

# Biofilm and substrate preference in the dreissenid larvae of Lake Erie

B.C. Wainman, S.S. Hincks, N.K. Kaushik, and G.L. Mackie

**Abstract:** Larval recruitment in the bivalves *Dreissena polymorpha* and *Dreissena bugensis* was measured on acrylic plastic plates with live mussels, mussel shells, or stones with primary biofilm and with the primary biofilm removed by scrubbing. Experiments were carried out at Nanticoke Thermal Generating Station on Lake Erie for nine 24-h periods between 21 July and 5 August 1993. Recruitment density ranged from a high of  $29\,300 \pm 34\,800$  mussels·m<sup>-2</sup>·day<sup>-1</sup> (mean  $\pm$  SD,  $n = 10$ ) for plates with shells and biofilm to a low of  $4640 \pm 540$  mussels·m<sup>-2</sup>·day<sup>-1</sup> ( $n = 10$ ) for the stone treatment that had the biofilm removed by scrubbing. Recruitment was not significantly different between treatments with dreissenid shells and live adult dreissenids though both of these treatments had more settling than the plates with mussel-sized stones. Our results show that dreissenid shell material is a particularly attractive substrate for dreissenid recruitment in Lake Erie and that biofilm removal reduces mussel recruitment by 10–20%.

**Résumé :** Nous avons mesuré le recrutement des larves des bivalves *Dreissena polymorpha* et *Dreissena bugensis* sur des plaques d'acrylique portant des moules vivantes, sur des coquilles de moules ou sur des pierres, en présence du film biologique primaire ou après enlèvement de ce film biologique par raclage. Les expériences ont été menées à la centrale thermique de Nanticoke, sur le lac Érié, pendant neuf périodes de 24 h, entre le 21 juillet et le 5 août 1993. La densité de recrutement allait d'un sommet de  $29\,300 \pm 34\,800$  moules·m<sup>-2</sup>·jour<sup>-1</sup> (moyenne  $\pm$  ET,  $n = 10$ ), pour les plaques portant les coquilles et le film biologique, à un plancher de  $4640 \pm 540$  moules·m<sup>-2</sup>·jour<sup>-1</sup> ( $n = 10$ ) dans le cas des pierres dont on avait enlevé le film biologique par raclage. Le recrutement n'était pas nettement différent entre les expériences avec les coquilles de dreissenidés et les adultes vivants, mais dans les deux cas le naissain était plus nombreux que sur les pierres de même taille. Nos résultats montrent que les coquilles de dreissenidés constituent un substrat particulièrement attirant pour le recrutement de ces mollusques dans le lac Érié, et que l'élimination du film biologique réduit de 10–20% le recrutement des moules.  
[Traduit par la Rédaction]

## Introduction

The zebra mussel (*Dreissena polymorpha*) and its close relative the quagga mussel (*Dreissena bugensis*) have become serious economic biofoulers since their accidental introduction into the Great Lakes (Hebert et al. 1991). The recruitment and aggregation of these exotic dreissenids has led to the occlusion of domestic and industrial pipelines, electrocorrosion of steel pipes (Mackie et al. 1989), water outages (LePage 1993), and fouling of trash bars, condensers, and heat exchangers (Kovalak et al. 1993). Estimates of damage caused by dreissenid infestations are in excess of \$5 billion before the year 2000 for the Great Lakes region (Armor and Wiancko 1993).

The Lake Erie dreissenids are diecious with gametes released directly into the water and fertilized externally. After a brief trocophore stage, free-swimming veliger larvae are produced (Walz 1975). At the time of settlement and metamorphosis (i.e., recruitment) the larval mussels secrete byssal threads that allow them to attach to most hard substrates. Once attached, mussels may detach from their byssal threads and move to another substrate (Martel 1993). However, the significance of this translocation behaviour to the overall population is difficult to assess. Inhibition of recruitment offers a promising means of controlling dreissenid infestation, since recruitment is the first step in biofouling. To efficiently control exotic mussel recruitment, colonization and the mechanisms of substrate selection must be understood.

Recruitment of many sessile marine invertebrates on or near conspecifics is critical as it can increase reproductive success, filter feeding efficiency, and competitive ability, and decrease juvenile and adult mortality (Knight-Jones and Stevenson 1950; Knight-Jones and Crisp 1953; Bayne 1969; Hidu 1969; Meadows and Campbell 1972; Tamburri et al. 1992; McGrath et al. 1988; reviewed by Williams 1964 and Rodriguez et al. 1993). There are few similar data for freshwater mussels but aggregations of zebra and quagga mussels are common, consisting of clumps of individual mussels attached to each other by byssal threads (Mackie et al. 1989) with populations in excess of 750 000 individuals·m<sup>-2</sup> (Kovalak et al. 1993).

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Studies have shown that free-swimming larvae of many marine invertebrates make active, consistent habitat choices and that recruitment is seldom random (reviewed by Bayne 1969; Meadows and Campbell 1972). For example, the recruitment of planktonic larvae can be affected by light, gravity, surface texture (Crisp 1974), current velocity, initial surface chemistry, and chemical cues from bacteria and microflora (Meadows and Campbell 1972; reviewed by Roberts et al. 1991).

Since 1935, when Zobell and Allen noted that surfaces were more attractive to settling larvae when microbial films were present (Bonar et al. 1990), there has been considerable research on the role of surface biofilms (reviewed by Tamburri et al. 1992) in inducing settlement. Studies have shown that in the marine environment bacteria and other microorganisms quickly colonize submerged surfaces (Maki et al. 1988, 1989, 1990). The surface biofilm initially consists of a glycoproteinaceous film. Soon after immersion a surface is colonized by bacteria, diatoms, and protozoa (the primary film). It has been shown that certain marine bacteria increase recruitment of a number of marine organisms (reviewed by Williams 1964; Meadows and Campbell 1972; Maki et al. 1988, 1990; Harrold et al. 1991; Johnson et al. 1991).

While this phenomenon is well known in marine research, there is no documented research on the effect of biofilms on the recruitment of zebra or quagga mussels. The nature of the stimulus that leads to the recruitment and aggregation of dreissenids is also unclear. The objectives of this study were to determine if surface biofilms affect dreissenid recruitment and to investigate if adult conspecifics or their shells influence the recruitment pattern of veligers.

## Methods

### Study site

All experiments were carried out in the forebay of the Nanticoke Thermal Generating Station (T.G.S.) at Nanticoke, Ontario, on Lake Erie (42°48'N, 80°03'W). Water in the forebay is pumped from Lake Erie through two 600-m tunnels from a depth of 13 m. The tunnels and the forebay are extensively colonized with dreissenids, and mussel fouling of condensers and pump intakes is a major problem for the generating station. Dreissenids collected from the walls of the forebay are 95–100% *D. bugensis*.

### Experimental methods

Six experimental treatments were chosen to evaluate the effect of biofilms and adult dreissenids on veliger recruitment. Each treatment consisted of a substrate, either live mussels, dreissenid mussel shell, or stones, held on 5 × 10 cm acrylic plastic (Plexiglas™, methyl methacrylate) plates by nylon 0.5-cm mesh fabric bags. For each type of substrate there was a biofilm and a biofilm-removed treatment. The substrate for the first treatment was live adult dreissenids with biofilm removed by scrubbing (see next section); the second treatment was identical except that the biofilm was not removed from the adults and the acrylic plate. These live-mussel treatments were used to investigate whether a substance from live adult dreissenids induced recruitment of veligers. The substrate in treatment 3 consisted of dreissenid shells on plates with biofilm

removed; the fourth treatment was identical to the third except that the biofilm was left intact on the shells and plate. Treatments 3 and 4 were used to determine if the dreissenid shell alone could change recruitment rates. Treatment 5 consisted of stones on an acrylic plate with biofilm; in treatment 6 the biofilm was removed from the stones and the plate. The stones in treatments 5 and 6 were intended to simulate the hydrodynamic qualities of adult mussels and act as a control group.

The adult dreissenids used in treatments 1 and 2 were collected attached to rocks in Nanticoke Harbour on 8 July 1993, before any apparent recruitment in eastern Lake Erie. These 2- to 3-cm zebra and quagga mussels were kept in the laboratory in recirculating 50-L aquaria where they were fed every 2–3 days with *Chlorella pyrenoidosa* to a concentration of 30 000 cells·mL<sup>-1</sup>. The water in the aquaria was replaced with fresh Lake Erie water every 3–4 days. Mussels used for the experiments were cut from their colonies with a scalpel and were used in the experiments only after they opened and extended their siphons. Four adult mussels were confined on each surface of the acrylic plastic plate in treatments 1 and 2.

### Preparation of dreissenid shells and stones

The mussel shells used in treatments 3 and 4 were collected from trash racks at Nanticoke T.G.S. that had been removed the previous year. The shells were cleaned by sonication for 3–4 h then scrubbed with a brush and Sparkleen™ and then rinsed five times in deionized water before use. In treatments 5 and 6, eight mussel valves were confined, inside surface down, on the surface of the acrylic plates.

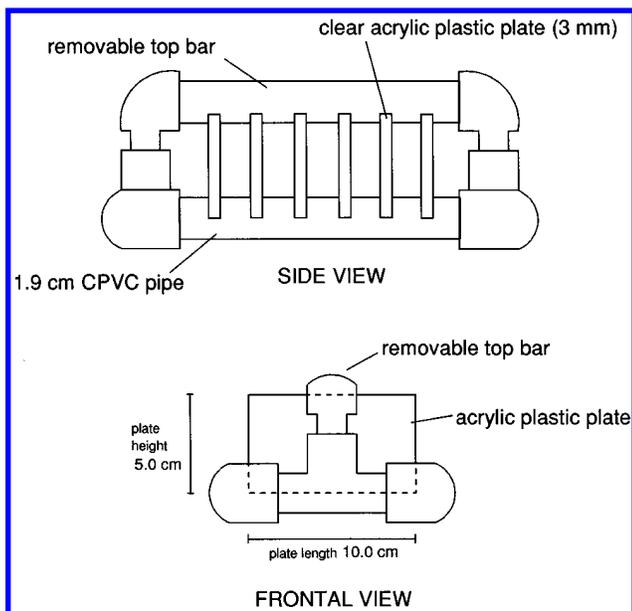
Stones used in treatments 5 and 6 were between 1 and 3 cm in diameter and approximately the size and shape (oblong and compressed) of the adult dreissenids used in treatments 1 and 2. Stones were cleaned in the same manner as the dreissenid shells. Four stones were confined on each face of the acrylic plates.

### Preparation of surface biofilms

Before experiments began, a biofilm was allowed to develop on the various substrates in 30-L aquaria for 4–6 days under the continuous light of a 40-W Gro and Sho™ fluorescent light placed 15 cm above the surface of the tank. Tanks were filled with water collected at Nanticoke T.G.S. Biofilm was grown on the substrate and acrylic plates after they had been contained in the nylon mesh bags.

Biofilm and other surface material in treatments 2, 4, and 5 were removed by the method of Tamburri et al. (1992) with a few modifications. In our experiments biofilm was removed from substrate surfaces and plates by scrubbing with a soft, nylon-bristled brush and Sparkleen (Fisher Scientific, Pittsburgh, Pa.), a moderately alkaline, free-rinsing detergent blend designed for manual washing. We elected to use a detergent rather than the sodium hypochlorite used by Tamburri et al. (1992) for two reasons. Hypochlorite ion is extremely toxic to zebra mussels (Martin et al. 1992) and we felt that its use could lead to unacceptable levels of mortality among the live mussels. Also, hypochlorite ion is extremely reactive and we felt that it may oxidize shell biochemicals critical to settlement. All materials that we had cleaned with detergent were rinsed five times with distilled water to remove any traces of soap or surfactant. It is likely that no residues were left on the cleaned surfaces because Sparkleen leaves no inhibitory or toxic

**Fig. 1.** Diagram of the experimental apparatus used for containing acrylic plastic plates. All parts of the apparatus are made of chlorinated polyvinyl chloride plastic (CPVC).



residues when assayed using the glassware inhibitory residue test (American Public Health Association, American Water Works Association, and Water Environment Federation 1992) by Scientific Associates Inc., St. Louis, Mo.

Once the shells and stones had been cleaned and rinsed they were stored for 18–24 h in deionized water before being installed in the racks. Live, scrubbed mussels were held for 18–24 h in a mixture of 50% unchlorinated, Guelph well water filtered through Whatman GF/F filters and 50% deionized water. No mussel mortality resulted from the cleaning process.

Groups of six plates (one of each treatment) were randomly placed in slotted racks constructed entirely of chlorinated polyvinyl chloride plastic (CPVC, see Fig. 1). Plates in racks were 3 cm apart and three racks (18 plates) were used on each experimental date.

For each experiment, racks were randomly suspended 1–1.5 m out from the corrugated metal wall of the forebay and 2 m below the surface of the water. Ten experiments were carried out between 20 July and 10 August 1993. Racks were placed in the water between 11:00 and 14:00 and removed 23.5–24.5 h later and air dried.

Newly recruited dreissenids were counted under 400 $\times$  magnification using a Wild-Heerbrug stereomicroscope. The newly recruited dreissenids showed up as pearlescent spheres against the darker background of the plates and substrates. We could not distinguish between the newly recruited mussels of the two dreissenid species in Lake Erie though this may now be possible (Martel 1995). During periods of intense settling, clumps of 10–15 newly recruited mussels had to be teased apart and counted. Only mussels found on the substrates and the large flat surfaces of the plates (not on the edges of the plates or on the mesh bags) were counted. An areal recruitment value in mussels per square metre for each treatment was arrived at by adding together recruitment on the substrate and the 100-cm<sup>2</sup> acrylic plastic plate and multiplying by 100.

The shell length of the recruits was measured on 20 July, 29 July, and 9 August 1993. Measurements were made at 160 $\times$  magnification using a Hi-pad digitizing tablet interfaced to an IBM personal computer (Roff and Hopcroft 1986). The length was measured as the longest distance between the umbone and the ventral margin. Mussels larger than 500  $\mu$ m were considered to be translocators (mussels that had metamorphosed and, perhaps, previously settled) and were excluded from the counts. No more than two translocators were ever detected per treatment.

To assess recruitment patterns over a longer term, additional experiments, using the same methods as outlined above, were carried out over 7 or 10 days rather than 24 h. These experiments commenced on 20 July 1993 when six racks were deployed in the forebay and ended when three racks were removed on each of 27 July and 30 July.

Recruitment preference data were analyzed by Friedman's test, which is appropriate for nonparametric randomized block analyses of variance (ANOVAs). Tukey's procedure was used for multiple comparisons of the data. All statistical tests were carried out using SYSTAT (Wilkinson 1990). Critical values for Friedman's test and Tukey's procedure were taken from Zar (1984). A probability level of  $p < 0.025$  was considered significant.

## Results

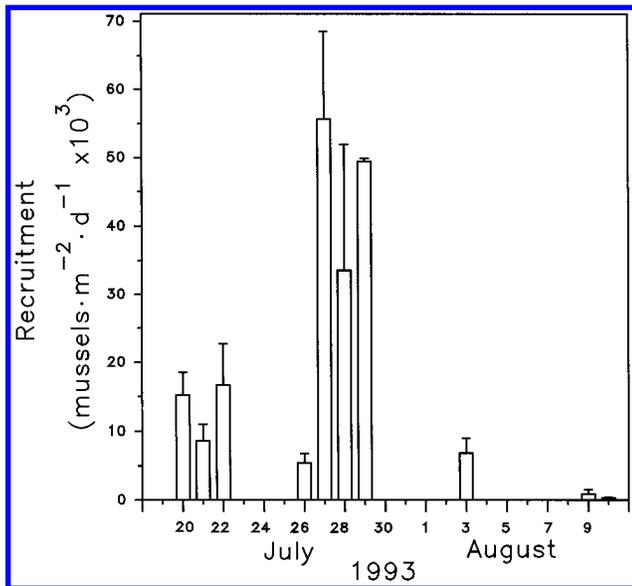
### Total dreissenid recruitment

Mussel recruitment was not detected on acrylic plates that had been deployed in the forebay of Nanticoke T.G.S. 4 weeks prior to the experiment then removed and inspected 1, 2, and 3 weeks prior to the beginning of the experiment. Recruitment was not detected on August 11, 12, or 13 so the experiments were terminated.

During our experiments, mean mussel recruitment on the six treatments (i.e., live mussels, mussel shells, and stones on acrylic plates either with biofilm or with biofilm removed) varied from a low of  $283 \pm 104$  mussels $\cdot$ m<sup>-2</sup> $\cdot$ day<sup>-1</sup> (mean  $\pm$  standard deviation,  $n = 18$ ) on 10 August to a high of  $55\,695 \pm 18\,940$  mussels $\cdot$ m<sup>-2</sup> $\cdot$ day<sup>-1</sup> ( $n = 17$ ) on July 27 (Fig. 2). On the first day of the experiment, 20 July 1993, the average rate of recruitment for all treatments was  $15\,200 \pm 3192$  mussels $\cdot$ m<sup>-2</sup> $\cdot$ day<sup>-1</sup> ( $n = 18$ ). The size of the newly settled mussels was  $390 \pm 51$   $\mu$ m ( $n = 40$ ) on 20 July,  $279 \pm 42$   $\mu$ m ( $n = 40$ ) on 29 July, and  $363 \pm 90$   $\mu$ m ( $n = 40$ ) on 9 August.

Two sets of three racks containing all six experimental treatments were suspended in the forebay of Nanticoke T.G.S. on July 20 to measure medium-term recruitment rates. One set of three racks was removed after 7 days (20 July – 27 July) and one set was removed after 10 days (20 July – 30 July). The three racks left in the water for 7 days attracted a total of 15 568 mussels (86 489 mussels $\cdot$ m<sup>-2</sup>) whereas the racks that were deployed for 10 days attracted 32 690 settlers (181 611 mussels $\cdot$ m<sup>-2</sup>). These values yield average daily recruitment rates of 12 300 and 18 200 mussels $\cdot$ m<sup>-2</sup> $\cdot$ day<sup>-1</sup> for the 7- and 10-day experiments, respectively. During the extended settlement experiments, other experiments were underway that measured daily recruitment. During the 7-day experiment daily recruitment was measured five times whereas during the 10-day experiment daily recruitment was measured seven

**Fig. 2.** Total daily recruitment of dreissenids on acrylic plastic plates and experimental substrates on 10 dates between late July and early August 1993. Error bars indicate standard deviation of the mean ( $n = 17$  or 18).



times. Average recruitment rates calculated from the daily recruitment experiments are estimated to be  $20\,300 \pm 18\,100$  mussels · m<sup>-2</sup> · day<sup>-1</sup> ( $n = 5$ ) during the 7-day experiment and  $26\,000 \pm 18\,600$  mussels · m<sup>-2</sup> · day<sup>-1</sup> ( $n = 7$ ) during the 10-day experiment. It was not possible to statistically compare recruitment measured daily with areal recruitment measured over an extended period, given the low number of replicates for the extended period data.

### Substrate preference during dreissenid recruitment

The maximum daily rate of recruitment measured was  $114\,800$  mussels · m<sup>-2</sup> · day<sup>-1</sup> ( $n = 3$ ) on the treatment with mussel shells and intact biofilm on 28 July. A number of treatments, particularly those without mussel shell or biofilm, failed to attract any settlers, particularly near the end of the experiment. Mean daily mussel recruitment for the whole experiment ranged from a maximum of  $29\,300$  mussels · m<sup>-2</sup> · day<sup>-1</sup> ( $n = 10$ ) for the mussel shells with intact biofilm to  $4\,640$  mussels · m<sup>-2</sup> · day<sup>-1</sup> ( $n = 10$ ) for stones with biofilm removed by scrubbing (Table 1).

In our experiments dreissenid recruitment was significantly greater in the treatments that included mussel shells or live mussels than in the control treatments with stones as a substrate ( $p < 0.001$ , Table 2). There was no significant difference between the number of veligers settling on mussel shells and the number settling on live mussels.

On average 10–20% fewer recruits were found on treatments where biofilm had been removed by scrubbing (Fig. 3A) than on the identical treatment where biofilm was intact (Fig. 3B). Any treatment with biofilm had significantly greater recruitment than its counterpart with biofilm removed ( $p < 0.002$ , Table 2). Daily recruitment numbers varied over two orders of magnitude so a plot of fractional recruitment (recruitment on one treatment divided by total recruitment for

**Table 1.** Dreissenid recruitment (mussels · m<sup>-2</sup> · day<sup>-1</sup>) on acrylic plastic plates and substrates with either intact biofilm or biofilm removed by scrubbing with detergent.

Treatment	Substrate number	Average recruitment	SD	Min.	Max.
Mussels	1	18 900	21 300	300	83 700
Mussels + biofilm	2	24 300	27 800	400	113 200
Shells	3	18 400	20 600	0	84 100
Shells + biofilm	4	29 300	34 800	100	114 800
Stones + biofilm	5	13 900	18 400	0	55 600
Stones	6	4640	540	0	21 600

**Note:** The substrates were as follows: mussels (live, adult dreissenids), shells (dreissenid shells), or stones (compressed, oblong stones 1–3 cm in diameter). Sample sizes were 10.

**Table 2.** Statistical analysis of dreissenid recruitment on acrylic plastic plates and substrates with either intact biofilm or biofilm removed by scrubbing by detergent.

	Mussels	Mussels + biofilm	Shells	Shells + biofilm	Stones	Stones + biofilm
Mussels	—	—	—	—	—	—
Mussels + biofilm	6.76*	—	—	—	—	—
Shells	1.35	8.11*	—	—	—	—
Shells + biofilm	5.16*	1.61	6.51*	—	—	—
Stones	9.89*	16.65*	8.54*	15.04*	—	—
Stones + biofilm	5.24*	12.00*	3.89	10.40*	4.65*	—

**Note:** The substrates were as follows: mussels (live adult dreissenids), shells (dreissenid shells), or stones (compressed, oblong stones 1–3 cm in diameter); each of these three substrates was a treatment with and without biofilm. Values reported are Friedman's  $\chi_r^2$ .

\* Significantly different at  $p < 0.025$ .

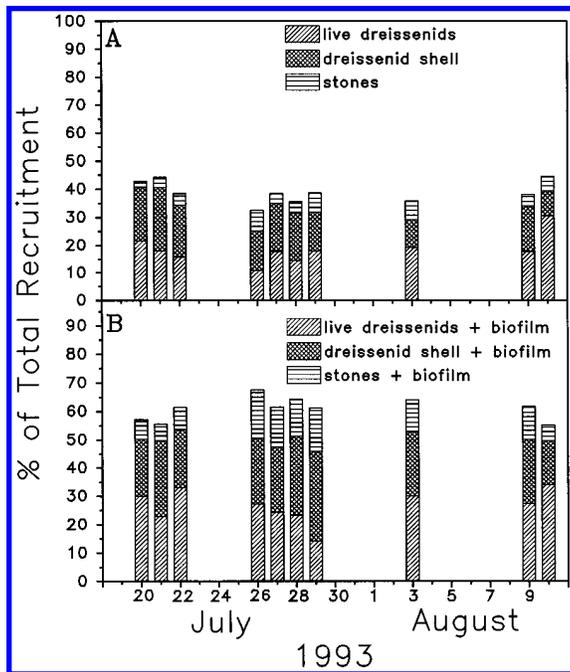
that experiment) was used to illustrate surface preference in dreissenids (Fig. 3).

## Discussion

Our experiment appeared to encompass a complete dreissenid veliger recruitment period that lasted about 3 weeks, beginning in late July 1993. Recruitment began quickly and the most intense recruitment occurred near the middle of the settling period. No recruitment was apparent 1, 2, or 3 weeks before experiments began and recruitment had fallen to near zero by the end of our experiments. Recruitment rates were extremely variable; for example, on July 26 dreissenid recruitment was  $5\,444$  mussels · m<sup>-2</sup> · day<sup>-1</sup> but it was  $55\,694$  mussels · m<sup>-2</sup> · day<sup>-1</sup> the next day.

Daily mussel recruitment rates appear to be lower when they are estimated from racks left out for 7 or 10 days. The daily settling rate estimated from racks left in the water for 7 consecutive days was  $12\,300$  mussels · m<sup>-2</sup> · day<sup>-1</sup> whereas the average daily recruitment rate measured five times during the same 7-day period was  $20\,300$  mussels · m<sup>-2</sup> · day<sup>-1</sup>. Similarly, daily recruitment estimated from racks left in the water for 10

**Fig. 3.** Substrate and biofilm preference in dreissenid larvae on 10 dates in July and August 1993. Recruitment was measured on acrylic plastic plates with live mussels, mussel shells, or stones with primary biofilm removed by scrubbing (A) and with the primary biofilm intact (B). Recruitment data are expressed as percentages of total daily recruitment.



consecutive days was  $18\,200\text{ mussels}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ , while the average recruitment rate measured seven times during the same 10-day period was  $26\,000\text{ mussels}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ . However, the standard deviation of the daily settling data is about  $18\,000\text{ mussels}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$  so the difference may not be significant. If estimates of settling rate are lower when plates are deployed over longer periods of time it may be due to settled mussels detaching after death or migrating before the racks are collected (see Connell 1985 and Doka 1995 for further discussion of post-veliger mortality). It may also be that a well-colonized surface inhibits settling.

The average shell length of our newly recruited dreissenids ranged from 279 to 390  $\mu\text{m}$ . In Lake Erie in 1992 Martel et al. (1994) found that the average length of newly settled dreissenids was about 250  $\mu\text{m}$ , so the average length of ours is 10–55% greater than that of the 1992 dreissenids. Our length data are consistent with the settlement of quagga mussel veligers, which are more than 20% longer than zebra mussels at settlement (Martel 1995). Mussels on racks of acrylic plates deployed before there was any settlement in 1993 and removed in January 1994 at the same site as our experiments appeared to be exclusively quagga mussels (W.T. Claxton, University of Guelph, Guelph, ON N1G 2W1, unpublished data). These observations of increased length at recruitment and the high density of quagga mussels recruited to substrates in the forebay of Nanticoke T.G.S. are consistent with the general takeover of habitat in eastern Lake Erie in 1993 by quagga mussels.

Our measurements of daily areal recruitment rates in 1993 are difficult to compare with measurements from 1992 by

Martel et al. (1994), since the interannual difference in potential rates of recruitment, which is influenced by a number of factors including veliger numbers, may be large. Also, Martel et al. (1994) did not measure recruitment by area but rather recruitment per  $12 \times 11\text{ cm}$  "scouring pad" per day. Assuming that the scouring pad collector is simply a flat surface of  $264\text{ cm}^2$  and that entrapment of veligers by the tortuous net of scouring pad fibres was minimal, then recruitment values for the data from Martel et al. (1994) range from 0 to  $95\,300\text{ mussels}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ . The upper limit of this range from Martel et al. (1994) is lower than what we measured for a preferred surface, such as shells with intact biofilm, but greater than what we measured for an unfavoured surface, such as stones with biofilm removed, so the scouring pad sampler is intermediate in its attractiveness to veligers. From a technical standpoint, however, there is little to recommend the scouring pad sampler because of possible entrapment of veligers and also because the scouring pad samples must be extracted, filtered, and preserved before being counted. Veligers settling on the plates used in this study were counted directly under the microscope without preservation.

We found that recruitment of dreissenids was intense and brief. In water that has been chlorinated to prevent mussel settlement in water intakes, settlement often begins with the appearance of large numbers of veliger larvae but it is weeks before significant recruitment occurs. Our work supports the contention of Claudi and Mackie (1994) that the duration of chlorination of raw lake water could be greatly decreased by focussing on the first signs of settling rather than on the first appearance of veligers, since the veligers themselves are not the problem. Martel et al. (1994) have shown that the appearance of late-stage veligers correlates well with recruitment, so either counts of late-stage veligers or direct measurements of recruitment could be used to synchronize chlorination with periods of recruitment and therefore decrease chlorine use.

We expected that late in the recruitment period veligers would settle indiscriminately on any available surface. However, in our experiments the preference of mussels for the substrates did not appear to change with time though we could not test directly for this phenomenon. We also expected that the discriminatory abilities of veligers would be less when large numbers were settling, but surface preferences appeared to be the same regardless of recruitment density (Fig. 3).

The presence of conspecific adults is known to be an important factor in the recruitment of many marine organisms. Investigations of barnacle larvae also suggest that surface bacterial films are important. Tamburri et al. (1992) found that both biofilm and conspecifics produce waterborne substances that are attractive to settlers. The results of our experiment support this observation. The amount of recruitment on live, adult mussels, shell from dead mussels, or stones was significantly increased by the presence of a 4- to 6-day biofilm. Though the nature of the interaction between settlers and biofilm is not clear our data suggest that biofilm removal will decrease the rate of colonization. Maki et al. (1989) suggested that a critical characteristic of larval preference is surface free energy. Barnacles have high settlement on surfaces with high critical surface energies (i.e., hydrophilic and highly wettable surfaces like glass). Maki et al. (1989) also found that the adhesive strength was greatest on substrates with high surface energies. Bryozoans, however, prefer surfaces with low

critical surface energies (hydrophobic and poorly wettable; Roberts et al. 1991). Surface energy is also important in bacterial attachment and colonization because the adsorption of macromolecules to solid substrates alters surface charge and energy (Fletcher and Marshall 1982). The adsorption of different proteins to a surface affects bacterial attachment (Fletcher and Marshall 1982). The biofilm in our experiments was not well characterized but surfaces with substantial biofilm in general have high surface energies, which suggests that dreissenids prefer similar surfaces to barnacles.

The physical removal of biofilm is not likely to be a practical method to control dreissenid recruitment even though we observed significant decreases in settlement. Biofilm removal by scrubbing only decreased settling and many mussels attached to the cleaned surfaces. Also, significantly enhanced mussel recruitment was seen even though our biofilm was only cultured for 4–6 days. Control of recruitment by biofilm removal would therefore involve physical removal of biofilm at intervals less than 4 days, which would be expensive and impractical and only about a 10–20% decrease in settling could be anticipated.

Though physical biofilm removal may be impractical, chemical removal of biofilm may be a viable control strategy. Chemical removal of biofilm is likely to be more effective at discouraging recruitment than physical removal of biofilm because biofilm could be continually removed with antibiofilm agents. In our experiments biofilm would have begun to form as soon as the plates were lowered into the water and cleaned treatments therefore may have provided a suitable surface for recruitment soon after being deployed. Certainly comparisons of the biofouling potential of surfaces must consider the recruitment-enhancing nature of surface biofilm.

There was no difference between the amount of recruitment on live mussels and mussel shells if both surfaces had an intact biofilm, or between live mussels and mussel shells if both surfaces had their biofilm removed. These results indicate that some physical or chemical factor in the shell was significant in encouraging recruitment and that the adult conspecifics did not appear to be producing recruitment-mediating compounds. In addition, settlers preferred both shells and live mussels, with or without biofilm, to stones for recruitment.

The preference of veligers for dreissenid shells suggests that removing mussels without removing all shells and associated material is an inadequate means for controlling settling and subsequent biofouling. To decrease recolonization all shells and associated material must be removed.

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