lake Wabamun, Alberta	28.9	7.1 (max)	Higher biomass, different composition	Rasmussen 1982
Vales Point Power Station, Lake Macquerie, Australia	>37	6-8	Higher density, lower taxa richness, changes to sea grass composition	Robinson 1987
Sheep River, Alberta		2-10	Higher density, lower taxa richness	Osborne and Davies 1987
Savannah River Plant, Aiken, South Carolina	46	25 (max)	*Chironomus sp.* dominates chironomid community, higher rates of emergence when reactor shutdown	Coler and Kondratieff 1989
Forsmark Nuclear Plant, Sweden	25	8-10	Lower diversity; favoured opportunist reproducers	Snoeijs 1989
Fairfield Reservoir, Freestone County, Texas	40-42	7.2 (avg)	Lower diversity, lower abundance (seasonal variation in responses); macroinvertebrates eliminated in peak summer	Wellborn and Robinson 1996
Savannah River Plant, Aiken, South Carolina	50	25 (max)	Higher density, lower EPT, higher richness	Lakly and McArthur 2000
Portage Creek, Michigan	25	13-18	Lower diversity, lower abundance, fewer EPT taxa	Nedeau et al. 2003
Pialassa Baiona, Italy	25-34		Lower species richness	Ponti et al. 2010
Nesjavellir Power Plant, Iceland *geothermal	>27	7-12	Reduced chironomid diversity	Snorrason et al 2011
Newton Lake, Jaspar County, Illinois	35	5-10	Higher density (winter), no difference in density (summer)	Metzke and Pederson 2012

FIGURES

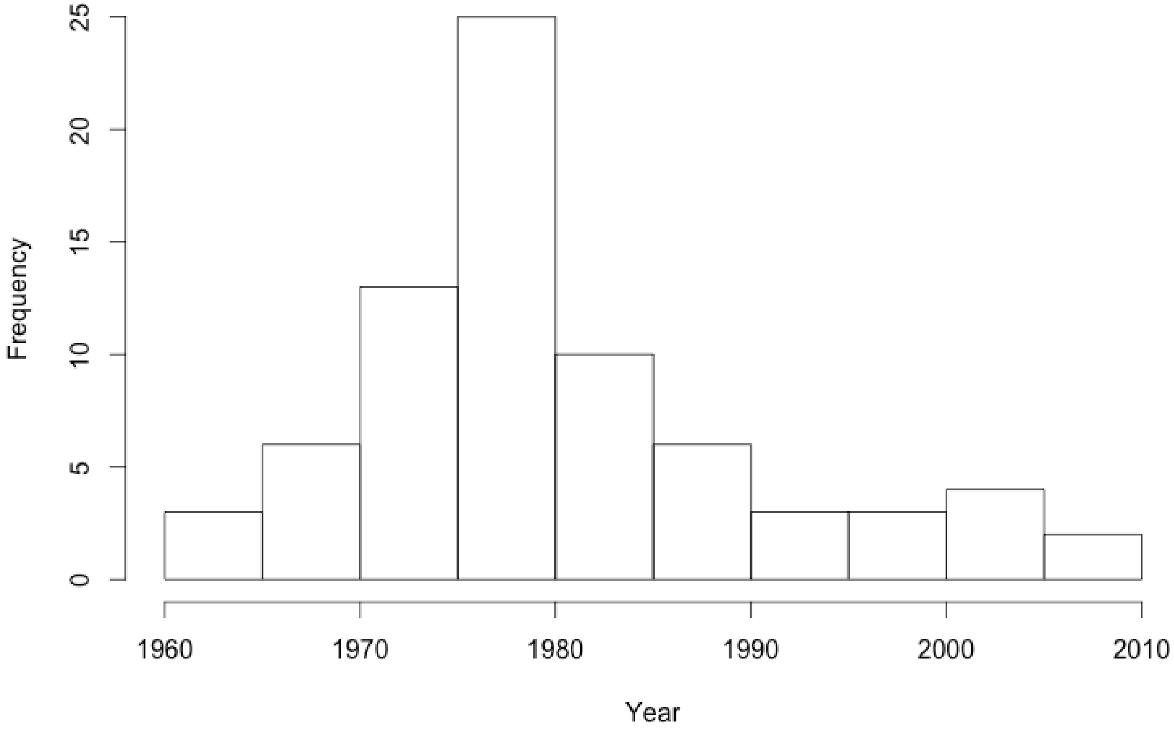

Figure 1: Frequency of peer-reviewed publications as a function of publication year, based on Google Scholar search for "Effects of thermal effluent on invertebrates" (n=75; accessed on 2 December 2013).

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CHAPTER 2: Effects of thermal effluent on the diversity and distribution of benthic invertebrates in the St. Lawrence River

INTRODUCTION

Water temperature strongly influences the structure and function of aquatic communities by altering rates of chemical and biological processes (Angermeier and Karr 1994, Coutant et al. 1999, Caissie 2006), thereby affecting the reproduction, growth, and metabolism of aquatic organisms – most of which are poikilotherms (Sweeney and Schnack 1977, Vannote and Sweeney 1980, Stanford and Ward 1983, Hauer and Benke 1991). As a result, aquatic animals generally have a specific range of temperatures they can tolerate, as well as precise thermal criteria and optima for life history parameters such as egg development and emergence time (Coutant 1977, Vannote and Sweeney 1980, Hauer et al. 1997, Magnuson et al. 1997). Consequently, temperature is a primary physical constraint on the distribution and abundance of organisms within an aquatic system (Brown et al. 2005).

Increasingly, human activities are affecting the temperature regimes of aquatic systems, with potential consequences for the diversity and abundance of local organisms. A significant anthropogenic source of temperature change in rivers is the release of thermal effluent, defined here as water used as an industrial coolant and subsequently released at an elevated temperature. A primary source of thermal effluent is power-generating stations, notably nuclear power plants. In addition to increasing the ambient temperature of the water, thermal plumes created downstream of power plant effluent cause local changes to flow rates (Snoeijs 1989), sedimentation (Massengill 1976), and physico-chemical water properties such as dissolved oxygen (Langford 1972, Aston 1973, Eloranta 1983). Studies evaluating the ecological impacts of thermal effluent have thus far yielded inconsistent results (i.e., deleterious, beneficial, and neutral effects on resident populations), reflecting variation in both interspecific responses among organisms and context-dependent influences across systems (Wellborn and Robinson 1996).

The goal of my research was to evaluate how the benthic macroinvertebrate community downstream of a nuclear power plant in Québec has been altered by thermal discharge. This case-study was based at the Gentilly-2 Nuclear Power Plant (G2NPP), on the south shore of the St. Lawrence River (SLR) (Langlois and Vaillancourt 1990). The first power plant located at this site, Gentilly-I, began operation in 1971 (Vaillancourt and Couture 1978). After refurbishment and the construction of a new discharge canal to direct the thermal effluent into the SLR, the present Gentilly-2 facility started operation in 1983 and was functional until it was decommissioned at the end of December 2012. For decades, the heated discharge from G2NPP was released to the environment at temperatures an average of 11.1ºC higher than ambient river temperature (Hydro-Québec 2006). This thermal enhancement prevented a multi-kilometer section of the river from freezing during the winter months (Langlois and Vaillancourt 1990), effectively mimicking a subtropical aquatic environment.

This site has been the focus of recent research efforts owing to the discovery of a new invasive species, the Asian clam (*Corbicula fluminea*), in the vicinity of the thermal plume (Simard et al. 2012). *C. fluminea* is a high-impact global invader, but is typically excluded from north temperate latitudes by low temperatures and ice cover which limit its survival and reproduction (McMahon 1999). Nevertheless, an established population was identified in Bécancour in 2009, appearing to use the G2NPP plume as a thermal refuge (Simard et al. 2012). It is well-documented that human activities, including changes to thermal regimes that remove thermal barriers, may alter habitats in such a way that they may become more vulnerable to invasions (Hobbs and Huenneke 1992, Moyle and Light 1996, Schindler 2001). Colonization of the thermal plume at G2NPP by *C. fluminea* seems to be another example of this phenomenon. In contrast to previous work at this site focusing on the *C. fluminea* population, the scope of my study was the characterization of the overall benthic invertebrate community in these artificially heated waters; however, I also address the role of *C. fluminea* in the community response to thermal effluent.

I used the G2NPP system to test hypotheses about the relationship between temperature and community properties, with the applied goal of interpreting how the invertebrate community has been altered downstream of G2NPP. Through field surveys of fixed, pre-determined stations in summer 2012, I tested predictions that 1) *Diversity is reduced at sites closer to the power plant*; 2) *Community composition is structured primarily by temperature, and secondarily by other environmental variables*; and 3) *Community abundance of organisms (density and biomass) is elevated at sites closer to the power plant.*

I expected species-specific differential responses to the influence of the hot-water discharge, although many taxa found in the SLR are broadly distributed in North America (Thorp and Covich 2009), and thus are able to survive in warmer waters than those naturally found in this region of Québec. It has been documented that warmer temperatures, within the tolerance ranges of the organisms, will result in earlier hatching, faster maturation, and more generations among aquatic invertebrates (Arthur et al. 1982, Lessard and Hayes 2003, Yanygina 2011). The consequence of these physiological enhancements could be a higher density of invertebrate organisms at the warmest sites. While many organisms in the SLR may be able to take advantage of the heated waters at G2NPP, it has been documented that in modified thermal environments, taxonomic evenness tends to decrease, depressing overall diversity, as the most thermophilic species are able to dominate (Gibbons and Sharitz 1981). In particular, *C. fluminea* has an evolutionary history in subtropical environments shared by no native species and very few other non-native species in the SLR. Furthermore, in its invaded range, *C. fluminea* often dominates the benthic macroinvertebrate community in terms of abundance (McMahon 1982).

In addition to thermal enhancement, my study site is a complex fluvial system that includes a discharge canal, natural depth gradients, and mixing dynamics with spatio-temporal variation (e.g., seasonal, tidal). As previous thermal effluent studies have suggested, changes to the structural habitat and physico-chemical properties of the river likely also have meaningful effects (Langford 1990). Therefore, my null hypotheses related to the effect of temperature were tested using a multivariate approach to determine both the direct and indirect effects of thermal enhancement on benthic invertebrates, as well as the impact of other physical changes associated with the discharge and natural river gradients.

METHODS

Data Collection

Field Site

The Gentilly-2 Nuclear Power Plant (G2NPP; 46º39’56”N, 72º35’64”W) was a pressured heavy-water CANDU reactor located on the south shore of the SLR immediately downstream of the Port of Bécancour, Québec (Langlois and Vaillancourt 1990). Coolant water used in the G2NPP nuclear reactors was taken up from the SLR and released to the river downstream through a discharge canal at a rate of 25 m^3/s when operating at peak capacity (Simard et al 2012). This discharge had temperatures elevated by an average of 11.1ºC and effects of the effluent have been observed up to 5 km downstream of the discharge source (Hydro-Québec 2006).

Field Survey

I sampled along a ~3.2 km transect downstream of G2NPP in the SLR. This distance encompassed the artificial discharge canal and most of the thermal plume (defined by year-round open water). I established 30 sampling stations along the length of the thermal plume perpendicular to the isotherms of the thermal gradient (Figure 1). Habitat type was restricted to open-water sites with a bottom of mixed sediments, outside of the shoreline macrophyte beds to control for the indirect effects of temperature on habitat structure owing to changes in plant production (e.g., Rasmussen 1982). Stations were spaced according to a geometric design, whereby stations #1-8 were separated by 30 m, #9-16 by 60 m, #17-24 by 120 m, and #25-30 by 240 m. I selected minimum intervals of 30 m between sampling stations in order to ensure that stations could be differentiated, based on the known positional error (<15m) of the Garmin *etrex* GPS unit used for navigation and site location. This spatial sampling design reflected the distance-decay of the thermal plume temperature gradient, with greater effort closer to the discharge source to assess community changes driven by temperature at a high-resolution.

I sampled the G2NPP transect twice in 2012, during spring (May 14-19) and summer (September 7-9) to ensure that seasonal differences in community patterns could be detected. Summer sampling needed to be delayed until early September, and conducted when the reactor was not operational, owing to an unexpected power plant shutdown between late July and late September 2012. At each station, 2 Petite Ponar benthic grabs were collected. I chose to maximize the number of sampling sites rather than within-site replicates, in order to better identify species distribution limits; consequently, owing to logistical constraints, only twin replicate samples were taken at each site. If a sediment sample obtained was deemed to be too small for comparison (<75% of full grab), a new benthic sample was collected. These samples were sieved through a 0.5 mm mesh on site, and the macroinvertebrates and coarse sediments retained in the sieve were fixed in 75% ethanol.

At each station, I also measured abiotic factors hypothesized to affect the distribution and abundance of organisms, particularly those expected to change with distance downstream from G2NPP. Temperature (ºC), specific conductivity (µS/cm), and dissolved oxygen (mg/L) were measured in the field using a YSI probe. Water transparency was measured using a Secchi tube at each station. Water flow velocity was measured using a digital flow meter (Swoffer Model 3000). Depth was measured using a weighted measuring tape. Sediments were analyzed by mean grain size composition (Phi scale), which I estimated for each sediment sample using methodology adapted from Jones and Ricciardi (2005) and Mudroch et al. (1997). Increasing values of Phi correspond to decreasing mean sediment size.

In addition, I deployed settling plates to obtain supplemental information on epibenthic organisms living in the thermal effluent that might not have been collected in the grabs, owing to substrate limitations and the delicate nature of some freshwater invertebrates. I suspended 10 plexiglass settling plates (676 cm^2) on weighted ropes from buoys ~ 150 m downstream of the outflow source in the discharge canal.

Sample Processing

I sorted samples in the laboratory using a dissection microscope to manually remove invertebrates from the coarse sediments. I then counted and identified the specimens to the lowest taxa possible within the time limits and expertise available, using identification keys from Jokinen (1992), Ricciardi and Reiswig (1994), Merritt and Cummins (1996), and Thorp and Covich (2009). Generally, I identified taxa to the family level, except Nematoda, Oligochaeta, Ostracoda, Turbellaria, Hydracarina, and Cyclopoida which could not be consistently identified to the family level. Previous research in the St. Lawrence has verified that differences in invertebrate environmental preferences are significant at the family level (Masson et al. 2010). Gammarid amphipods (*Echinogammarus ischnus* and *Gammarus fasciatus*), dreissenid zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*), and the Asian clam *Corbicula fluminea* were identified to the species level, thereby distinguishing some well-known non-native species from their functionally-similar native counterparts. Empty mollusc shells, insect (e.g., caddisfly) cases, and other indirect measures of invertebrate presence were excluded from the abundance counts.

Evidence of bryozoan species were found, but these colonial animals could not be meaningfully quantified as individuals. Bryozoa were identified by either their resting stages (statoblasts) or by the presence of colonial body fragments and counted separately, where the number of statoblasts or colony fragments was considered a proxy for colony abundance (Hartikainen et al. 2009). *Lophopodella carteri*, *Cristatella mucedo*, *Pectinatella magnifica*, and *Lophopus crystallinus* were identified and quantified by their statoblasts, which are easily recognizable (Ricciardi and Reiswig 1994). *Urnatella gracilis* was identified and quantified by its colonial body fragments, where an individual body fragment was defined to include all branches originating from a shared pedal base.

Invertebrate community biomass was measured as the preserved wet weight of invertebrates in a sample. Each preserved sample was first allowed to air-dry for 8 minutes to remove excess ethanol. Samples collected in May and September were each weighed precisely 22 weeks after preservation to avoid potential error due to changes in biomass over time after storage in ethanol. The Asian clam *C. fluminea* was weighed separately from the other invertebrates using the same protocol in order to identify the proportion of benthic invertebrate biomass that could be attributed to this relatively large invasive species known to dominate communities by mass (McMahon 1982).

Additional Data Sources

Data were obtained from 8 temperature loggers (thermographs) deployed by Québec Ministry of Sustainable Development, Environment, Wildlife and Parks (Développement Durable de l'Environnement de la Faune et des Parcs) between 2010 and 2012 (Figure 1). The loggers recorded bottom temperatures hourly, and were fixed in place by bricks. Summary data from 2010-2012 were analyzed to compare long-term temperature ranges and variability along the thermal gradient downstream of G2NPP.

Data Analyses

Invertebrate abundance and biomass measures were standardized per square metre (rather than per individual grab), for ease of data comparison and interpretation. Data analyses were conducted using 'R' Statistical Computing Software (R Core Team, 2013), unless otherwise noted. Environmental data collected during September, when G2NPP was shutdown for repairs and no thermal effluent was being discharged, were not considered representative of the typical influence of the thermal plume on the river at that time of year, and thus were excluded from certain analyses where appropriate. It is unlikely that the shutdown significantly affected community-level patterns, particularly among long-lived species, however this limitation had to be considered during data analysis and interpretation.

The G2NPP Downstream Environment

I used data collected in May to examine environmental patterns, when G2NPP was fully operational and the artificial thermal gradient was in effect. To put these data in the context of long-term thermal exposure, I used boxplots to compare temperature distributions at each of the temperature logger sampling locations over the annual cycle of the thermal regime according to season (i.e., *Winter*: December-March; *Spring*: April-May; *Summer*: June-August; *Fall*: September-November).

Community Diversity and Evenness (Hypothesis 1)

Patterns in overall diversity metrics along the gradient were investigated. The sampling transect was divided into three segments (i.e., *canal*: sites #1-12; *mid-plume*: sites #13-25; *downstream*: sites #26-30). EstimateS software was used to calculate diversity rarefaction curves to compare Simpson's diversity among the segments while controlling for differences in community abundance (Colwell 2013). The rarefaction analysis used sample-based abundance data, and sampled with replacement (100 randomizations) to ensure accurate error estimates. Rarefaction curves were compared using 95% confidence intervals.

To evaluate the individual components of diversity, the 'R' package *vegan* (version 2.0-8; Oksanen et al. 2013) was used to calculate total species richness and Simpson's evenness for each sample, which were investigated as a function of their distance downstream of G2NPP. In order to verify richness patterns, given the bias that differences in invertebrate abundance among sites might confer, an Abundance-based Coverage Estimator (ACE) was used to estimate richness values controlling for abundance, for comparison with the raw richness values.

Generalized Additive Models (GAMs) were generated using the 'R' package *mgcv* (1.7-24, Wood 2006) to identify the environmental variables most strongly associated with fluctuations in richness and evenness components. GAMs were used as they are robust to the highly non-linear, non-monotonic nature of the data, and do not assume a parametric structure between response and predictor variables (Guisan et al. 2002). Owing to limits in degrees of freedom, only 3 predictor variables could be used in a given model without sacrificing statistical power. The environmental variables expected to explain the most variation in the biological community (i.e., temperature, depth, sediment size, transparency, and distance downstream; verified by a canonical correspondence analysis, see "Community Composition") were used to build the richness and evenness models in order to ensure the most biologically relevant inferences. Environmental variables found to have significant correlations (Pearson's $r > 0.6$) were excluded from co-occurring in a given model (i.e., temperature and depth; temperature and distance downstream). Model selection was conducted using Akaike Information Criterion (AIC) values, where the lowest AIC value was used to identify the most parsimonious model (Burnham and Anderson 2002).

Community Composition (Hypothesis 2)

After establishing overall patterns in community diversity, I conducted a canonical correspondence analysis (CCA) on May environmental and biological data using the 'R' *vegan* package (version 2.0-8; Oksanen et al. 2013), to identify variation in environmental drivers associated with variation in the biological community composition. Mean invertebrate abundance for the replicate grabs at each site (n=2) was calculated for each family. Since CCA can be sensitive to rare organisms (Borcard et al. 2011), taxonomic groups that occurred in fewer than 5% of grabs (<3) were excluded from this analysis. The analysis was conducted with and without *C. fluminea* to examine the influence of this species' exceptional environmental preferences on the ordination results. To test the global significance of each CCA model, and the significance of individual CCA axis components, permutation procedures (n=999) were performed.

Community Abundance and Biomass (Hypothesis 3)

Community abundance (density) and biomass data were plotted to illustrate changes in the number of organisms and the biomass of organisms as a function of distance downstream from the G2NPP discharge source. *C. fluminea* abundance and biomass were distinguished from other members of the benthic invertebrate community. Previously, *C. fluminea* density and biomass at G2NPP were modelled as a function of environmental variables (Castañeda 2013). In the present study, to determine the environmental variables most strongly associated with fluctuations in changes in the biomass of the other benthic macroinvertebrates excluding *C. fluminea*, I used a Generalized Additive Model (GAM) in the 'R' package *mgcv* (Wood 2006). The model used biomass as a measure of abundance owing to its biological significance associated with ecosystem productivity (Hauer and Lamberti 2011). Models were evaluated using AIC values as described above (see "Community Diversity and Evenness").

Bryozoan Community

Bryozoan statoblast abundances and *U. gracilis* fragment abundance were examined across the downstream thermal gradient from the G2NPP discharge source. Statoblasts and colony fragments were considered proxies for bryozoan abundance, as they are non-floating forms and thus likely produced locally (Ricciardi and Reiswig 1994).

RESULTS

The G2NPP Downstream Environment

May environmental data characterized the canal and downstream environment as heterogeneous with a strong temperature gradient (Figures 2, 3, 4). Ambient water temperature was elevated to approximately 22ºC-25ºC up to ~500 m from the power plant, corresponding to the stretch of the thermal plume in the discharge canal, which constituted thermal enhancement of 8-10ºC (Figure 2). Temperature decreased precipitously as the canal water mixed with the SLR, attaining natural river temperatures of 14ºC approximately 1250 m downstream. Notable outliers included three sites outside of the 500 m canal which experienced colder temperatures resulting from direct exposure to the SLR, and two sites approximately 1500 m downstream which were warmer than surrounding sites. Surface and bottom temperatures revealed a thermocline in the immediate vicinity of the discharge canal that may be a seasonal phenomenon, as it was undocumented in previous summer sampling (Castañeda 2013).

The rapid decline in temperature downstream of the power plant was corroborated by temperature logger data, especially during winter (Figure 3). Closest to the discharge source (logger #1), mean monthly temperatures were generally above 10ºC in winter, whereas temperature declined sharply at sites downstream of logger #2. Spring and fall exhibited a less drastic decline in temperature with distance downstream; patterns in both seasons were similar, with monthly averages ranging between 5-25ºC. High within-site variability in spring and fall relative to winter or summer temperature regimes likely reflected a transition from low to high temperatures (and vice versa) rather than stochasticity. In summer, extremely high and variable temperatures were recorded at logger #1, with monthly averages in excess of 30ºC. Although the decline in temperature with distance downstream was less sharp during summer than other seasons, distance downstream is generally a proxy for decreasing temperature.

In addition to temperature, other physico-chemical characteristics appeared to be affected by the discharge canal (Figure 4). There were also distinct troughs in values of flow, transparency, and specific conductivity ~750 m downstream where the canal has opened to the flow of the SLR (Figure 4 a,b,c, respectively). Sediment size was at a maximum at the thermal effluent discharge point, where there is significant scour and an artificial gravel bottom, followed by some of the smallest sediment sizes in the remainder of the canal (up to 500 m) and particularly near the opening of the discharge canal to the open SLR thereafter (Figure 4d). Sediment sizes were more homogeneous downstream with a slight decline towards the end of the transect. Dissolved oxygen spanned a wide range of values within the canal from 9.8-11.3 mg/L (0-750 m), while downstream values were more homogeneous ~10.4 mg/L (750-3200 m) (Figure 4e). Depth initially increased with downstream distance until ~1500 m where it reached a maximum of ~7 m, and thereafter became shallower (Figure 4f). Thus, while temperature is the variable with the strongest downstream gradient with downstream distance, this is a complex system with overall highly variable physico-chemical characteristics.

Community Diversity and Evenness (*Hypothesis 1*)

A total of 41 taxonomic groups were represented in the 120 grabs collected during the two study periods (Appendix Table A1). After four months of deployment, settling plates were covered extensively with periphyton (> 90%), however an additional species of bryozoan colony (*Plumatella reticulata*) was identified using this indirect sampling method.

Rarefied Simpson's Diversity Index revealed a trend of diversity increasing downstream, with the canal sites exhibiting the lowest diversity in both seasons (Figure 5). However, overlap among the 95% confidence intervals on the rarefaction curves indicates that variability is too high to identify true differences in diversity among the components of the plume in the seasons sampled.

Subsequent assessment of the components of diversity revealed that mean taxonomic richness and Simpson's evenness exhibited concave up and down relationships with distance downstream from the plant, respectively (Figure 6). Richness was highest in the discharge canal and at the furthest sites downstream, while middle sites from 1000-2500 m were lowest (Figure 6). This pattern did not differ appreciably between May and September. However, the smoothed ACE model predicted that there was an overall positive trend in richness as a function of distance downstream in May, suggesting that measured richness in the canal was exaggerated, likely biased by higher abundances collected at these sites (Appendix, Figure A1). Conversely, mean Simpson's evenness was lowest in the discharge canal and furthest downstream and highest in the middle sites (Figure 6). Decreased evenness was found to be attributable to three dominant groups: *C. fluminea*, Oligochaeta, and Chironomidae (Appendix, Figure A2). When these groups were removed, differences in evenness among sites were reduced.

GAMs predicted taxonomic richness and evenness as a function of physical and structural habitat variables (Tables 1, 2). Richness was best explained by distance downstream from the G2NPP discharge source (a proxy for thermal exposure; r = -0.77), sediment size (Phi) and depth

(Table 1). Peak richness was predicted where depth was ~300 cm, corresponding to most locations in the discharge canal, and where sediments were fine (positive correlation between richness and Phi scale). In contrast, the variables temperature and sediment formed the most parsimonious model for community evenness (Table 2). Evenness exhibited a negative relationship with temperature, peaking at ~16ºC.

Community Composition (*Hypothesis 2*)

The CCA succeeded in separating sites on the basis of biological and environmental data collected in May, and yielded a globally significant model (permutation test, p = 0.001; Figure 7a). CCA1 explained the most variation in the data (26.64%, p=0.001) and was largely driven by the negative relationship between temperature and distance downstream. This axis separated *C. fluminea* from the other invertebrates and separated sites #1-3, where *C. fluminea* most dominated the community assemblage and structural environment, from downstream sites. CCA2 (18.59%, p=0.001) represented gradients in depth, flow, and sediment size. This second axis separated the remaining invertebrates and sampling sites that were not primarily dominated by *C. fluminea*. Due to its apparent dominating effect on the analysis, I re-examined the CCA output in the absence of *C. fluminea*. When *C. fluminea* was excluded from the analysis (Figure 7b), the importance of temperature and distance downstream became associated with the secondary axis CCA2 (11.20%, p=0.002). The new CCA1 (27.71%, p=0.001) reflected the inversely correlated gradients of depth and sediment grain size (Phi scale).

Several invertebrates other than *C. fluminea* were associated with increases in temperature and found at sites close to the power plant (sites #1-11), including elmid beetle larvae and ostracods (Figure 7b). Oligochaete worms showed some association with sites with higher temperatures, but they were largely ubiquitous like other tolerant taxa (e.g., nematodes and chironomids). A series of sites mid-way down the plume (~sites #15-26) that were characterized by increased depth, increased flow, and larger sediments supported hydropsychid caddisfly larvae, dreissenid mussels, and gammarid amphipods. Some families, such as unionid and sphaeriid bivalves were associated primarily with sites farthest downstream (e.g., #27-30).

Community Abundance and Biomass (*Hypothesis 3*)

The abundance of invertebrates was positively associated with proximity to the thermal discharge source, with *C. fluminea* comprising a large proportion of the community in the discharge canal (Figure 8). The *C. fluminea* population density was maximal in the vicinity of the discharge source, and negligible at sites >500 m downstream. In May, *C. fluminea* density declined sharply with distance from the discharge sources, while in September, its abundance declined gradually across the canal and then rapidly at sites downstream of 500 m. The total abundance of other invertebrates was high throughout the canal, declined beyond the canal, and then returned to relatively large numbers at 2500 m downstream. Similar patterns were observed for biomass, with the exception of *C. fluminea*, for which dominance by biomass was even more substantial than by density (Figure 9). Biomass patterns did not differ substantially between May and September.

The GAM that best predicted the pattern of benthic invertebrate biomass (excluding C. fluminea) included sediment size (Phi) and distance downstream from the G2NPP discharge source as explanatory variables (Table 3). In particular, biomass was found to increase in finer sediments.

Bryozoan Community

Bryozoan statoblast abundance (recorded for *L. carteri*, *C. mucedo*, *P. magnifica*, and *L. crystallinus*) had a strong positive relationship with proximity to the G2NPP thermal discharge, with an appreciable presence at the downstream extent of the transect in May (Figure 10). The invasive Asian species *L. carteri* dominated the bryozoan statoblast community, particularly in the vicinity of the thermal discharge canal. Native bryozoans *C. mucedo* and *P. magnifica* were moderately abundant in the canal and at the end of the transect. A noteworthy finding is the European species *L. crystallinus*, which is rare in its native range and previously unrecorded in the St. Lawrence River; three statoblasts from this species were retrieved from different sites within the canal (~100-500m downstream). Colonies of an additional native bryozoan, *Plumatella reticulata*, were not obtained in benthic grabs but were found growing in the canal on the settling plates deployed.

Another non-native bryozoan, the subtropical species *U. gracilis*, was also recorded from the St. Lawrence River for the first time. Its abundance was strongly positively associated with proximity to the discharge source (Figure 11). No evidence of its occurrence (colony fragments) was found beyond the canal. Similar fragment abundances were observed in both May and September .

DISCUSSION

The G2NPP Downstream Environment

The thermal plume generated by G2NPP effluent has altered the nearby SLR and its invertebrate communities in numerous ways. Natural river temperatures near Bécancour in May range between 11ºC-14ºC (St. Lawrence Global Observatory, 2013), which correspond approximately to the temperatures I recorded in May 2012 at many of the sites >600m downstream (outside the discharge canal) in the sampled plume. The warmest sites sampled were subjected to a thermal enhancement of ~10ºC above ambient at that time of year. However, although temperature exhibited a strong gradient, there were numerous other physico-chemical changes downstream of G2NPP, many of which were associated with the abrupt physical transition between the discharge canal and the open SLR. Precipitous changes to flow, sediment grain size, transparency, and conductivity were apparent as water flowed out of the discharge canal and mixed with the river. Although this project was initiated as a thermal study, it became clear that structural habitat changes to this system (e.g., the canal as a microhabitat) and natural environmental heterogeneity (e.g., variation in depth and sediment type) also had important roles in governing invertebrate distribution and abundance.

Community Diversity and Evenness (*Hypothesis 1*)

Diversity appeared to increase with distance downstream, with canal sites exhibiting lower rarefied Simpson's Diversity than mid-plume or downstream sites, which seemed to provide support for *Hypothesis 1*. However, these differences were not statistically significant. Using a rarefied diversity index might not have been a useful metric in this case, owing to high variability among grouped sites, and confounding patterns in richness and evenness. Evidence contrary to *Hypothesis 1* came from the high taxonomic richness observed in the discharge canal, however, communities near the downstream end of the plume were characterized in the same way as the G2NPP discharge canal (high richness, low evenness); therefore, this community structure seems to be related to the relatively shallow, fine-sediment conditions in both of these environments, rather than the result of the influence of the thermal effluent. Furthermore, there is evidence from the ACE model that the observed taxonomic richness may be exaggerated at sites with high invertebrate abundance, e.g., in the canal relative to downstream sites in May. For all these reasons, patterns in taxonomic richness must be interpreted with caution.

It is also notable that communities located in the canal area exhibited the lowest evenness. A major driver of this pattern was the exceptional dominance of *C. fluminea* in the canal; but other dominant groups were the oligochaete (mainly tubificid) worms and chironomid larvae, whose disproportionate abundances at sites throughout the thermal plume were noted in an earlier report (Hydro-Québec 2006). When these three groups were removed from evenness calculations, differences in evenness were much reduced. Given that temperature and sediment were significant predictors of evenness in the plume, it is unsurprising to see patterns driven by the dominance of thermophilic *C. fluminea* and fine-sediment organisms such as oligochaetes and chironomids.

Biological diversity (taxonomic richness, evenness) is commonly a source of inference about community stability (MacArthur 1955, McCann 2000) and provision of ecosystem services (Cardinale et al. 2002, S Giller et al. 2004, Cardinale et al. 2006). Thus, patterns in diversity indices downstream of G2NPP could yield insight into how the thermal effluent has affected the biological integrity of nearby organismal communities. It appears that the elevated richness at the warmest sites may be a sampling artifact related to increased invertebrate abundance at those sites, plus the dominance of a few tolerant species means that these sites might not be as functionally rich as their high taxonomic richness values may suggest. This provides evidence in support of *Hypothesis 1*, which states that overall diversity declines in proximity to the G2NPP.

However, a more meaningful interpretation of diversity indices requires consideration of community composition.

Community Composition (*Hypothesis 2*)

In contrast to predictions made in *Hypothesis 2*, temperature was only of primary importance for explaining variation in *C. fluminea* and was less important for the other members of the benthic invertebrate community. Climate change studies generally predict such range shifts, involving the expansion of thermophilic species into heated waters and the withdrawal of cold-adapted species (Hauer et al. 1997, Sagarin et al. 1999, Walther et al. 2002). When *C. fluminea* was excluded from the analysis, the remaining benthic invertebrates were primarily structured along multiple other physico-chemical gradients (e.g., natural depth gradients, changes to flow, changes to sediment size composition), reflecting *C. fluminea*'s exceptional thermal preferences (see also Simard et al. 2012; Castañeda 2013).

Other organisms that were abundant in the discharge canal included elmid beetle larvae, ostracods, and (as previously mentioned) oligochaete worms. Conditions in the canal promoted periphyton growth on bottom substrates (as suggested by its rapid overgrowth of deployed settling plates), which provides a food source for grazers like elmids (Thorp and Covich 2009). Ostracods can be filter feeders and deposit feeders, and thus might benefit from suspended microbial communities that are stimulated by the combination of detritus and high temperatures. Oligochaete worms tended to be abundant throughout the plume, but extremely high abundances were recorded in the discharge canal in particular; they likely benefited from both the soft sediment for burrowing and enhanced organic content of the surface sediments (Thorp and Covich 2009).

Another group that was abundant in the plume, but could not be included in the CCA analysis, were bryozoans. Many freshwater bryozoan species thrive in relatively warm waters (15-28ºC; Thorp and Covich 2009). As indicated by local statoblast abundance, the bryozoan community was dominated by the non-native species *Lophopodella carteri,* which occurs elsewhere in the St. Lawrence River (Ricciardi and Reiswig 1994), but was found in unprecedented abundance in the G2NPP canal. Fragments of an entoproct bryozoan *Urnatella gracilis* were also found in high abundance within the canal throughout the year and, unlike the other bryozoans, were restricted exclusively to warm sites nearest the discharge source. *U. gracilis* is a globally distributed warm-water species found throughout much of the United States (Thorp and Covich 2009), but has not been previously recorded in the St Lawrence River (Ricciardi and Reiswig 1994; A. Ricciardi, pers. comm.) and is considered a poor candidate for introduction via ballast water (Grigorovich et al. 2003). It typically grows on hard substrates, which may be provided by *C. fluminea* shells, in addition to the natural rocks found in the canal and the gravel added just below the discharge outflow. The abundance of filter-feeding bryozoans and *C. fluminea* suggests that particulate food, perhaps in the form of bacterial mats, is concentrated in the canal.

Mid-plume sites located beyond the opening of the discharge canal were unique in both habitat characteristics and their biological community. They were subject to strong mixing with natural SLR water, were deeper, and were subject to stronger current than other sites. Thus, they were populated by organisms that prefer such conditions, including hydropsychid caddisflies, gammarid amphipods (primarily *Gammarus fasciatus*), and dreissenid mussels.

Sites located the furthest downstream had communities characterized by unionid mussels, sphaeriid clams, and mayfly larvae (Ephemeridae, specifically *Hexagenia sp.*) which thrive in soft-bottomed sediments - similar to those also found in the canal. The positive correlation of these families with increasing downstream distance, despite the occurrence of preferred sediment types in the canal, suggests that their distribution is limited by high temperatures. However, when I excluded 'distance downstream' as an environmental predictor from the CCA analysis, the organisms typical of downstream sites no longer clustered separately from the central grouping of organisms, suggesting that there are characteristics of the downstream sites that met their habitat needs, aside from the inverse relationship between distance and temperature. These species may require food or propagules from the open river to sustain their populations. It is also possible that these species experienced competition or interference from *C. fluminea*, as they occupy a similar functional role as soft-sediment burrowing animals (Vaughn and Spooner 2006).

These groupings of habitat types and macroinvertebrate assemblages demonstrate that the area defined as the G2NPP thermal plume not only includes a thermal gradient, but also encompasses heterogeneous habitats, both naturally occurring and modified. Most notable is the organismal pattern associated with the abrupt transition between the canal and open river environments, which highlights the significance of natural environmental variation in determining community composition, even superseding substantial anthropogenic impacts like thermal enhancement. Such patterns have been documented before, in the heavily modified St. Lawrence River basin and elsewhere (e.g., Pinel-Alloul et al. 1996, Masson et al. 2010). An alternative perspective is that, as a heavily developed, industrial waterway, the biota of the St.

Lawrence River may have already faced selection pressures that have removed intolerant taxa and genotypes (Langford 1990). Identifying distributions of specific taxonomic groups within the plume gives insight into community changes resulting from environmental modification that biotic indices (like richness) alone cannot illuminate (Lakly and McArthur 2000).

Community Abundance and Biomass (*Hypothesis 3*)

Although *C. fluminea* has a distribution that is clearly restricted by temperature, these clams dominated the community biomass in close proximity to the thermal discharge, and comprised a significant proportion of these communities by numerical abundance as well. A potential source of bias in this analysis is that biomass was measured as preserved wet weight, and thus included mollusc shells in the measurement. A site with numerous *C. fluminea* would therefore have a disproportionate biomass relative to a site with soft-bodied organisms. However, there are other relatively large and heavy molluscs (e.g., native unionid mussels, gastropods) that can occupy a soft-bottom, mixed sediment environment (identified as the primary environmental predictor of abundance using a biomass model) like the canal, and indeed do so elsewhere in the plume. Thus *C. fluminea* dominance by biomass can likely be attributed to competitive exclusion or differential habitat preferences rather than a measurement bias. Elimination of molluscs near thermal effluent has already been documented in studies conducted before and after the opening of the Gentilly-1 Power Plant. They found that the initial discharge of thermal effluent into the surrounding SLR resulted in a mass die-off of molluscs, including the prolific European faucet snail *Bithynia tentaculata* (Vaillancourt and Couture 1978), which remains notably absent to this day (Hydro-Québec 2006).

C. fluminea reached its maxima at the closest sites to the discharge source, whereas other invertebrates peaked in abundance and biomass further downstream, generally ~500m, near the confluence of the discharge canal and the natural river, providing overall support for *Hypothesis 3* which predicted that abundance (density and biomass) would be enhanced closer to G2NPP. This may represent an optimal zone in a canal/river trade-off, in which animals are able to benefit from elevated growth and reproduction in the heated waters while maintaining access to resources offered by the downstream flow of the unaffected SLR water.

Although changes in abundance in response to thermal effluent are typically attributed to physiological responses to temperature, in this system, higher abundances (biomass) of invertebrates were primarily found at sites with smaller sediments. However, sedimentation can be affected by thermal effluent, both via modifications to flow regimes and the structural habitat (Massengill 1976) and via enhanced productivity, which enhances organic deposition (Coutant and Brook 1970). Considering the other community metrics examined, both the discharge canal and the furthest downstream sites in the transect possessed relatively fine sediments, constituting the most rich, abundant, and massive communities. These results must be interpreted with caution, as soft sediments are also most easily sampled with a Ponar grab, which was the primary sampling technique used in my study; however, extensive care was taken to ensure that grabs of equal volume were collected at each site. Perhaps soft sediment environments, either naturally occurring or resulting from human modification of rivers, have higher standing stocks because burrowing is easier, yielding more biomass in the interstitial spaces, or perhaps because of the richness of organic matter in these environments. Highly abundant bryozoans are further indicators of a productive community, suggesting a link to enhanced productivity (Hartikainen et al. 2009).

***Lophopus crystallinus*: A Non-native Bryozoan in the St. Lawrence River?**

The discovery of *L. crystallinus* statoblasts is of particular interest. This cryptogenic species is considered native to Europe and North America. However, evidence of its North American distribution is limited to a few historical accounts in the eastern United States. Furthermore, there have been no known preserved specimens from North America, and there have been no verifiable records since the 19th century (Wood and Marsh 1996). It was not collected in a survey of freshwater bryozoans in the SLR and surrounding regions in eastern Canada conducted over 20 years ago (Ricciardi and Reiswig 1994). However, statoblasts of this species were identified in the ballast tanks of foreign ocean vessels entering the St. Lawrence Seaway in the early 2000s (Kipp et al. 2010). Therefore, the most plausible explanation for the occurrence of this cryptogenic species in my study area is that it was introduced relatively recently to the river by ships.

Given that *L. crystallinus* is a holarctic species that can tolerate cold temperatures (Hill et al. 2007, Massard and Geimer 2008), it is not likely to be restricted to the plume, but statoblasts may have become concentrated in the discharge canal owing to the funnel effect of the outflow area. Although long-considered threatened in its native ranges in Europe and the United States, *L. crystallinus* is apparently not as rare as previously believed. In the UK, for example, it has recently been found to occupy a wider distribution than previously documented, suggesting that it is either expanding its range or has remained undetected in many areas owing to a lack of search effort and difficulties in locating and identifying colonies (Hill et al. 2007).

Research Limitations

While the results of my study offer novel, high-resolution insight into community trends downstream of G2NPP, they also must be interpreted with caution. While many possible explanatory variables were explored, a potentially important one was not directly measured: primary production. Data on primary productivity may have allowed more insight into possible reasons for community change and mechanisms for ecosystem-level consequences of the thermal enhancement. Changes to communities via changes to primary production have been recorded in some previous thermal effluent sites (e.g., Osborne and Davies 1987); in some cases they have been identified as a more important driver of community responses than temperature itself. Even here, while I cannot demonstrate causation, groups thriving in the canal tended to be heat-tolerant filter feeders and grazers, which would benefit greatly from enhanced primary production. Indeed, extensive periphyton growth was observed on settling plates deployed in the canal for 4 months as an exploratory study, although I am unable to quantitatively compare periphyton in the canal to downstream sites. Proper measurements of primary production require temporally and spatially replicated samples using dedicated equipment, which was beyond the scope of my study.

Overall, there was also little variation between general patterns of community characteristics in May and September, although there were a few changes to community composition. This consistency between spring and late summer suggests that community patterns remain similar over the course of the seasons at G2NPP. Alternatively, the temporary closure of G2NPP for repair during late summer may have minimized the usual seasonal effects of temperature. The significance of seasonal temperature cycles in compounding thermal stress from effluent has been documented at numerous sites in the literature (e.g., Snoeijs 1989). Therefore, while data collected in May provided insight into invertebrate community patterns at G2NPP in spring, more dramatic differences or patterns more directly attributable to temperature may have been found if summer sampling had been replicated during peak temperatures with the power plant operating at 100% capacity.

Finally, in order to establish causation and identify true community change under the influence of the thermal effluent relative to ‘natural’ conditions, one would need an unheated and hydrologically similar stretch of the river to act as a comparator. Owing to habitat heterogeneity, and the unique structure of the artificial discharge canal, this was not possible. While many associations between environmental change and the biological community have been identified, and their potential implications noted here, further experimental work would be required to untangle the mechanisms involved. Thus, this study focused on spatial community comparisons using a gradient approach, and allows for the evaluation of changes in future years, especially given the recent decommissioning of G2NPP.

TABLES

Table 1: Generalized Additive Models for taxonomic richness of benthic invertebrates collected downstream of the Gentilly-2 Nuclear Power Point discharge point in May 2012, in order of best fit based on AIC values. ** indicates statistically significant predictor variables (α=0.05).

Model predictor variables	p-value	AIC
Distance downstream **Sediment Size (Phi)** **Depth**	4.51E-15 ** 0.000801 ** 0.0078 **	91.81
Sediment size (Phi) **Distance downstream**	0.0012 ** 2.46E-13 **	108.73
Distance downstream **Sediment Size (Phi)** **Transparency**	2.79E-9 ** 0.0058 ** 0.43	110.04
Distance downstream **Depth** **Transparency**	7.65E-5 ** 0.00105 ** 0.013 **	110.95
Distance downstream **Transparency**	4.3E-9 ** 0.016 **	116.71

Table 2: Generalized Additive Models for Simpson's evenness of benthic invertebrates collected downstream of the Gentilly-2 Nuclear Power Plant discharge point in May 2012, in order of best fit based on AIC values. ** indicates statistically significant predictor variables (α=0.05).

Model predictor variables	p-value	AIC
Temperature	0.00024 **	-42.57
Sediment Size (Phi)	0.082	
Temperature	0.00090 **	-41.58
Sediment Size (Phi)	0.22	
Transparency	0.66	
Distance downstream	0.012 **	-39.05
Sediment Size (Phi)	0.15	
Depth	0.32	
Distance downstream	0.00017 **	-38.72
Sediment size (Phi)	0.061	
Distance downstream	0.012 **	-38.58
Transparency	0.12	
Depth	0.24	

Table 3: Generalized Additive Models for biomass of benthic invertebrates collected downstream of the Gentilly-2 Nuclear Power Plant discharge point in May 2012. ** indicates statistically significant predictor variables (α=0.05). * indicates marginally significant predictor variables ($0.05 < p < 0.1$).

Model Predictor Variables	p	AIC
Sediment Size (Phi)	0.032 **	174.26
Distance Downstream	0.25	
Distance Downstream	0.24	174.58
Depth	0.20	
Transparency	0.068*	
Distance Downstream	0.0064**	175.11
Depth	0.13	
Distance Downstream	0.13	175.87
Sediment Size (Phi)	0.072*	
Transparency	0.26	
Distance Downstream	0.037**	176.41
Transparency	0.16	

FIGURES

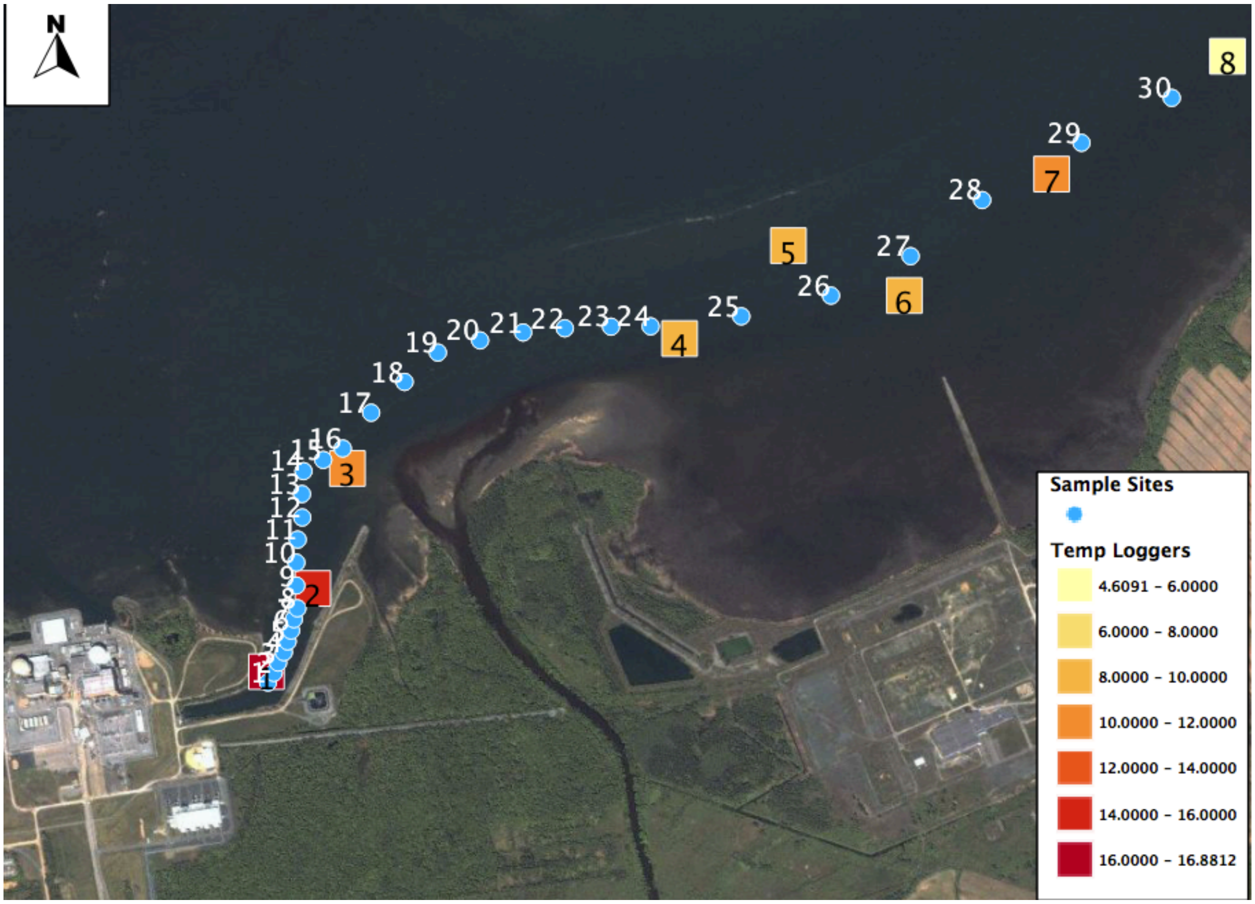

Figure 1: Geographic locations of sampling stations (blue circles; #1-30) along 3.2 km transect downstream of the Gentilly-2 Nuclear Power Plant outflow surveyed during May and September 2012. Locations of temperature loggers deployed by Québec Ministry of Sustainable Development, Environment, Wildlife and Parks in 2010-2012 are indicated by yellow-red squares colour-coded for maximum winter temperature (#1-8). Image credit: Quantum GIS, Yahoo Satellite 2013.

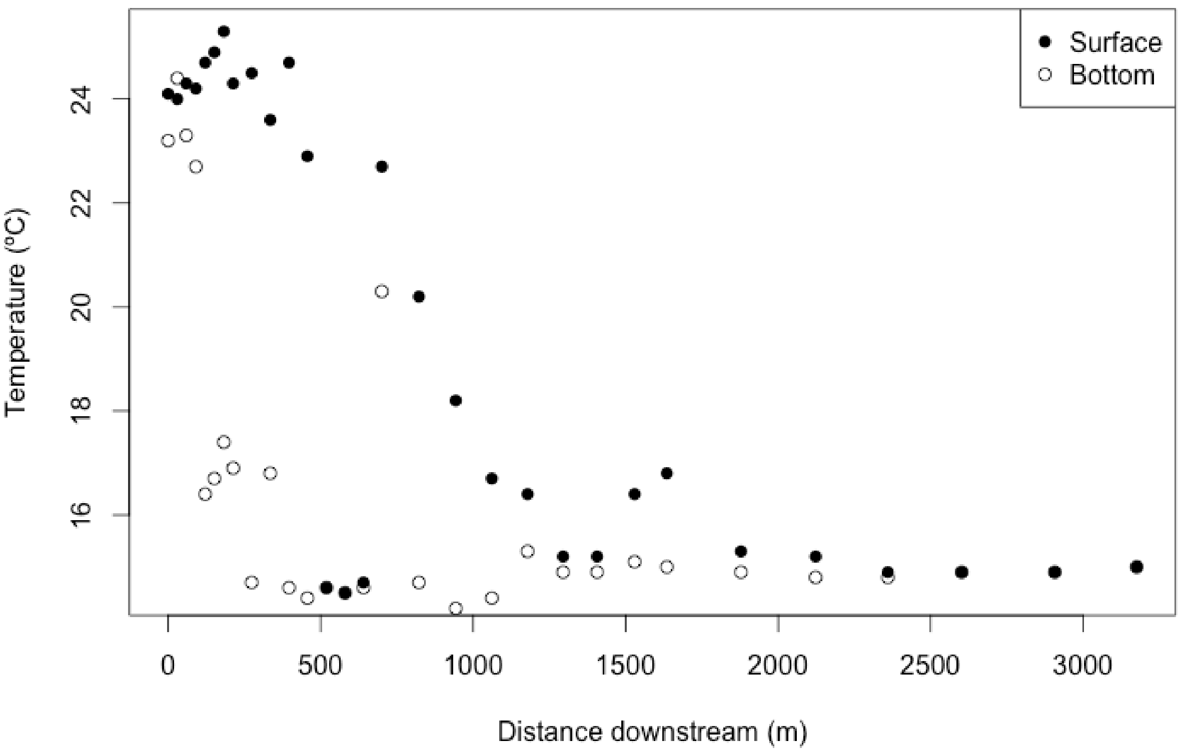

Figure 2: Surface and bottom temperatures (ºC) as a function of distance downstream of the Gentilly-2 Nuclear Power Plant along the sampling transect in May 2012.

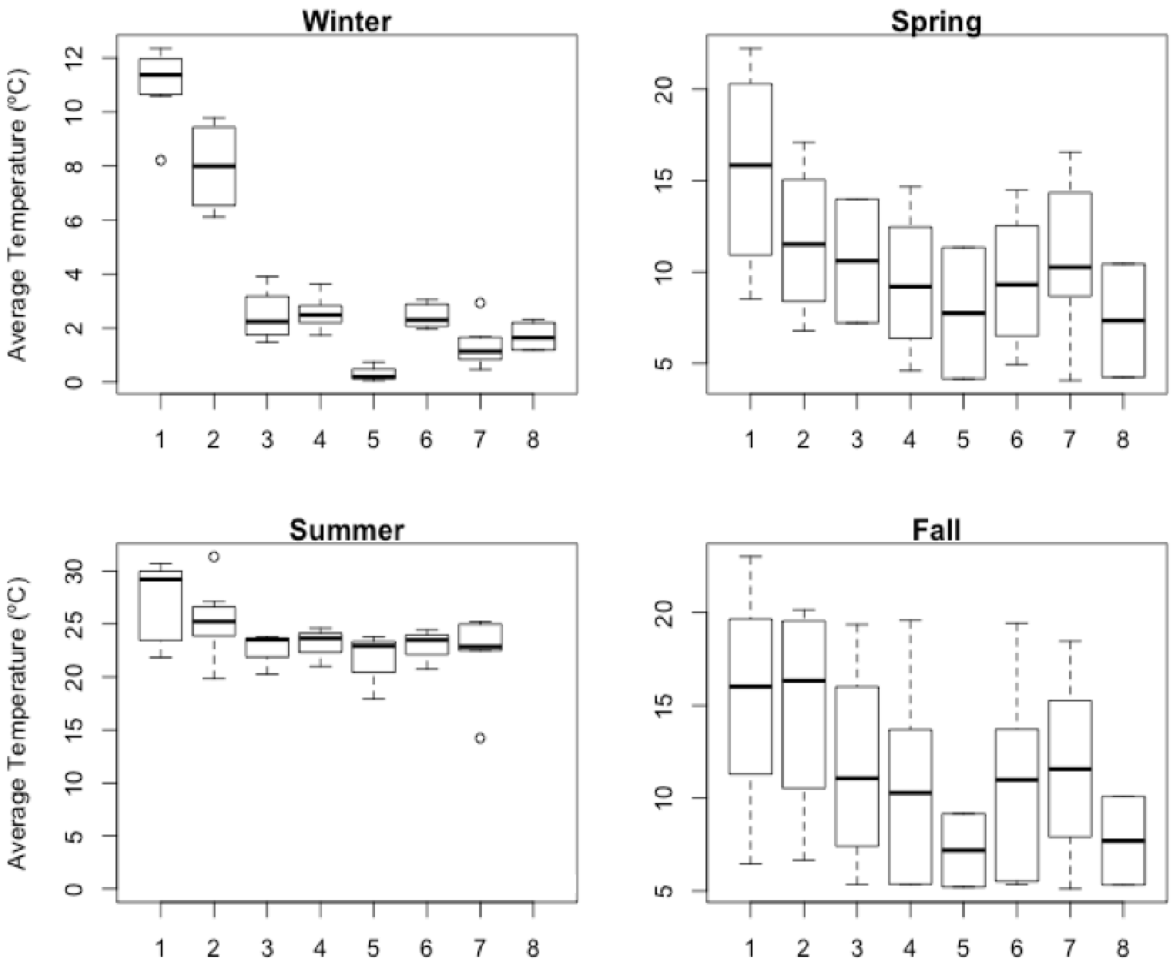

Figure 3: Average temperatures based on hourly recordings from 8 loggers downstream of the Gentilly-2 Nuclear Power Plant along the sampling transect (where #1 is closest to G2NPP discharge and #8 is furthest downstream; Figure 1). Recordings took place between 2010-2012 in winter (December-March), spring (April-May), summer (June-August), and fall (September-November). Logger #8 was not operational during summer months.

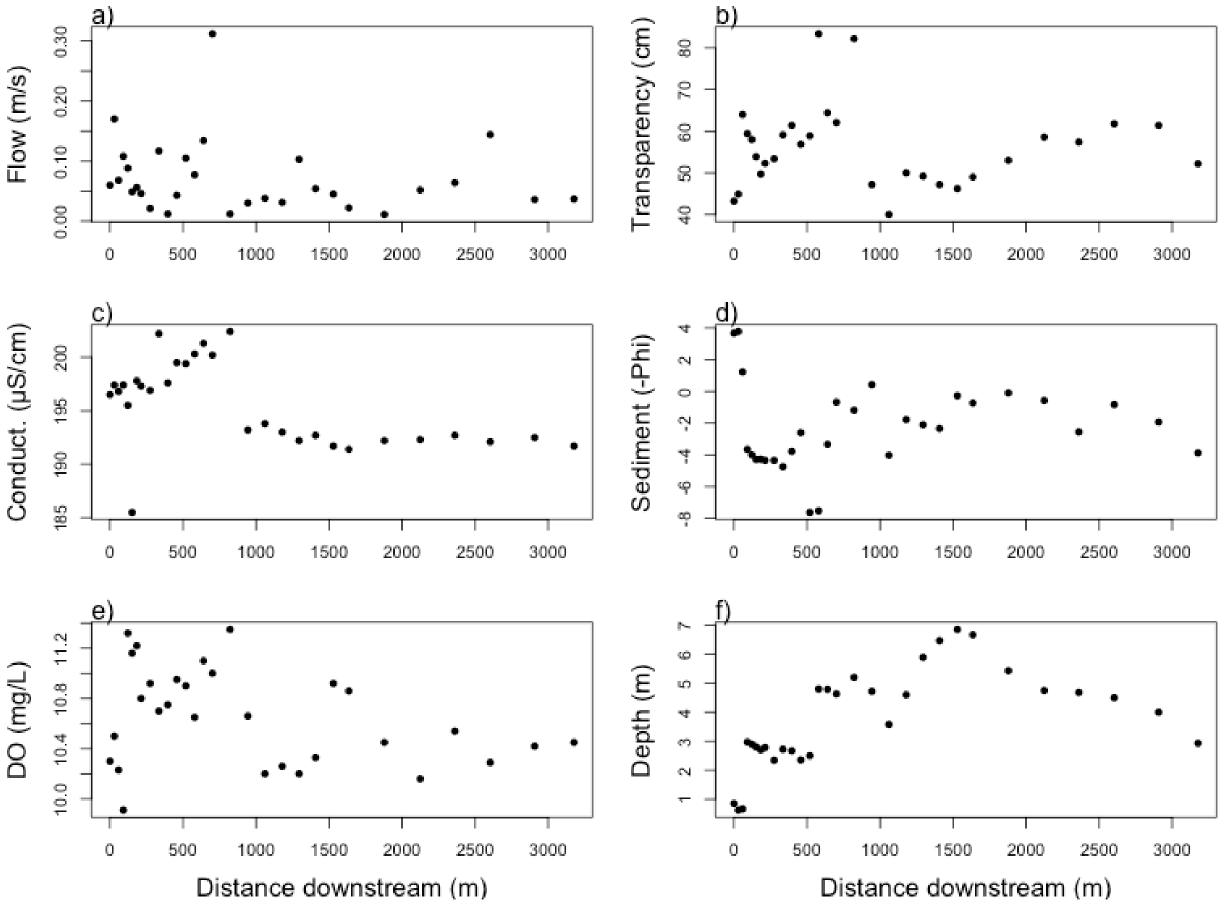

Figure 4: Physico-chemical variables as a function of distance downstream of the Gentilly-2 Nuclear Power Plant along the sampling transect in May 2012: **a)** flow (m/s), **b)** transparency (cm), **c)** specific conductivity (uS/cm), **d)** sediment size (-Phi), **e)** dissolved oxygen (mg/L), **f)** depth (m).

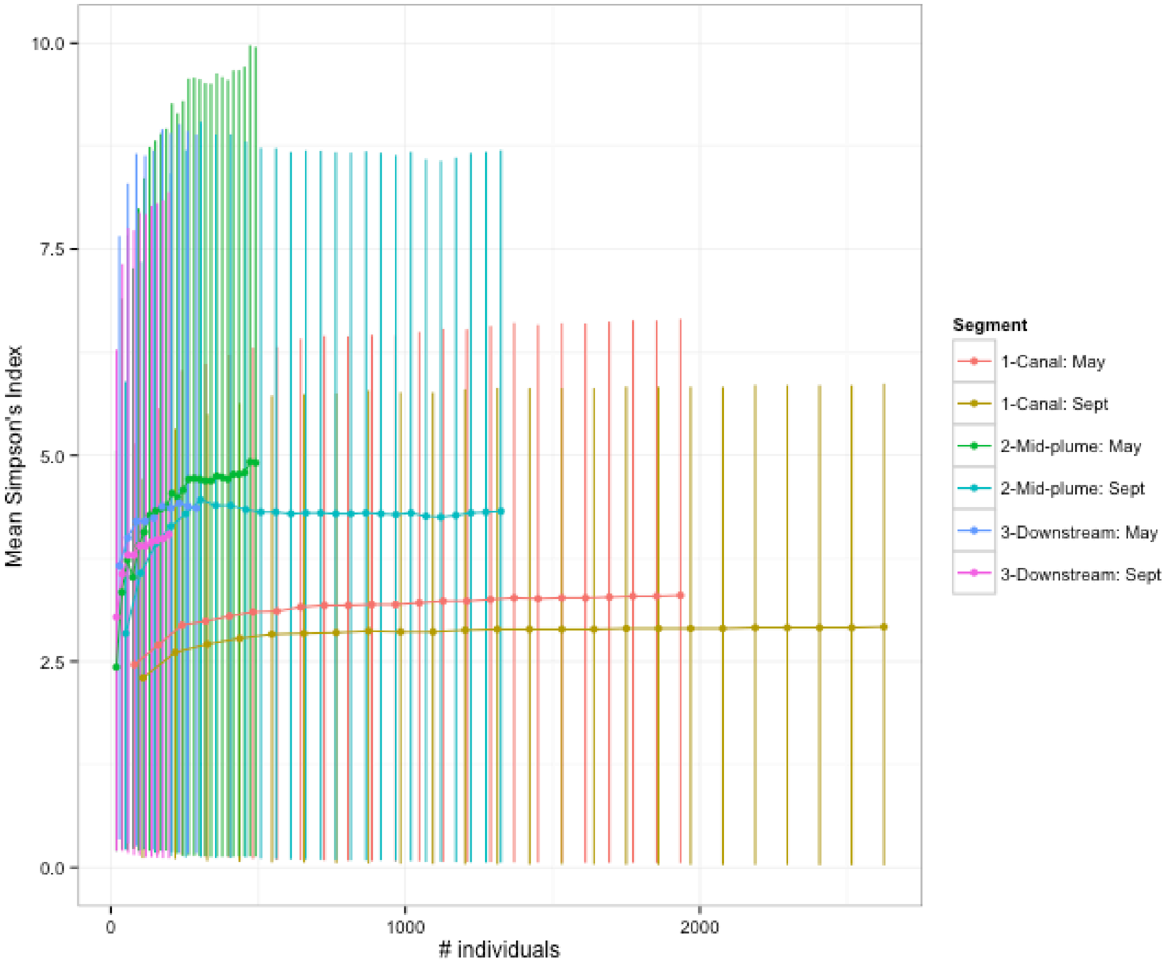

Figure 5: Mean rarefied Simpson's Index for 3 segments of the thermal plume downstream of the Gentilly-2 Nuclear Power Plant thermal discharge: canal (sites #1-12), mid-plume (sites #13-25), downstream (sites #26-30) in May and September 2012. Vertical bars represent 95% confidence intervals.

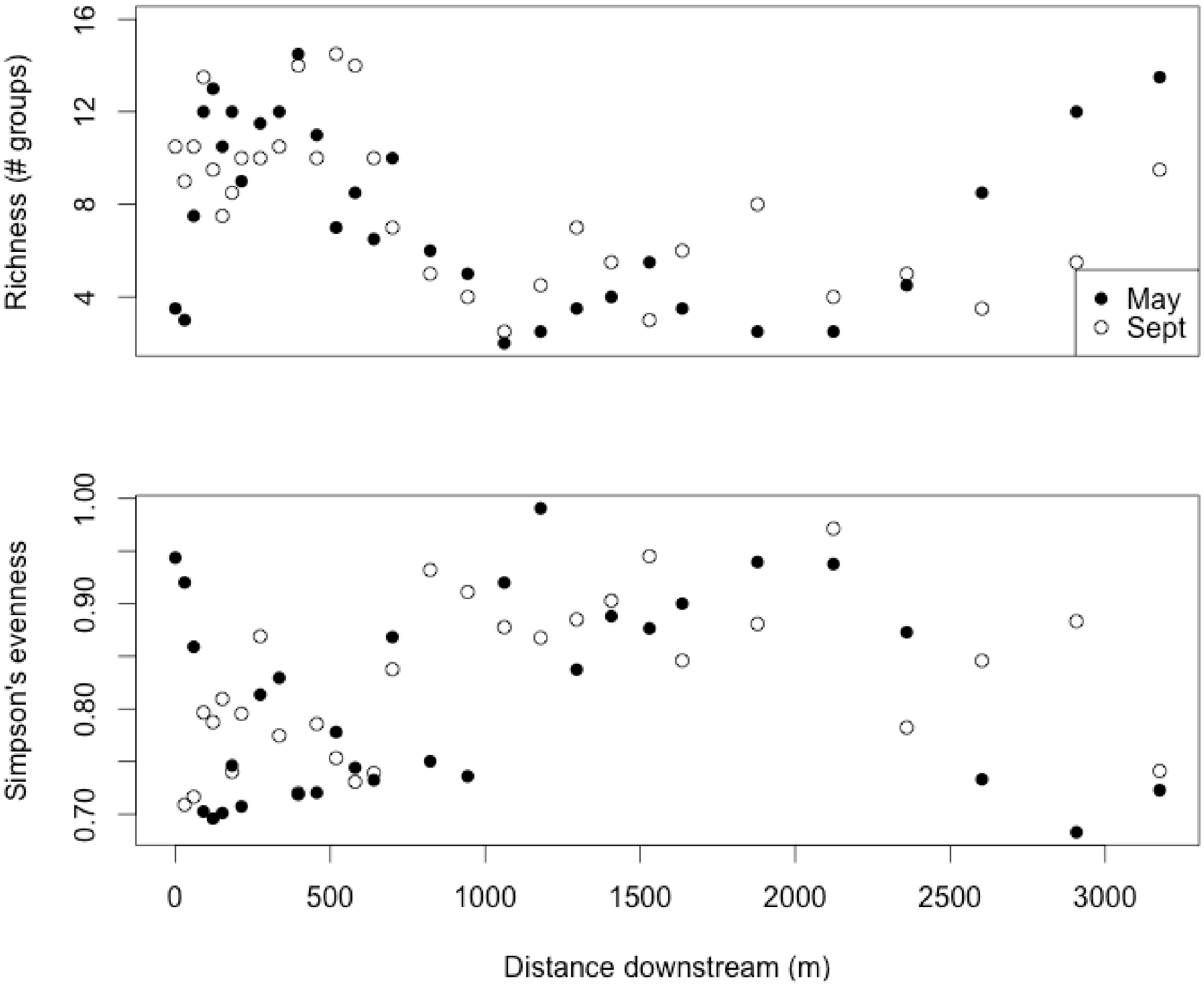

Figure 6: Mean taxonomic richness (# taxonomic groups / m^2) and mean Simpson's evenness (inverse Simpson's evenness / m^2) as a function of distance downstream of the Gentilly-2 Nuclear Power Plant in May and September 2012.

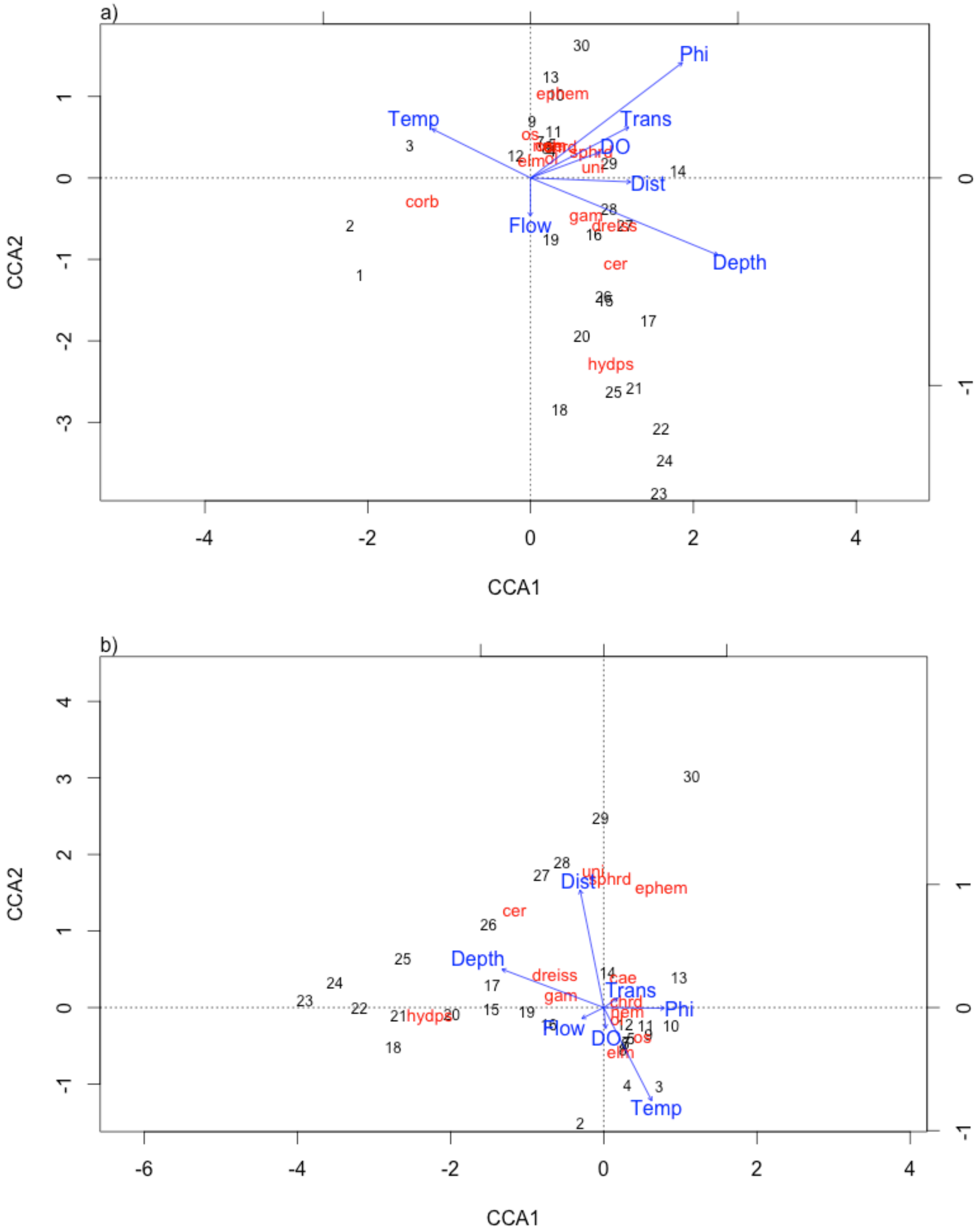

Figure 7: Canonical Correspondence Analysis biplots **a)** including and **b)** excluding *C. fluminea* for May 2012. Blue arrows represent environmental factors: Temp = temperature, DO = dissolved oxygen, Flow = flow velocity, Depth = water depth, Dist = distance from outflow, Phi = sediment size (Phi scale), Trans = transparency. Black numbers represent sampled sites. Red labels indicate taxa: uni = Unionidae mussels, sphrd = Sphaeriidae clams, ephem = Ephemeridae nymphs, cer = Ceratopogonidae larvae, dreiss = Dreissenidae mussels, gam = Gammaridae amphipods, cae = Caenidae nymphs, hydps = Hydropsychidae larvae, chrd = Chironomidae larvae, nem = Nematoda worms, ol = Oligochaeta worms, os = Ostracoda, elm = Elmidae larvae.

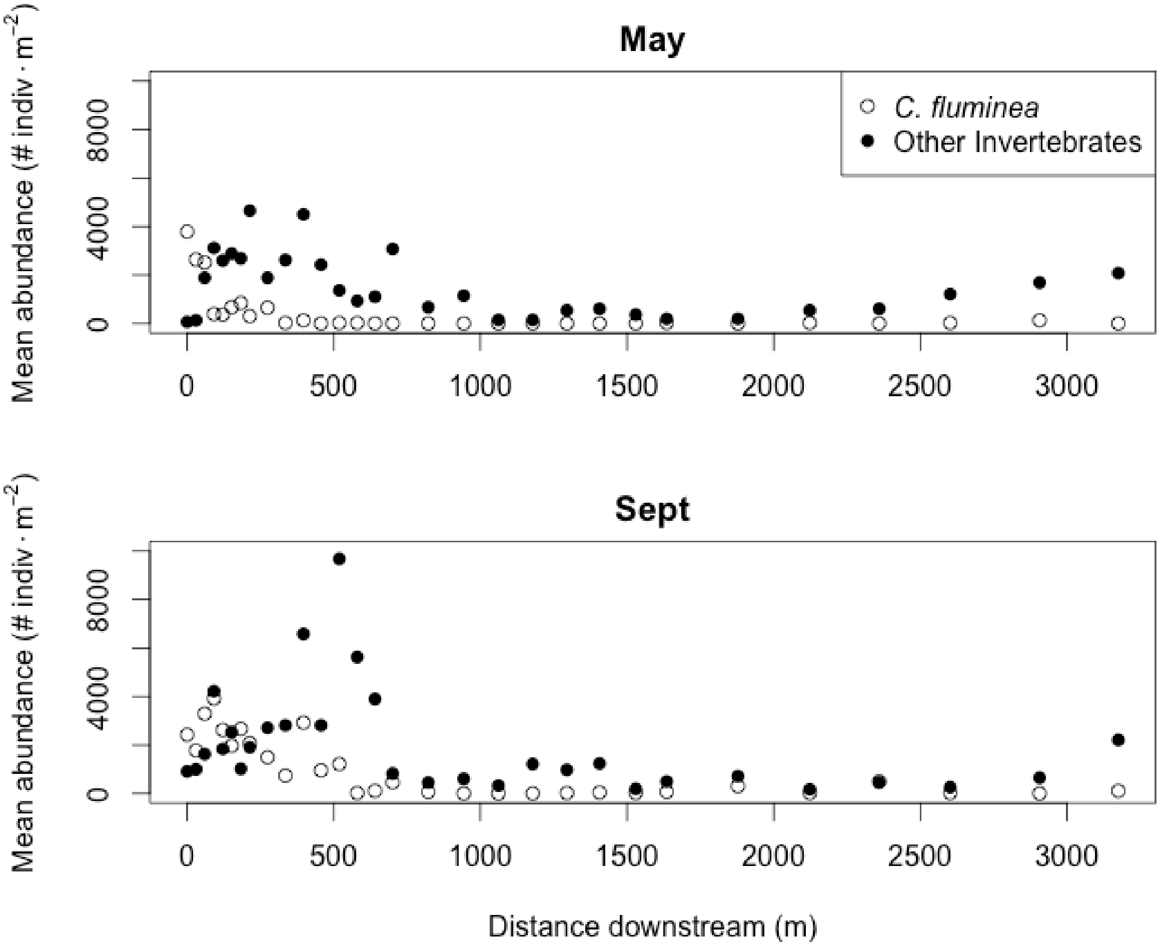

Figure 8: Invertebrate abundance as a function of distance downstream from the Gentilly-2 Nuclear Power Plant in May and September 2012.

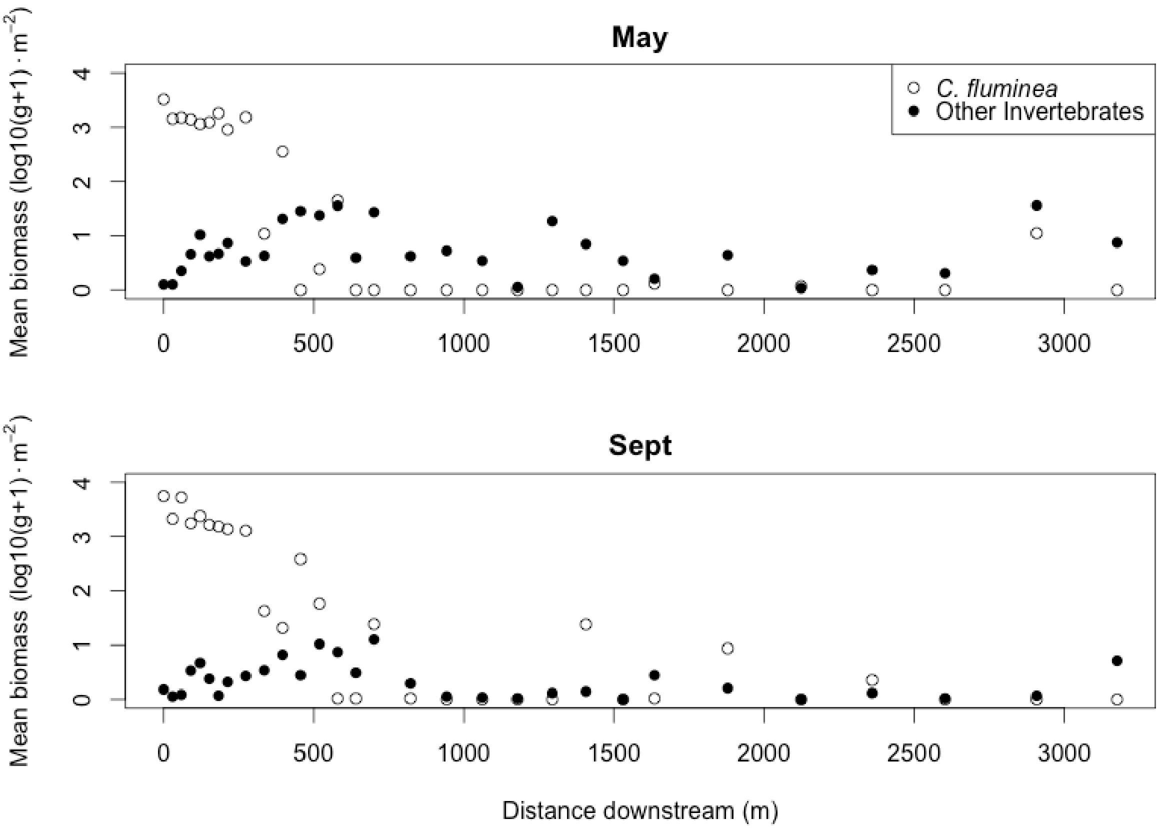

Figure 9: Invertebrate biomass ($\log_{10}$ transformed) as a function of distance downstream from the Gentilly-2 Nuclear Power Plant discharge point in May and September 2012.

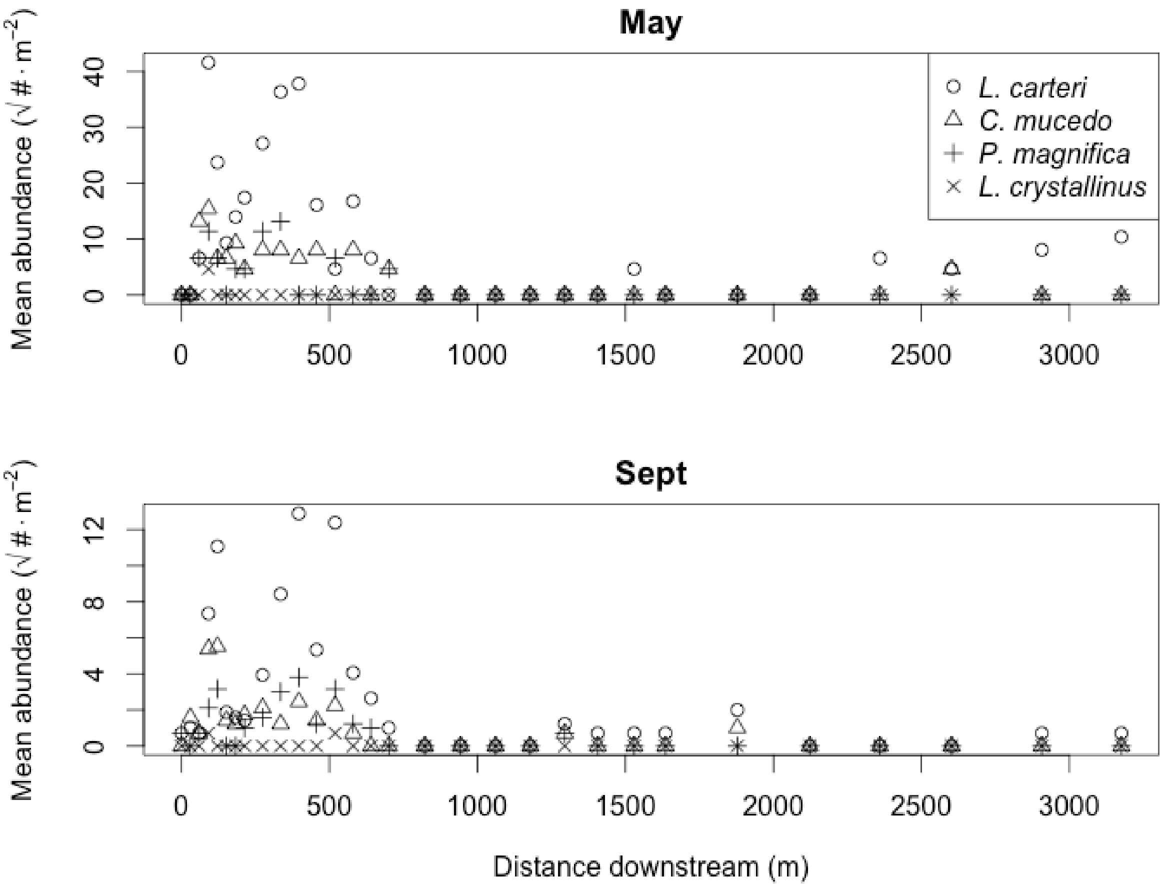

Figure 10: Statoblast abundance (square-root transformed) as a function of distance downstream from the Gentilly-2 Nuclear Power Plant discharge point in May and September 2012. Four species are represented: *L. carteri* = *Lophopodella carteri*, *C. mucedo* = *Cristatella mucedo*, *P. magnifica* = *Pectinatella magnifica*, *L. crystallinus* = *Lophopus crystallinus*.

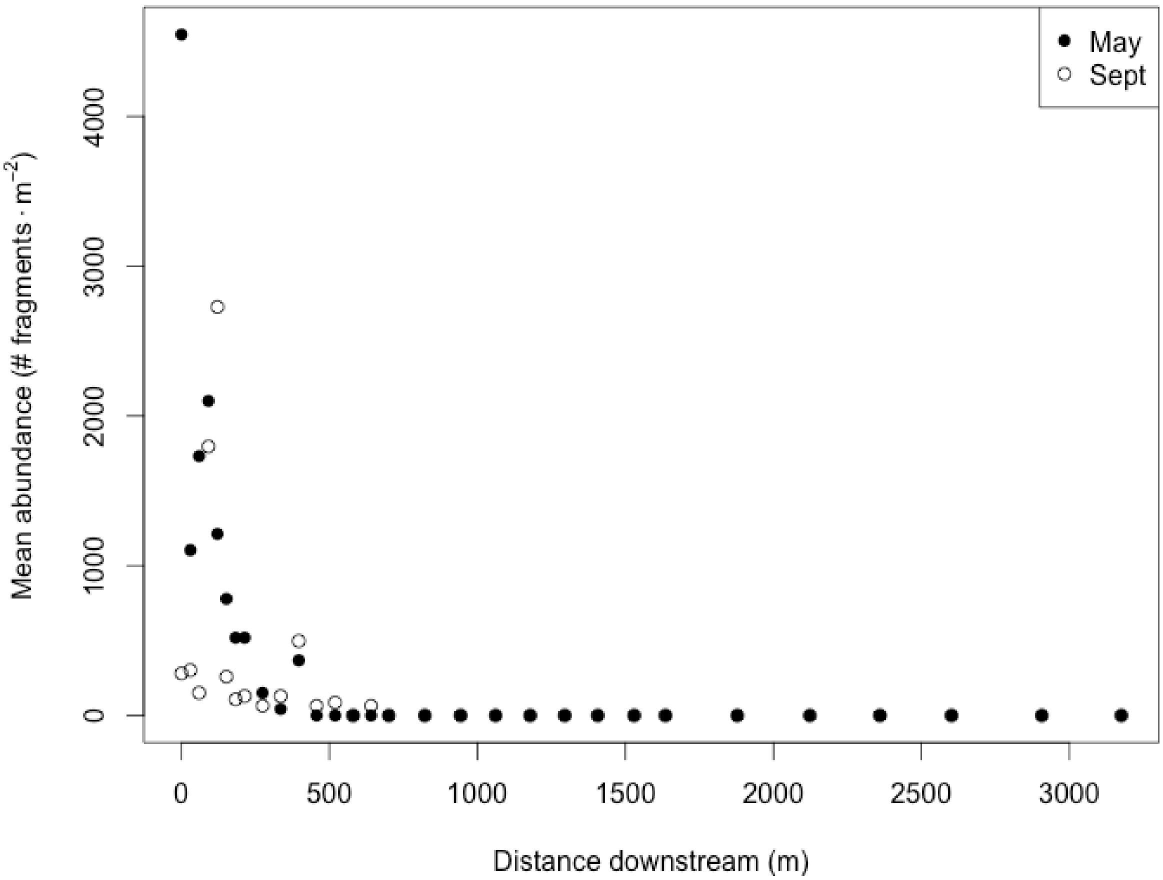

Figure 11: *Urnatella gracilis* abundance (colonial fragments) as a function of distance downstream from the Gentilly-2 Nuclear Power Plant discharge point in May and September 2012.

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CONCLUSIONS

Studies of thermal effluent are often initiated out of concern for the alteration of aquatic habitats, and they tend to underestimate the adaptability of organisms to temperature changes, particularly over long acclimation periods. Furthermore, changes to other environmental variables may be of equal or greater consequence than temperature. In general, diversity indices and characteristics of community composition downstream of G2NPP reflected physical habitat characteristics other than temperature - such as natural depth gradients and variation in sediment type. In particular, the microhabitat of the discharge canal was defined by structural habitat characteristics that shaped its biological community. In summary, invertebrate community changes occurred along the G2NPP thermal gradient, but they seemed to be better explained by habitat heterogeneity along the plume than by the thermal alterations.

Consistent with other studies, at G2NPP temperature was an important predictor only for a minority of taxa, most notably the Asian clam, *C. fluminea,* which was amongst the most dominant species in terms of abundance. Consequently, *C. fluminea* had a significant effect on observed metrics. It is evident that a dominant species, such as an invasive invertebrate with unique habitat preferences, can influence water quality assessments whose inferences are based on invertebrate community characteristics. The specific invader in question matters as well. For example, dreissenid mussels dominate benthic communities throughout the St. Lawrence River, but were excluded from much of the G2NPP site likely due to a combination of unsuitable substrate and elevated temperatures.

Other thermophilic taxa in the discharge canal included bryozoans. This is an understudied group of organisms that, despite their ubiquity and potentially high abundance (Francis 2001, Wood 2001), are often ignored in freshwater invertebrate community assessments. The presence of two non-native species (*L. carteri* and *U. gracilis*) and a cryptogenic species (*L. crystallinus*), two of which were conspicuously abundant, highlights the need for careful consideration of 'obscure' taxa as indicators of community change.

This study was timely because of the permanent closure of G2NPP in late December 2012, allowing that section of the river to revert to its natural thermal regime. Consequently, the *C. fluminea* population suffered a mass die-off over winter and was eliminated by June 2013 (Castañeda et al. 2013, unpublished). My research described the biological patterns established by long-term thermal effluent exposure and a major species invasion, and establishes a baseline against which adaptation and succession of invertebrate populations can be measured in future years.

Owing to the well-documented significance of the G2NPP plume as a thermal refuge for the Asian clam, I predict *C. fluminea* will not recolonize this site in the future unless a cold-tolerant strain is introduced (Simard et al. 2012, Castañeda 2013). However, despite the mass die-off at G2NPP, previous research suggesting that *C. fluminea* has a lower thermal limit for survival of 2ºC (Mattice and Dye 1975) is currently being re-evaluated (Müller and Baur 2011). Indeed, *C. fluminea* populations have recently been found established in north temperate lakes in the absence of thermal plumes (e.g., Lake George NY; Margaret Modley, Lake Champlain Basin program, pers. comm.). Ongoing experiments are investigating whether these populations survive in cold climates via phenotypic plasticity, or because they constitute a unique cold-adapted genotype; in any case, these organisms are a potential source for re-invasion of the St. Lawrence River.

The closure of G2NPP has caused directional water flow to cease in the discharge canal, which I have characterized as a unique microhabitat. Although there is no longer an extreme modified thermal regime, the physical structure of the canal will continue to create discontinuity in this river system. The canal will likely remain a warm, shallow, soft sediment environment, but may no longer be hospitable to most filter feeders, so the abundance of bryozoans may be reduced. The community may be further transformed through recolonization by organisms which were previously excluded by temperature or by competition with *C. fluminea*. However, over longer time periods, I expect the benthic community to reflect a lentic assemblage, in the absence of substantive water flow through the canal.

There are surprisingly few studies which have evaluated long-term effects of thermal effluent exposure, or focused on the recovery of post-thermal bodies of water. One study examined the potential for recovery on the Savannah River Site in South Carolina after thermal enhancement (Lakly and McArthur 2000); a stream formerly exposed to thermal effluent (11 years post-thermal) was found to be more similar to nearby streams currently exposed to thermal pollution than it was to comparable natural streams. The authors concluded that the effluent effects compound over time, as species richness and density may not recover to a previous or "natural" state unless or until the natural fluid dynamics of the system return. For years after effluent exposure, they found that communities remained functionally and structurally distinct. Research for the purposes of restoration should be a priority in this field. Without long-term studies, and follow-up research after decommissioning, the consequences of decades of exposure to thermal effluent cannot be evaluated, and the resilience of aquatic systems to these perturbations will remain unknown.

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APPENDIX

Table A1: Taxonomic groups identified downstream of the Gentilly-2 Nuclear Power Plant thermal discharge point in May and September 2012.

Phylum	Class	Order	Family	Genus/Species
Nematoda				
Annelida	Oligochaeta			
Arthropoda	Ostracoda			
Arthropoda	Maxillopoda	Cyclopoida		
Arthropoda	Arachnida (Hydracarina)			
Arthropoda	Insecta	Diptera	Chironomidae	e.g., *Chironomus sp.*
Arthropoda	Insecta	Diptera	Ceratopogonidae	
Arthropoda	Insecta	Coleoptera	Elmidae	e.g., *Dubiraphnia sp.*
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	*Hexagenia sp.*
Arthropoda	Insecta	Ephemeroptera	Caenidae	
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	
Arthropoda	Insecta	Trichoptera	Hydropsychidae	
Arthropoda	Insecta	Trichoptera	Odontoceridae	
Arthropoda	Insecta	Trichoptera	Hydroptilidae	
Arthropoda	Insecta	Trichoptera	Polycentropodidae	
Arthropoda	Insecta	Trichoptera	Leptoceridae	
Arthropoda	Insecta	Anisoptera	Gomphidae	
Arthropoda	Insecta	Anisoptera	Aeshnidae	
Arthropoda	Insecta	Anisoptera	Protoneuridae	
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus fasciatus, Echinogammarus ischnus*
Mollusca	Bivalvia	Veneroida	Corbiculidae	*Corbicula fluminea*
Mollusca	Bivalvia	Veneroida	Sphaeriidae	*Sphaerium sp., Pisidium sp.*
Mollusca	Bivalvia	Veneroida	Dreissenidae	*Dreissena bugensis, D. polymorpha*
Mollusca	Bivalvia	Unionoida	Unionidae	
Mollusca	Gastropoda	Basommatophora	Ancylidae	*Ferrissia sp.*
Mollusca	Gastropoda	Basommatophora	Physidae	
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	*Fossaria sp.*
Mollusca	Gastropoda	Heterostropha	Valvatidae	*Valvata tricarinata*
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Amnicola sp.*
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Elimia sp., Pleurocera sp.*
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	
Bryozoa	Phylactolaemata		Lophopodidae	*Lophopodella carteri*
Bryozoa	Phylactolaemata		Lophopodidae	*Lophopus crystallinus*
Bryozoa	Phylactolaemata		Cristatellidae	*Cristatella mucedo*
Bryozoa	Phylactolaemata		Pectinatellidae	*Pectinatella magnifica*
Bryozoa	Phylactolaemata		Barentsiidae	*Urnatella gracilis*
Bryozoa	Phylactolaemata	Plumatellida	Plumatellidae	*Plumatella reticulata*

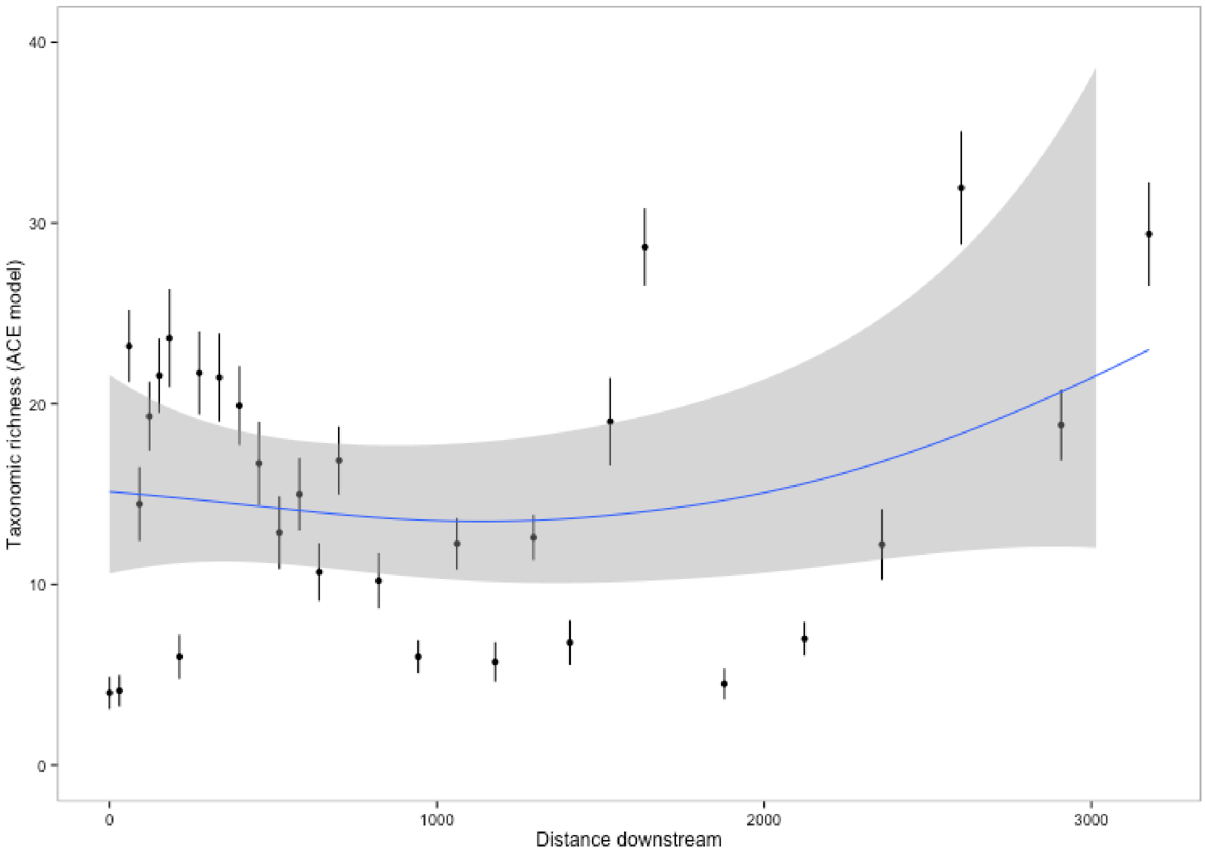

Figure A1: Taxonomic richness predicted by the Abundance-based Coverage Estimator (ACE) as a function of distance downstream of the Gentilly-2 Nuclear Power Plant in May 2012, fitted with a smoother.

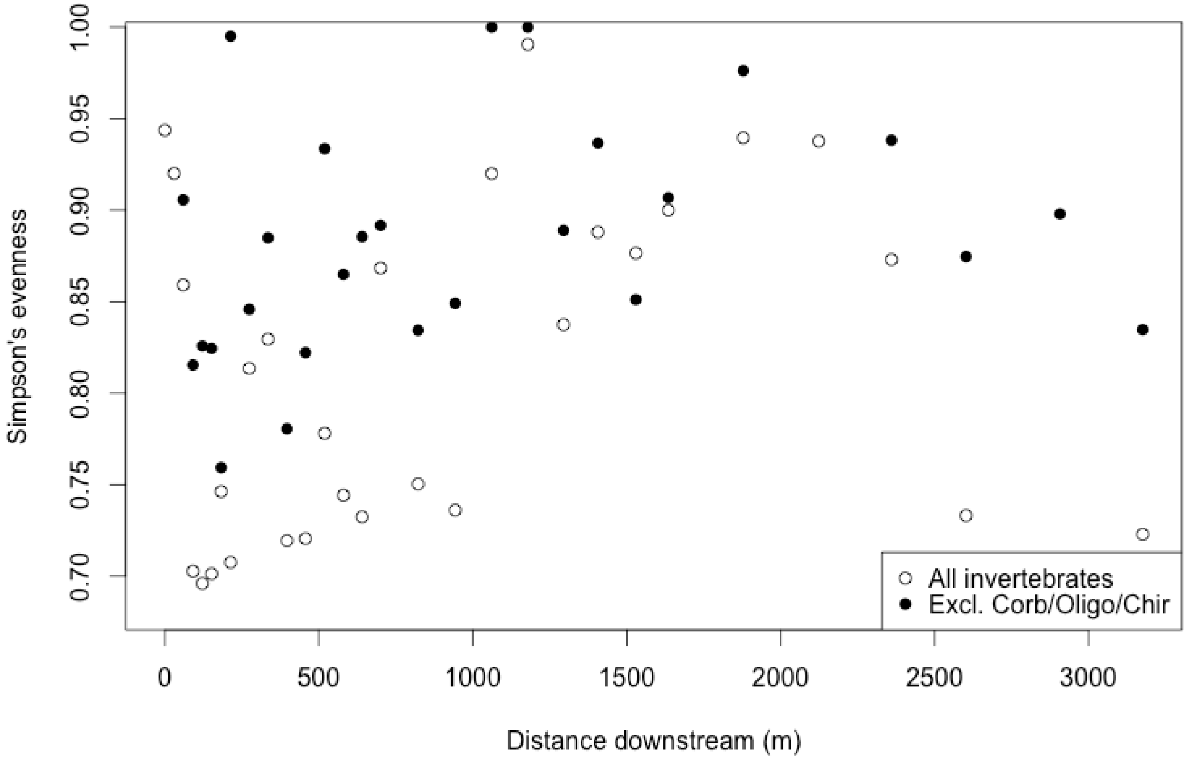

Figure A2: Mean Simpson's evenness (inverse Simpson's evenness / m^2) as a function of distance downstream of the Gentilly-2 Nuclear Power Plant in May 2012, with all invertebrates included (open circles) and excluding three dominant groups: *Corbicula fluminea*, Oligochaeta, Chironomidae (solid circles).