

Article

Do freshwater gastropods avoid the benthic cyanobacterium *Lyngbya wollei*?

Anna Visconti,^{1,2†} Antonella Cattaneo,^{1*} Pierre Gagnon,³ and Christiane Hudon³

¹ Groupe de recherche interuniversitaire en limnologie et en environnement aquatique (GRIL) and Département des Sciences Biologiques, Université de Montréal, Montreal, QC, Canada

² ISE Istituto per lo Studio degli Ecosistemi, Verbania Pallanza (VB), Italia

³ Water Science and Technology Branch, Environment Canada, Montreal, QC, Canada

*Corresponding author: antonia.cattaneo@umontreal.ca

†Current address: annavisconti79@gmail.com

Received 12 March 2014; accepted 18 July 2014; published 3 December 2014

Abstract

We experimentally assessed the mechanisms underlying the reduction in gastropod abundance in areas dominated by the filamentous, toxin-producing cyanobacterium *Lyngbya wollei*, which has replaced the large beds of *Vallisneria americana* in some areas of the St. Lawrence River. We hypothesized that the reduction in gastropod abundance was due to adult avoidance of cyanobacterial mats in favour of filamentous chlorophytes or vascular macrophytes. Of the 4 gastropod taxa offered a choice between *L. wollei* and filamentous chlorophytes, 3 (*Pleurocera acuta*, *Amnicola limosa*, and *Gyraulus parvus*) were either indifferent or even attracted to the cyanobacterium, and only *Viviparus* sp. preferred the chlorophytes. *Lyngbya wollei* exhibited higher nitrogen (N) contents (5–6%) and a lower carbon to nitrogen (C:N) ratio (6.1–7.6) than filamentous chlorophytes (3–5% N, C:N ratio 8.2–11.6). When offered a choice between *L. wollei* and the ribbon-leaved *V. americana* supporting either natural or partially removed epiphyte cover, *P. acuta* and *A. limosa* preferred the macrophyte with its natural epiphyte cover. Epiphytes on *V. americana* were twice as abundant and had a lower C:N ratio than natural epiphytes on *L. wollei*. Additional experiments exposing juvenile *Bithynia tentaculata* and *Physa gyrina* to different filamentous substrata showed similar growth but lower survival when gastropods were reared with *L. wollei* rather than with chlorophytes. Overall, our results showed that direct avoidance of *L. wollei* by adult gastropods did not explain their decline in areas dominated by cyanobacterial mats. Instead, gastropod decline likely resulted from habitat degradation coincident with reduced macrophyte abundance combined with decreased survival of juveniles exposed to *L. wollei*.

Key words: *Amnicola*, *Bithynia*, filamentous algae, Gastropods, *Gyraulus*, *Lyngbya wollei*, *Physa*, *Pleurocera*, *Rhizoconium*, substratum selection, *Viviparus*, *Vallisneria*

Introduction

Gastropods represent an important component in benthic assemblages of lakes and rivers in terms of density, biomass, and diversity. Herbivorous gastropods rely on aquatic vegetation for both food and substratum and represent a key link in trophic webs transferring matter and energy to top predators (fish, birds, mammals). High gastropod densities are commonly associated with aquatic

plants because both faunal abundance and species composition tend to be correlated with macrophyte bed size (Cyr and Downing 1988, Strayer and Malcom 2007). Although feeding selectivity varies among taxa, most pulmonate gastropods graze preferentially on epiphytic algae (Russell-Hunter 1978, Lodge 1986) whereas prosobranchs include deposit-feeders of sediment and detritus (Schreiber et al. 1998, Dorgelo and Leonards 2001) feeding occasionally on seston (e.g., *Viviparus* and *Bithynia*;

Brown et al. 1989, Brendelberger 1995a). Consumption of vascular macrophytes mostly occurs in the absence of other food resources (Lombardo and Cooke 2002). Moreover, gastropod feeding and substratum preferences may be related to body size, with large taxa sometimes consuming macrophytes while small individuals are restricted to detritus and periphyton (Lombardo and Cooke 2002).

Feeding on microcystin-producing planktonic cyanobacteria is detrimental to gastropods (Gérard et al. 2005), and presence of microcystin in gastropod tissues has been observed in the field (Kotak et al. 1996, Zurawell et al. 1999). Experimental exposition of *Lymnea stagnalis* (Gastropoda, Pulmonata) to purified extracts of the MC-LR microcystins induced a reduction in egg production (Gérard et al. 2005). A 10-year decline in gastropod assemblages in a French lake has been partly attributed to periodic cyanobacterial blooms (Gérard et al. 2008).

In contrast with the hepatotoxins (microcystins) produced by planktonic cyanobacteria, filamentous benthic cyanobacteria belonging to the genus *Lyngbya* produce saxitoxin analogues (lyngbyatoxins, LYNGTX, LWTX-1, and LWTX-6; Foss et al. 2012, Lajeunesse et al. 2012). The neurotoxic effects of lyngbyatoxins were recently documented for amphipods living at sites colonized by *Lyngbya wollei* mats ($\approx 100 \mu\text{g}$ LWTX-1/g dry mass (DM); Gélinas et al. 2013), but their impact on gastropod fitness and growth has not been documented. Indirect evidence is provided by the reported 2–5-fold rise in mortality of the estuarine herbivorous gastropod *Cerithidea californica* following an experimental shift in the composition of intertidal algal assemblages toward dominance of (unidentified) benthic cyanobacteria (Armitage and Fong 2004).

Lyngbya wollei is a benthic cyanobacterium that forms mats of filaments documented in a variety of lakes, reservoirs, and rivers in North America (Hudon et al. 2014). Replacement of macrophyte assemblages dominated by *Vallisneria americana* and filamentous chlorophytes by *L. wollei* mats has been documented in the St. Lawrence River (Vis et al. 2008). Areas dominated by *L. wollei* were characterized by a sharp drop in biomass of total invertebrates (9-fold), especially gastropods (11-fold), in comparison with areas dominated by *V. americana* and chlorophytes (Hudon et al. 2012). In addition, low abundance of invertebrate prey coincided with reduced fish species richness and slow growth of juvenile fish in comparison with *V. americana*-dominated wetlands (Hudon et al. 2012). The reduction in gastropod abundance could result from the reduced complexity of the habitat following the decline of *V. americana* or from a direct negative effect of *L. wollei* mats (toxicity, low food quality or quantity). Because gastropods are able to respond to organic clues generated by other organisms

(Haynes and Taylor 1984, Brönmark 1985), they may avoid *L. wollei*, which produces saxitoxins (Foss et al. 2012, Lajeunesse et al. 2012) and odour and taste metabolites (Watson et al. 2008).

This experimental study addressed 3 questions: (1) Do gastropods avoid *L. wollei* in favour of morphologically similar but nontoxic filamentous chlorophytes? (2) Do gastropods prefer a physically firm substratum like the ribbon-like leaves of the macrophyte *V. americana* over flimsy cyanobacterial filaments? (3) Is gastropod choice affected by epiphyte abundance and quality (elemental composition) on different substrata?

To test these questions, we performed short-term, substratum choice laboratory experiments offering different gastropod taxa and size a choice between *L. wollei* and various filamentous chlorophytes (question 1) or between *L. wollei* and the submerged vascular macrophyte *V. americana* (question 2), taking into account the epiphyte cover (question 3). Gastropod avoidance of *L. wollei* in favour of filamentous chlorophytes could stem from either repulsion for cyanobacterial toxins or attraction to nutritious food. In addition, gastropod avoidance of *L. wollei* in favour of the macrophyte *V. americana* could reflect a preference for a sturdy, firm substratum. Finally, we predicted that gastropods would prefer a substratum with abundant epiphytic cover. Throughout the substratum choice experiments, we accounted for gastropod subclass and size because the ecological response of these groups may be different.

In addition to short-term substratum choice experiments, we also investigated the effects of *L. wollei* on gastropod recruitment, comparing growth and survival of juveniles reared in the presence of either this cyanobacterium or filamentous chlorophytes.

Methods

Field collection

Gastropods and aquatic vegetation were collected on 4 dates between July and September 2012 at different locations in 2 fluvial lakes of the St. Lawrence River near Montreal (Lake Saint-Pierre: 46.17451 N, -72.802652 W, and Lake Saint-Louis: 45.42764 N, -73.846065 W; Table 1). Abundant gastropods were found only in areas devoid of *L. wollei*, which ruled out testing the possible effect of previous exposure to the cyanobacterium. Additional samples of filamentous chlorophytes were collected in an urban pond (Beaubien park: 45.51573 N, -73.608688 W) located close to Université de Montréal. Gastropods were collected using either a rake or a D-frame net (500 μm mesh) passed through the submerged vegetation (Tourville Poirier et al. 2010).

Table 1. Organisms used in the experiments of substratum choice by gastropods. Organisms were either collected in St. Lawrence River fluvial lakes Saint-Louis (LSL) or Saint-Pierre (LSP) or in a pond in urban Park Beaubien (PB) near Université de Montréal. Experiments for which each organism was used are specified.

Gastropods				
Subclass	Taxon	Size (length, cm)	Site	Experiment
Prosobranchia	<i>Pleurocera acuta</i> (Rafinesque)	>1	LSP	I; IIa; IVb
	<i>Ammicola limosa</i> (Say)	<0.5	LSP; LSL	IIIa–c; IVa
	<i>Bithynia tentaculata</i> (Linnaeus)	—	LSP	Growth/Survival
	<i>Viviparus</i> sp. (Montfort)	>1	LSP	IIb
Pulmonata	<i>Gyraulus parvus</i> (Say)	<0.5	LSP; LSL	IIIb–d
	<i>Physa gyrina</i> (Say)	—	LSP	Growth/Survival
Vegetation				
Phylum	Taxon	Filament width (µm)	Site	Experiment
Cyanobacteria	<i>Lyngbya wollei</i> (Farl. ex Gomont)	50	LSP	All
Chlorophyta	<i>Hydrodictyon</i> (Roth)	35	LSP	I
	<i>Oedogonium</i> (Link)	30	LSP	I
	<i>Spirogyra</i> (Link)	65	LSL	IIIa–b
	<i>Rhizoclonium</i> (Kuetzing)	95	PB	II; IIIc–d
Macrophyta	<i>Vallisneria americana</i> (Michx)	5334	LSP	IVa–b

Laboratory experiments

Macrophytes and metaphyton

The different types of vegetation used for the experiments are commonly found in St. Lawrence River fluvial lakes (Table 1). *Lyngbya wollei*, a benthic filamentous cyanobacterium, was contrasted with several taxa of filamentous chlorophytes: *Spirogyra*, *Rhizoclonium*, *Oedogonium*, and *Hydrodictyon*. We also used the rosette, ribbon-forming macrophyte *Vallisneria americana*, which is the dominant submerged macrophyte in the St. Lawrence River. Aquatic vegetation (cyanobacteria, chlorophytes, macrophytes) was kept in filtered (Whatman GF/C) and oxygenated water under laboratory light conditions ($\sim 13 \mu\text{E m}^{-2} \text{s}^{-1}$) for 1–4 d before the experiments.

Quantity and quality of food offered to gastropods was assessed by measuring chlorophyll *a* (Chl-*a*) content and elemental composition as carbon (C) and nitrogen (N) tissue content of the vegetation types tested in each experiment. Loosely attached epiphytes were removed from weighted subsamples of *L. wollei* and *V. americana* by gently shaking in water. Epiphytic algae thus resuspended were concentrated by centrifugation and then frozen and freeze-dried. After removing the epiphytes, subsamples of the different types of vegetation were similarly frozen, freeze-dried, and ground to powder. Chl-*a* content was measured in subsamples after 24 h extraction in 95% ethanol and reading their absorbance spectrophotometrically before and after acidification (Nusch 1980). Percent C and N in vegetation and epiphyte subsamples were determined with a Fisons Elemental Analyser 1108 CHNS-O.

Gastropods

Gastropods were held in laboratory aquaria under ambient temperature (20–24 °C) and light ($\sim 13 \mu\text{E m}^{-2} \text{s}^{-1}$) and used within 1–4 d of collection. Individuals were sorted by taxa (based on Pennak 1978, Clarke 1981, Thorp and Covich 2010) and size class (Table 1) and kept fasting in filtered (Whatman GF/C) oxygenated water at least 24 h prior to the experiment. The gastropod taxa used for the experiments are commonly encountered in the St. Lawrence River fluvial lakes. *Pleurocera acuta* (Cerithiacea, Pleuroceridae), *Ammicola limosa* (Rissoacea, Hydrobiidae), *Bithynia tentaculata* (Bithynidae), and *Viviparus* sp. (Viviparidae) are Prosobranchia (gill-breathing snails), while *Gyraulus parvus* (Planorbacea, Planorbidae) and *Physa gyrina* (Physacea, Physidae) belong to the Pulmonata subclass (lung-breathing snails; Table 1).

All experiments were carried out in filtered water from the sites where *L. wollei* were collected. At these sites, water was turbid (Secchi depth 30–50 cm) with moderate conductivity (257–266 $\mu\text{S cm}^{-1}$) and nutrient concentrations (total phosphorus: TP = 29–82 $\mu\text{g L}^{-1}$ P; total nitrogen: TN = 377–950 $\mu\text{g L}^{-1}$ N), and relatively high dissolved organic carbon (DOC = 4–6 mg L^{-1} C).

Choice experiments: experimental setup

Four experiments (I–IV) were performed to test the substratum preference of gastropods of different taxa and size exposed to various vegetation types (Table 2). All experiments offered a choice between *L. wollei* and another substratum (Table 2): (1) filamentous chlorophytes (either *Hydrodictyon*, *Oedogonium*, *Rhizoclonium*, or *Spirogyra*)

Table 2. Summary of the choice experiments carried out using various gastropod taxa offered a choice between *Lyngbya wollei*, different types of filamentous chlorophytes, or the macrophyte *Vallisneria americana*. For each experiment (Exp.), date, gastropod taxon, number of individual per aquarium, and number of aquaria are specified. We indicated also the number of aquaria under natural light (L) or dark (D) conditions, the duration (days), and the frequency of individual counts on each substratum.

Exp.	Start date	Gastropod taxon (individuals × no. aquaria)	Vegetation	Light/Dark (no. of aquaria)	Duration (day 1–2) Count frequency
I	26 Jul	<i>Pleurocera acuta</i> (25 × 8)	<i>L. wollei</i> vs. <i>Oedogonium</i> (90%)/ <i>Hydrodictyon</i> (10%)	L (4)/D (4)	Day 1: t_0, t_1-t_5 ; Day 2 t_6-t_{10}
IIa	31 Jul	<i>P. acuta</i> (25 × 1)	<i>L. wollei</i> vs. <i>Rhizoclonium</i>	L (1)/D (1)	Day 1: t_0, t_1-t_{10} ; Day 2 $t_{11}-t_{15}$
IIb	31 Jul	<i>Viviparus</i> sp. (30 × 1)	<i>L. wollei</i> vs. <i>Rhizoclonium</i>	L (1)/D (1)	Day 1: t_0, t_1-t_{10} ; Day 2 $t_{11}-t_{15}$
IIIa	14 Aug	<i>Ammnicola limosa</i> (50 × 4)	<i>L. wollei</i> vs. <i>Spirogyra</i>	L (4)	Day 1: t_0, t_1
IIIb	14 Aug	<i>Gyraulus parvus</i> (50 × 4)	<i>L. wollei</i> vs. <i>Spirogyra</i>	L (4)	Day 1: t_0, t_1
IIIc	15 Aug	<i>A. limosa</i> (50 × 4)	<i>L. wollei</i> vs. <i>Rhizoclonium</i>	L (4)	Day 1: t_0, t_1
IIId	15 Aug	<i>G. circumstriatus</i> (50 × 4)	<i>L. wollei</i> vs. <i>Rhizoclonium</i>	L (4)	Day 1: t_0, t_1
IVa	11 Sep	<i>A. limosa</i> (50 × 4)	<i>L. wollei</i> vs. <i>V. americana</i>	L (4)	Day 1: t_0, t_1-t_5 ; Day 2 t_6-t_{10}
IVb	11 Sep	<i>P. acuta</i> (20 × 4)	<i>L. wollei</i> vs. <i>V. americana</i>	L (4)	Day 1: t_0, t_1-t_5 ; Day 2 t_6-t_{10}

by large (Experiments I and II) and small (Experiment III) gastropods; (2) firm vascular plant substratum (*Vallisneria americana*; Experiment IV); or (3) different quantity and quality of epiphytic cover (Experiment IV).

Mats (1 g wet mass) of different vegetation types were positioned in the corners of clear glass aquaria (15 × 25 cm) filled with 4 L (to about 10 cm depth) of filtered water from the collection site. The position of each substratum in the different corners of successive aquaria was randomly allocated. At the start of each experiment (t_0), gastropods were put at the center of the aquarium. The number of gastropods assigned to each aquarium (Table 2) was established to account for the natural density range (ind g^{-1} dry vegetation) of different taxa and size groups in the St. Lawrence River (after Tourville Poirier et al. 2010). After a 2–4 h period to allow gastropod movement toward the different substrata, the number of individuals observed on each vegetation mat and on the aquarium walls was recorded. In each experiment, the frequency of repeated observations depended on gastropod size. For large gastropods (*P. acuta* and *Viviparus* sp.), which could be easily counted without disturbing the mats, 5 counts were made over 80 minutes on 2 consecutive days (16 h gap, t_1-t_5 and t_6-t_{10} on day 1 and 2, respectively; Table 2). For small taxa (*A. limosa* and *G. parvus*), counts were made only once at the end of the experiments (t_1) because they involved removal and disruption of the mats (Table 2).

Experiments I and II contrasted ambient light and dark laboratory conditions by covering half of the aquaria with an opaque box because gastropod feeding and mobility may be influenced by light (Lombardo et al. 2010). Once the lack of a significant effect of light–dark treatment was established, subsequent experiments were carried out exclusively under ambient laboratory light.

Growth and survival experiments

Growth and survival of juvenile *Physa gyrina* (Pulmonata) and *Bythinia tentaculata* (Prosobranchia) exposed to either *L. wollei* or filamentous chlorophytes were assessed to determine the effects of substratum on early gastropod life stages. Egg clutches deposited by adult gastropods kept in the laboratory were isolated in separate Petri dishes filled with filtered and oxygenated water from the collection site. Upon hatching, juvenile gastropods from each clutch were divided into 2 equal groups and raised either in the presence of *L. wollei* or of chlorophytes, which were mostly *Rhizoclonium* but occasionally *Cladophora* (10%) or *Hydrodictyon* (10%), depending upon availability. Growth and survival measurements were carried out on 7 different clutches of *Physa gyrina* (groups of 5–55 individuals) and 2 clutches of *Bythinia tentaculata* (groups of 11 and 45 individuals). Water was changed weekly, and 0.1g (wet mass) of freshly collected filamentous algal substratum was provided.

Algae were carefully cleaned of epiphytes, protozoans, insect larvae, and detritus under a dissecting microscope. The number of surviving juveniles was recorded weekly, and subsamples were photographed under a camera mounted on a dissecting microscope without removing the gastropods from their culture dish. Gastropod size (shell length, mm), digitally measured from photographs (Image Pro Plus; Media Cybernetics Manufacturing, Warrendale, PA, USA), was recorded over 4–8 consecutive weeks (depending on the hatching date) from July to September. To ascertain the condition of surviving juveniles at the end of the experiments, individuals were frozen, freeze-dried, exposed to concentrated hydrochloric acid fumes for 24 h, and ground to powder prior to analysis of C and N tissue content with a Fisons Elemental Analyser 1108 CHNS-O.

Statistics

Experimental counts were checked for outliers by testing the homogeneity of the finest level of replication with Pearson chi-square tests or stratum (aquarium)-adjusted Pearson chi-square tests. A single outlying aquarium was detected with this method and removed from Experiment IIIa results. Most experiments exhibited over-dispersed animal counts fitted with negative binomial regression, except Experiment IIIb and IIIc counts, which could be modelled as Poisson variates. Our models involved combinations of vegetal substrata, light conditions, and epiphyte presence as fixed effects, with interaction, in 1- or 2-way analyses. When interaction between substrata and epiphyte effects were statistically significant ($p < 0.05$), the 2 factors were combined into one. Because the effect of light–dark conditions was never significant, light and dark gastropod counts were pooled before preference analysis. Statistical analyses of gastropod preferences were performed with SAS, version 9.3 (SAS Institute 2011).

Survival of juvenile snails raised in the presence of chlorophytes and *L. wollei* was compared using Kaplan-Meier estimates followed by a log-rank test stratified by sample (clutch; SAS, v9.4).

Results

Different kinds of vegetation had contrasting morphological characteristics and provided widely different tissue elemental composition (Table 3). *Lyngbya wollei* was characterized by large (50 μm wide) filaments with a thick sheath (10 μm) colonized by a range of epiphytes. *Lyngbya wollei* had consistently the highest N tissue content (5–6%) and lowest C:N ratio (6–7.6) of all vegetal substrata, with only slight variation among dates. *Rhizoclonium* was morphologically similar to *L. wollei*, with stout (95 μm wide) unbranched filaments, whereas *Hydrodictyon*, *Oedogonium*, and *Spirogyra* were less robust than the previous 2 taxa and had less epiphyte cover. Filamentous chlorophytes had generally lower N content (3.4–5.4%) and higher C:N ratios (8–12) than *L. wollei* (Table 3). Gastropod selection for *L. wollei* was also tested against the ribbon-forming (5 mm blade width) macrophyte *Vallisneria americana*, which supported abundant epiphytes. Epiphytes of *L. wollei* represented a plentiful (in terms of organic C g^{-1} dry plant) yet relatively poor-quality food resource (N content, 0.5–1.4%; C:N ratios, 7.5–12.4), possibly owing to the large amounts of mineral sediment and N-poor detritus caught within the mat. This contrasted with epiphytes on *V. americana*, which were 2-fold more abundant (as $\mu\text{g g}^{-1}$ Chl-*a* dry plant) and had a lower tissue C:N ratio (6.8) than epiphytes growing on *L. wollei* (Table 3).

Substratum choice experiments

All experiments offered a choice between *L. wollei* and an alternative substratum to gastropods of different size and taxon.

Experiment I. In the presence of *L. wollei* and *Oedogonium* (mixed with about 10% *Hydrodictyon*), the prosobranch *P. acuta* chose *L. wollei* in 78% of cases ($p < 0.001$; Fig. 1a).

Experiment II. No significant preference ($p = 0.06$) was detected when large *P. acuta* (Experiment IIa) were offered a choice between *L. wollei* and *Rhizoclonium*, with 56% and 44% of individuals selecting each type of vegetation, respectively (Fig. 1b). Given the same substratum choice, the large prosobranch *Viviparus* sp. (Experiment IIb) exhibited a significant preference ($p < 0.001$) for *Rhizoclonium* (79%) over *L. wollei* (Fig. 1c).

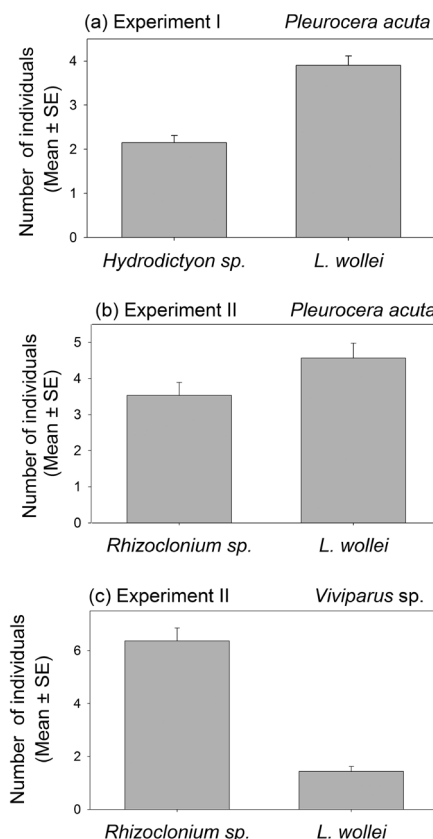


Fig. 1. (a) Experiment I: Substratum choice for large *Pleurocera acuta* (mean \pm SE) offered a choice between *Lyngbya wollei* and *Oedogonium* (mixed with 10% *Hydrodictyon*). The number of individuals found on each substratum differed significantly ($p < 0.0001$). (b) and (c) Experiment II: Substratum choice for large *Pleurocera acuta* (a; mean \pm SE) and *Viviparus* sp. (b; mean \pm SE) offered a choice between *Lyngbya wollei* and *Rhizoclonium*. The number of individuals found on each substratum differed significantly for *Viviparus* ($p < 0.0001$) but not for *P. acuta* ($p = 0.059$). Individuals found on aquarium glass surfaces were excluded in all experiments.

Table 3. Elemental composition (mean, SD when n = 3) of the different types of vegetation used in the experiments. For epiphytes, biomass per g of dry substratum as Chl-*a* and as organic carbon (C) is also provided. N = nitrogen.

Taxon	Date	C (%)	N (%)	Chl- <i>a</i> (%)	C: N	C: Chl- <i>a</i>	Epiphyte biomass/plant DM	
							Chl- <i>a</i> ($\mu\text{g g}^{-1}$)	C (mg g^{-1})
Cyanobacteria								
<i>Lyngbya wollei</i>	25 Jul	33.5 (3.7)	5.3 (0.4)	0.59 (0.02)	6.3 (0.3)	59 (8)		
<i>Lyngbya wollei</i>	14 Aug	38.0	5.1	0.39	7.6	117		
<i>Lyngbya wollei</i>	11 Sep	36.0 (1.1)	5.9 (0.3)	0.95 (0.58)	6.1 (0.1)	46 (21)		
Chlorophytes								
<i>Hydrodictyon</i>	25 Jul	31.4	3.5	0.62	9.0	51		
<i>Oedogonium</i>	25 Jul	33.4	3.5	—	9.5	—		
<i>Rhizoclonium</i>	30 Jul	49.2	4.2	0.14	11.6	341		
<i>Rhizoclonium</i>	14 Aug	35.0	3.4	0.56	10.3	63		
<i>Spirogyra</i>	14 Aug	44.2	5.4	1.29	8.2	34		
Macrophytes								
<i>V. americana</i>	11 Sep	36.3 (0.8)	3.7 (0.3)	0.59 (0.10)	9.7 (0.7)	63 (9)		
Epiphytes								
Epi- <i>L. wollei</i>	25 Jul	5.4 (0.6)	0.7 (0.1)	0.05 (0.01)	7.5 (0.2)	113 (8)	645 (46)	73 (6)
Epi- <i>L. wollei</i>	14 Aug	17.1 (1.4)	1.4 (0.3)	0.08 (0.04)	12.1 (1.9)	282 (118)	440 (82)	118 (23)
Epi- <i>L. wollei</i>	11 Sep	6.7	0.5	0.02 (0.001)	12.4	402	444 (96)	146
Epi- <i>V. americana</i>	11 Sep	10.4	1.5	0.13 (0.3)	6.8	74	1178 (75)	87

Experiment III. Small prosobranchian *A. limosa* (Experiment IIIa) and small pulmonate *G. parvus* (Experiment IIIb) did not show any significant preference between *L. wollei* and *Spirogyra* (Fig. 2a and 2c; $p = 0.50$ and $p = 0.89$, respectively). When the same gastropod taxa were offered a choice between *L. wollei* and *Rhizoclonium* (Experiments IIIc and III d), both grazers showed a marked preference (72% of observations for *A. limosa*, $p < 0.001$; 67% for *G. parvus*, $p = 0.006$) for the filamentous cyanobacterium (Fig. 2b and 2d).

Experiment IV. When small *A. limosa* (Experiment IVa) and large *P. acuta* (Experiment IVb) were offered a choice between *L. wollei* and the macrophyte *V. americana*, both grazers showed a preference for *V. americana* with natural epiphytic cover. For small *A. limosa* (Fig. 3a), the preference for *V. americana* was clear regardless of the presence/removal of the epiphytic cover (74% and 62%, respectively; $p < 0.0001$); moreover, the preference for natural epiphytic cover was significant for both substrata (79% for *L. wollei* and 69% for *V. americana*; $p < 0.0001$). Large *P. acuta* did not have a marked preference between substrata; the preference for natural epiphytic cover was significant only on *V. americana* (68%, $p = 0.0002$; Fig. 3b).

Only results for gastropods that made a clear selection between vegetation types have been presented; however, in each experiment, some individuals were found on the

aquarium walls. In the experiments contrasting *L. wollei* with filamentous chlorophytes (Experiments I–III), large gastropods (*P. acuta* and *Viviparus* sp.) were found on

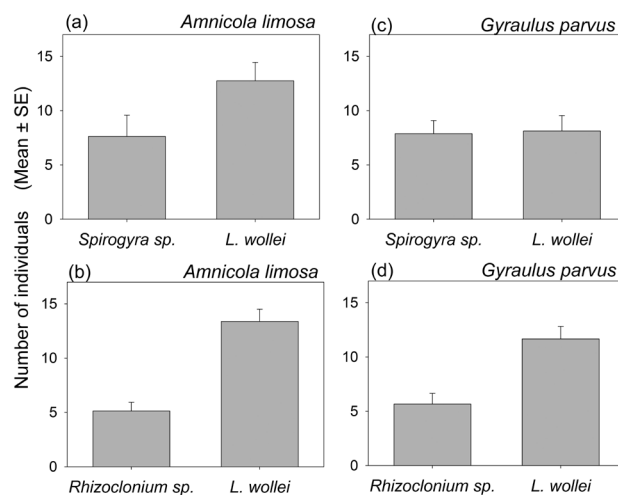


Fig. 2. Experiment III: (a) and (b) Substratum choice for small *Amnicola limosa* (mean \pm SE) and (c) and (d) *Gyraulus parvus* (mean \pm SE) offered a choice between *Lyngbya wollei* and either (a) and (c) *Spirogyra* or (b) and (d) *Rhizoclonium*. The number of individuals found on each substratum differed significantly ($p < 0.01$) only for experiments with *Rhizoclonium*. Individuals found on aquarium glass surfaces were excluded.

aquarium glass surfaces more often (mean = 48%, SE = 3, n = 10) than small taxa (*A. limosa* and *G. parvus*; mean = 28%, SE = 3, n = 8). Similarly, when offered a choice between *L. wollei* and *V. americana* (Experiment IV), large *P. acuta* remained on aquarium glass surfaces more frequently (mean = 51%, SE = 3, n = 4) than small *A. limosa* (mean = 28%, SE = 1, n = 4).

Survival and growth experiment

Each experiment on growth of juvenile *B. tentaculata* (2 clutches) and *P. gyrina* (7 clutches) was initiated when juveniles reached a size of ~1 mm (i.e., 4–10 d after hatching). Neither species showed a significant difference in growth rate when exposed to *L. wollei* or to filamentous chlorophytes (Fig. 4a and 4b). *Physa gyrina* mean growth rate was 0.22 mm week⁻¹ on chlorophytes and 0.21 mm week⁻¹ on *L. wollei*. Mean growth rate of *B. tentaculata* was only 0.07 and 0.11 mm week⁻¹ on chlorophytes and on *L. wollei*, respectively. *Physa gyrina* tissue elemental

composition was not significantly different (2-sample t-test, p = 0.46) under the 2 rearing treatments, with C:N ratios averaging 4.88 (SE = 0.14, n = 5) for juveniles in presence of *L. wollei* and 5.21 (SE = 0.25, n = 5) for those in presence of chlorophytes. Elemental composition of *B. tentaculata* tissue was not analyzed because too few organisms were available at the end of the experiment.

In the presence of filamentous chlorophytes, juvenile survival of *P. gyrina* was overall 1.5 higher than in the presence of *L. wollei*. Differences between treatments were significant (Kaplan-Meier estimates followed with a log-rank test, p = 0.028; Fig. 4c). A similar pattern was observed for *B. tentaculata*, but the availability of only 2 clutches prevented statistical analysis (Fig. 4d).

Discussion

Vast macrophyte beds supporting an abundant and diverse gastropod fauna typically cover large areas of the St. Lawrence River fluvial lakes (Vincent et al. 1991, Tessier et al. 2008). The aim of our study was to explore the 11-fold decline in gastropod biomass recently observed in areas of this river where *L. wollei* replaces macrophyte beds (Hudon et al. 2012). Overall, the results of our experiments did not support the view that gastropods avoid *L. wollei* mats. On the contrary, gastropod substratum choice (with the exception of *Viviparus* sp.) was directed strongly toward *L. wollei* when compared with different taxa of filamentous chlorophytes (Fig. 1–3). Camacho and Thacker (2006) showed experimentally that amphipods fed preferentially on *Rhizoclonium* rather than on *L. wollei*, although *L. wollei* saxitoxins stimulated their consumption. Large marine Opisthobranchs readily consume *Lyngbya majuscula*, a morphologically similar marine counterpart to *L. wollei*, despite its neurotoxin content (Capper et al. 2006). The preference toward *L. wollei* in our experiments might be linked with its nutritional quality, which was higher than for the chlorophytes, as suggested by N content (5.1–5.9 vs. 3.4–5.4%) and C:N tissue ratio (6.1–7.6 vs. 8.2–11.6). Similarly, experimental food preferences of *Radix* and *Bithynia* increased with decreasing C:N ratios and with increasing N content (Brendelberger 1995b).

Our second question was whether gastropods would select a physically firm and broad substratum facilitating movement and feeding. *Amnicola limosa* showed a clear preference for *V. americana* leaves over *L. wollei* filaments, but *P. acuta* choice for the macrophyte was significant only when offered with its natural epiphytic cover. Both *A. limosa* and *P. acuta* exhibited a clear preference for substrata colonized by epiphytes, as expected by our third question regarding an effect of epiphyte quantity and quality (tissue elemental

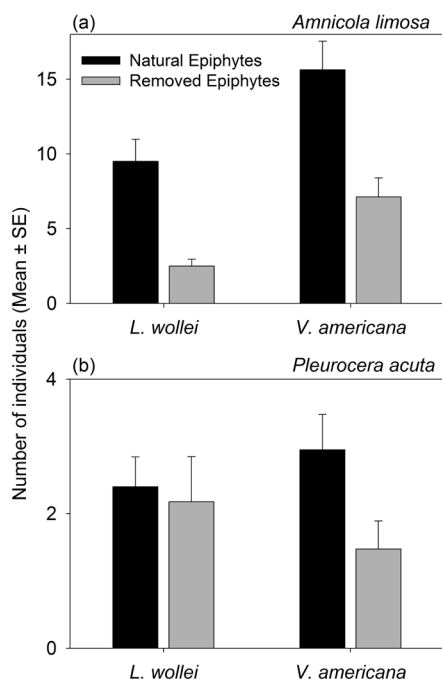


Fig. 3. Experiment IV. (a) Substratum choice for small *Amnicola limosa* (mean ± SE) and (b) large *Pleurocera acuta* (mean ± SE) offered a choice between *Lyngbya wollei* and *Vallisneria americana*, supporting either their natural epiphytes (black bars) or after manual epiphyte removal (grey bars). The number of *A. limosa* found on *V. americana* were significantly higher than on *L. wollei* ($p < 0.0001$). Presence of natural epiphyte cover was significant on both vegetation types ($p < 0.0001$). For *P. acuta*, the choice between *L. wollei* and *V. americana* was not significant ($p = 0.927$). Presence of natural epiphyte cover was instead highly significant ($p = 0.0002$), especially on *V. americana* (interaction vegetation–epiphyte cover $p = 0.03$). Individuals found on aquarium glass surfaces were excluded.

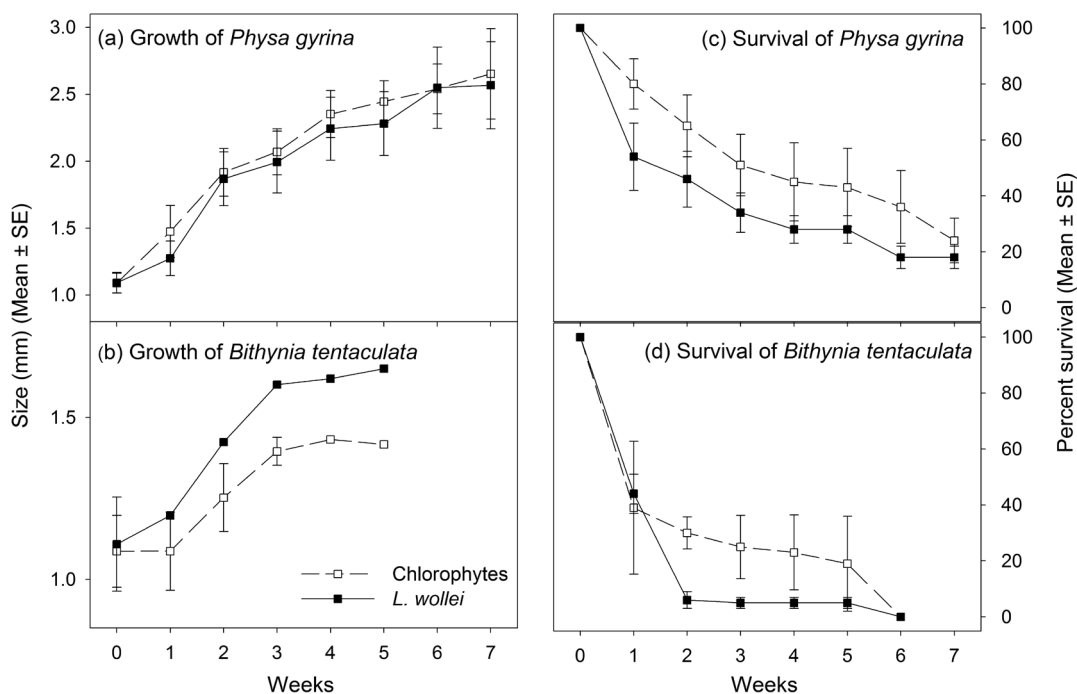


Fig. 4. (a and b) Growth and (c and d) survival curves for (a and c) juvenile *Physa gyrina* (weekly mean \pm SE, $n = 7$ clutches); (b and d) juvenile *Bithynia tentaculata* (weekly mean \pm SE, $n = 2$ clutches) raised in presence of either *L. wollei* (full line and symbols) or chlorophytes (dotted line, open symbols).

composition) on gastropod substratum choice. Epiphyte removal decreased gastropod selection more markedly for *V. americana* than for *L. wollei*. Epiphytes on the macrophyte represented a better food source than those on the cyanobacterium because of their higher biomass (as Chl-*a*) and lower C:N ratio. Utilization of epiphytes by gastropods is well known and has been demonstrated in experiments and by stable isotope analyses (Lodge 1986, Jaschinski et al. 2011).

We suggested that substratum choice would be modulated by gastropod subclass and size because these groups have different ecological characteristics (Brown and Lydeard 2010). Gastropod choice did not vary between subclasses because the prosobranch *A. limosa* and the pulmonate *G. parvus* responded similarly in 2 parallel experiments. Instead, the effect of size was clear; choices between substrata were more evident for small-bodied snails than for large taxa, which were more often found on the aquarium glass. This difference could be related to the different ecologies of these genera. Small-bodied *Amnicola* and *Gyraulus* are typically found on macrophytes or metaphyton whereas *Pleurocera*, and especially *Viviparus*, are bottom dwellers (Pennak 1978). Gastropod movement and feeding rates can be influenced by light (Lombardo et al. 2010), but this effect was not significant in our experiments.

In summary, our substratum choice experiments indicate that gastropods are not deterred by *L. wollei* but definitely prefer macrophytes with natural epiphyte cover. These observations suggest that gastropod decline in areas of the St. Lawrence River colonized by *L. wollei* is more likely related to the concurrent reduction in macrophytes than to any direct, negative effect of this cyanobacterium. Macrophyte biomass was 3-fold lower in areas colonized by *L. wollei* than elsewhere in the same fluvial lake, and epiphyte cover was halved. Gastropod biomass was reduced 5-fold per gram of macrophyte and 11-fold on areal basis (Tourville Poirier et al. 2010, Hudon et al. 2012). Gastropod density is positively related to macrophyte biomass (Cyr and Downing 1988, Strayer and Malcom 2007). Dense macrophyte beds could reduce fish predation (Crowder and Cooper 1982, Marklund et al. 2002) and lessen invertebrate disturbance by waves and current (Madsen and Warncke 1983, Green 2005) while trapping detrital food (Rooke 1986).

The experiments on gastropod juveniles raise the possibility, however, that gastropod waning in areas colonized by *L. wollei* could be linked to the negative effects of this cyanobacterium on gastropod recruitment. In our rearing experiments, juvenile growth rates and tissue elemental composition of *B. tentaculata* (Prosobranchia) and *P. gyrina* (Pulmonata) were similar in

presence of *L. wollei* and of filamentous chlorophytes; however, survival was halved in juveniles exposed to *L. wollei*. This reduction in survival could be explained by accelerated toxin accumulation, which was observed in juvenile gastropods exposed to microcystin (Gérard et al. 2005, Lance et al. 2006).

The lack of avoidance by gastropods of cyanobacteria despite their negative effects on recruitment is not unique to our study. The estuarine herbivorous gastropod *Cerithidea californica* was not deterred by cyanobacterial mats regardless of a 2–5-fold rise in mortality in their presence in the field (Armitage and Fong 2004). Similarly, *Limnaea stagnalis* did not avoid the microcystin-producing *Planktothrix agardhii* when offered a choice between this cyanobacterium and nontoxic lettuce leaves (Lance et al. 2006), despite documented effects of the toxin on its fecundity (Lance et al. 2007).

Previous studies have shown that species richness and abundance of gastropod assemblages can exhibit both rapid (within-year) and long-term (10-year) responses to water level changes and cyanobacterial blooms (Gérard et al. 2008). In the St. Lawrence River, the recently documented occurrence of toxin producing *L. wollei* (Lajeunesse et al. 2012) represents a symptom of ecosystemic degradation resulting from the cumulative impacts of eutrophication, contamination, water level variations, and climate change (Hudon et al. 2014). Under those conditions, the overall drop in the carrying capacity of this habitat for fish (Hudon et al. 2012) is consistent with the disappearance of the preferred substratum for adult gastropods and decreased survival of their juveniles.

Acknowledgements

We thank Jean-Pierre Amyot, David Lévesque, and Maxime Wauthy for excellent field and laboratory support. Bernadette Pinel-Alloul and Louse Cloutier provided advice for gastropod rearing and identification. Funding was provided by a Merit scholarship program for foreign students of the Quebec Research Fund (FRQNT) to A.V., by a Natural Sciences and Engineering Research Council of Canada (NSERC) Grant (A.C.), and by the St. Lawrence Centre of Environment Canada (C.H.).

References

- Armitage AR, Fong P. 2004. Upward cascading effects of nutrients: shifts in a benthic microalgal community and a negative herbivore response. *Oecologia*. 139:560–567.
- Brendelberger H. 1995a. Growth of juvenile *Bithynia tentaculata* (Prosobranchia, Bithynidae) under different food regimes - a long-term laboratory study. *J Mollus Stud*. 61:89–95.
- Brendelberger H. 1995b. Dietary preference of 3 fresh-water gastropods for 8 natural foods of different energetic content. *Malacologia*. 36:147–153.
- Brönmark C. 1985. Interactions between macrophytes, epiphytes, and herbivores: an experimental approach. *Oikos*. 45:25–30.
- Brown KM, Lydeard C. 2010. Mollusca: Gastropoda. In: Thorp JH, Covich AP, editors. *Ecology and classification of North American freshwater invertebrates*, 3rd ed. San Diego (CA): Academic Press. p. 277–307.
- Brown KM, Varza DE, Richardson TD. 1989. Life histories and population dynamics of two subtropical snails (Prosobranchia: Viviparidae). *J N Am Benthol Soc*. 8:222–228.
- Camacho FA, Thacker RW. 2006. Amphipod herbivory on the freshwater cyanobacterium *Lyngbya wollei*: chemical stimulants and morphological defenses. *Limnol Oceanogr*. 51:1870–1875.
- Capper A, Tibbetts IR, O'Neil JM, Shaw GR. 2006. Dietary selectivity for the toxic cyanobacterium *Lyngbya majuscula* and resultant growth rates in two species of opisthobranch mollusc. *J Exp Mar Biol Ecol*. 331:133–144.
- Clarke A H. 1981. *The freshwater molluscs of Canada*. Ottawa (Canada): National Museum of Natural Sciences, National Museums of Canada.
- Crowder LB, Cooper WE. 1982. Habitat structural complexity and the interaction between bluegills and their prey. *Ecology*. 63:1802–1813.
- Cyr H, Downing JA. 1988. Empirical relationships of phytomacrofaunal abundance to plant biomass and macrophyte bed characteristics. *Can J Fish Aquat Sci*. 45:976–984.
- Dorgelo J, Leonards PEG. 2001. Relationship between C/N ratio of food types and growth rate in the snail *Potamopyrgus jenkinsi* (E. A. Smith). *J N Am Benthol Soc*. 20:60–67.
- Foss AJ, Philips EJ, Yilmaz M, Chapman A. 2012. Characterization of paralytic shellfish toxins from *Lyngbya wollei* dominated mats collected from two Florida springs. *Harmful Algae*. 16:98–107.
- Gélinas M, Lajeunesse A, Gagnon C, Gagné F. 2013. Temporal and seasonal variation in acetylcholinesterase activity and glutathione-S-transferase in amphipods collected in mats of *Lyngbya wollei* in the St-Lawrence River (Canada). *Ecotox Environ Safte*. 94:54–59.
- Gérard C, Briant L, Rouzic BL. 2005. Variation in the response of juvenile and adult gastropods (*Limnaea stagnalis*) to cyanobacterial toxin (microcystin-LR). *Environ Toxicol*. 20:592–596.
- Gérard C, Carpentier A, Paillisson J-M. 2008. Long-term dynamics and community structure of freshwater gastropods exposed to parasitism and other environmental stressors. *Freshwater Biol*. 53:470–484.
- Green JC. 2005. Velocity and turbulence distribution around lotic macrophytes. *Aquat Ecol*. 39:1–10.
- Haynes A, Taylor BJR. 1984. Food finding and food preference in *Potamopyrgus jenkinsi* (E. A. Smith) (Gastropoda: Prosobranchia). *Arch Hydrobiol*. 100:479–491.
- Hudon C, Cattaneo A, Tourville Poirier A-M, Brodeur P, Dumont P, Mailhot Y, Amyot P, Despatie SP, de Lafontaine Y. 2012. Oligotrophication from wetland eutrophication alters the riverine trophic network and carrying capacity for fish. *Aquat Sci*. 74:495–511.

- Hudon C, deSève M, Cattaneo A. 2014. The increasing occurrence of the benthic filamentous cyanobacterium *Lyngbya wollei*: a symptom of freshwater ecosystems degradation. *Freshwater Sci.* 33:619–638.
- Jaschinski S, Brepohl DC, Sommer U. 2011. The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus* L.): stable isotope and fatty acid analyses. *Aquat Sci.* 73:91–101.
- Kotak BG, Zurawell RW, Prepas EE, Holmes CFB. 1996. Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Can J Fish Aquat Sci.* 53:1974–1985.
- Lajeunesse A, Segura PA, Gélinais M, Hudon C, Thomas K, Quilliam MA, Gagnon C. 2012. Detection and confirmation of saxitoxin analogues in freshwater benthic *Lyngbya wollei* algae collected in the St. Lawrence River (Canada) by liquid chromatography–tandem mass spectrometry *J Chromatogr A.* 1219:93–103.
- Lance E, Briant L, Bormans M, Gérard C. 2006. Interactions between cyanobacteria and Gastropods: I. Ingestion of toxic *Planktothrix agardhii* by *Lymnaea stagnalis* and the kinetics of microcystin bioaccumulation and detoxification. *Aquat Toxicol.* 79:140–148.
- Lance E, Paty C, Bormans M, Briant L, Gérard C. 2007. Interactions between cyanobacteria and gastropods: II. Impact of toxic *Planktothrix agardhii* on the life-history traits of *Lymnaea stagnalis*. *Aquat Toxicol.* 81:389–396.
- Lodge DM. 1986. Selective grazing on periphyton: a determinant of fresh-water gastropods microdistributions. *Freshwater Biol.* 16:831–841.
- Lombardo P, Cooke GD. 2002. Consumption and preference of selected food types by two freshwater gastropod species. *Arch Hydrobiol.* 155:667–685.
- Lombardo P, Miccoli FP, Giustini M, Cicolani B. 2010. Diel activity cycles of freshwater gastropods under natural light: Patterns and ecological implications. *Ann Limnol - Int J Lim.* 46:29–40.
- Madsen TV, Warncke E. 1983. Velocities of currents around and within submerged aquatic vegetation. *Arch Hydrobiol.* 97:389–394.
- Marklund O, Sandsten H, Hansson LA, Blindow I. 2002. Effects of waterfowl and fish on submerged vegetation and macroinvertebrates. *Freshwater Biol.* 47:2049–2059.
- Nusch EA. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Arch Hydrobiol Beith Ergebn Limnol.* 14:14–36.
- Pennak RW. 1978. *Freshwater invertebrates of the United States.* 2nd ed. New York (NY): John Wiley and Sons.
- Rooke JB. 1986. Seasonal aspects of the invertebrate fauna of three species of plants and rock surfaces in a small stream. *Hydrobiologia.* 134:81–87.
- Russel-Hunter WD. 1978. Ecology of freshwater pulmonates. In: Fretter V, Peake J, editors. *The Pulmonates Vol. 2A. Systematics, evolution and ecology.* Orlando (FL): Academic Press. p. 335–383.
- Schreiber ESG, Glaister A, Quinn GP, Lake PS. 1998. Life-history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in lake Purrumbete, Victoria, Australia. *Mar Freshwater Res.* 49:73–78.
- SAS Institute. 2011. *SAS/STAT 9.3 User's Guide.* Cary (NC): SAS Institute Inc.
- Strayer DL, Malcom HM. 2007. Submersed vegetation as habitat for invertebrates in the Hudson River Estuary. *Estuar Coasts.* 30:253–264.
- Tessier C, Cattaneo A, Pinel-Alloul B, Hudon C, Borcard D. 2008. Invertebrate communities and epiphytic biomass associated with metaphyton and emergent and submerged macrophytes in a large river. *Aquat Sci.* 70:10–20.
- Thorp JH, Covich AP. 2010. *Ecology and classification of North American freshwater invertebrates.* 3rd ed. San Diego (CA): Academic Press.
- Tourville Poirier A-M, Cattaneo A, Hudon C. 2010. Benthic cyanobacteria and filamentous chlorophytes affect macroinvertebrate assemblages in a large fluvial lake. *J N Am Benthol Soc.* 29:737–749.
- Vincent B, Rioux H, Harvey M. 1991. Factors affecting the structure and epiphytic gastropod communities in the St. Lawrence River (Quebec, Canada). *Hydrobiologia.* 220:57–71.
- Vis C, Cattaneo A, Hudon C. 2008. Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. *J Phycol.* 44 38–44.
- Watson SB, Ridal J, Boyer GL. 2008. Taste and odour and cyanobacterial toxins: impairment, prediction, and management in the Great Lakes. *Can J Fish Aquat Sci.* 65:1779–1796.
- Zurawell RW, Kotak BG, Prepas EE. 1999. Influence of lake trophic status on the occurrence of microcystin-LR in the tissue of pulmonate snails. *Freshwater Biol.* 42:707–718.