Zebra Mussel's Calcium Threshold and Implications for its Potential Distribution in North America

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Introduction

Since the discovery of zebra mussels (*Dreissena polymorpha*) in the Great Lakes in 1988 (Griffiths *et al*. 1991), several studies have assessed which regions or water bodies could become colonized in various parts of North America. Most of these studies recognized ambient calcium concentration as a key factor affecting the mussels' potential distribution (e.g. Mellina & Rasmussen 1994; O'Neill 1996), and based their assessments in part on estimates of the minimum calcium concentration needed to support a reproducing population, which we refer to as the mussels' "calcium threshold." Curiously, studies based on either European or North American data have generally reported different calcium thresholds. In an analysis of over 500 European lakes in regions that had been occupied by zebra mussels for over 50 years, zebra mussels were found only in waters with at least 28.3 mg/l of calcium (Ramcharan *et al*. 1992; Padilla 1997). In 527 lakes in Belarus, zebra mussels were found only in lakes with more than 25.4 mg/l of calcium (Karatayev 1995). In North America, on the other hand, zebra mussel populations have been reported from several sites with calcium concentrations between 13 and 25 mg/l (Strayer 1996; Mellina & Rasmussen 1994; M. Hauser, pers. comm. 1997), and studies have estimated calcium thresholds as low as 8.5 mg/l (Hincks & Mackie 1997).

Three hypotheses could explain these differences:

1. European and North American zebra mussels may be genetically distinct (due, for example, to founder effect in the establishment of the North American population), with the North American mussels having a lower calcium threshold.
2. In the regions examined by the European studies, zebra mussels may be limited to waters with greater than 25-28 mg/l of calcium by some undetermined, co-varying environmental factor, rather than by calcium.
3. Zebra mussels found at sites in North America with less than 25-28 mg/l of calcium may be non-reproducing "sink" populations resulting either from larvae drifting in from reproducing populations established at upstream sites with higher calcium levels, or from mussels repeatedly introduced by anthropogenic transport. The apparent North American calcium threshold would thus represent the concentration needed for settlement and growth, with the apparent European threshold representing the concentration needed for successful gonad development and gametogenesis, fertilization, or embryonic or early larval development.

Regarding the first hypothesis, if the genetic diversity of North American zebra mussel populations had been reduced by founder effect relative to the genetic diversity of European source populations, we would expect the resulting phenotypic range to be narrower (or at least no broader) in North America than in Europe. Theoretically, selection pressures in a novel environment could result in a broadening of environmental range. However, there has been little time for natural selection to act: zebra mussels are thought to have arrived in North America less than two decades ago, and, as discussed below, they were reported from waters with <25 mg/l of calcium within three years of their estimated date of arrival. Thus genetic difference seems an unlikely explanation for the broader reported calcium range in North America.

Regarding the second hypothesis, it is possible that the observed European distribution pattern is caused by some other unknown abiotic or biotic factor rather than by variation in calcium concentrations. However, until a specific potential factor is identified, this hypothesis remains untestable.

Here, we investigate the third hypothesis, that zebra mussel populations at sites with <25-28 mg/l calcium do not successfully reproduce and are sustained by larval recruitment or anthropogenic introduction from other sites with higher calcium levels. We consider this question by:

1. Reviewing data from experimental studies for evidence of zebra mussels' calcium requirements;

2. Reviewing all published cases of zebra mussel populations in waters with less than 28 mg/l of calcium to determine whether there are populations in higher-calcium, upstream waters that could be serving as larval sources;
3. Reviewing all reports of zebra mussel occurrences in North America to determine whether there are any established populations in isolated, low calcium waters; and
4. Reviewing all assessments to date of potential zebra mussel distributions in North America to determine how the use of different calcium thresholds would affect estimates of potential distribution.

Measurement and Reporting of Calcium Concentrations

Calcium occurs in fresh waters in solution as the free ion (Ca^{++}), as calcium carbonate ($CaCO_3$), and in colloidal complex with sediments and organic matter. Standard analytical methods define "dissolved calcium" as calcium measured in a sample after filtration through a 0.45 μm membrane filter, and "total calcium" as calcium measured in an unfiltered sample after vigorous digestion (US EPA 1983; Eaton *et al*. 1995). In practice these measures are likely to be close unless total calcium levels are quite high, and in some cases the same data is reported both as dissolved and as total calcium[1] (Pederson, pers. comm. 1998; Kirschner, pers. comm. 1998). Calcium concentrations are sometimes expressed in terms of the equivalent in mg/l of calcium carbonate, which can be converted to concentration as calcium by multiplying by 0.4, the ratio of the molecular weight of calcium to that of calcium carbonate (Cole 1975; Eaton *et al*. 1995). In this report, we treat concentrations reported as total or dissolved calcium, or converted from concentrations as calcium carbonate, as equivalent measures and report them simply as calcium concentrations.

Hardness is also reported for many waters. Hardness is the sum of various cations of sulfates, chlorides and carbonates, with calcium and magnesium ions usually accounting for most of the hardness (Cole 1975). In current standard analytical practice, "total hardness" is defined as the sum of calcium and magnesium concentrations expressed as calcium carbonate (Eaton *et al*., 1995). However, because these ions can vary in relative concentrations, measurements of hardness cannot be directly converted into calcium concentrations. While harder waters usually do have higher calcium concentrations, this may not be the case in soda lakes and some other alkaline waters in arid regions (Cole 1975).

We found that data on calcium concentrations were not available for many water bodies, and in other cases only one or a few measurements were available. Calcium levels can be highly variable in some water bodies, changing with location or depth and over various time scales.[2] They generally vary more in hardwater than in softwater lakes, in part because when calcium is near saturation levels increased photosynthetic activity can substantially increase the precipitation of calcium carbonate from the epilimnion (Wetzel 1975). In seven meromictic lakes in Washington state, calcium levels measured at various depths ranged from 50% below to 300% above the surface

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1 For example, calcium measurements made in 1987 and 1988 in Chatauqua Creek at Barcelona, New York were reported to the US EPA's STORET database as dissolve d calcium by one agency and as total calcium by another agency. We found many ot her examples of this.

2 A number or researchers suggested in personal communications that calcium co ncentrations may be elevated near concrete structures, but we were unable to find a ny quantification of this or any other research into this possible effect (Nichols. pers. comm. 2001).

values for those lakes (Edmondson 1963). In Lawrence Lake in southern Michigan, calcium concentrations during periods of summer and winter stratification were around 70-85 mg/l at 12 m depth and around 60-70 mg/l at the surface, with a brief dip to 40 mg/l at the surface during early spring ice melt (Wetzel 1975). In Glen Lake in Ontario, calcium concentrations fluctuated annually from around 22-24 mg/l in the winter to around 19-21 mg/l in the late spring and early summer (Neary & Leach 1992), and in hypereutrophic Wintergreen Lake in Michigan, calcium levels at one-meter depth varied from above 50 mg/l in the winter to around 20 mg/l in June to near zero in early August (Wetzel 1975). The range of calcium concentrations that can be found within individual water bodies must be kept in mind when assessing calcium data relative to zebra mussel distributions.

A further complexity is that zebra mussels' calcium requirements probably vary with changes in other environmental factors. For example, several studies have concluded that zebra mussels' calcium threshold varies with pH, usually declining with increasing pH (Ramcharan *et al.* 1994; Hincks & Mackie 1997; Nierzwicki-Bauer, pers. comm. 2001). Zebra mussels also require magnesium, and their better survival in natural waters with higher calcium concentrations may perhaps be due to the presence of magnesium in those waters, rather than higher calcium levels *per se* (Nichols, pers. comm. 2001). Zebra mussels may also obtain some calcium from their diet: mollusks typically meet between 70-80% of their calcium needs through absorption of the free ion from the water column, and the rest through food (Vinogradov *et al.* 1993).

The Zebra Mussel's Life Cycle

The development, growth or survival of a zebra mussel could be affected by ambient calcium concentrations thoughout its life cycle. Gametogenesis generally begins in the fall or winter, with spawning (release of eggs) starting in the spring when water temperatures rise above 12° C, although most spawning occurs above 17-18° C (Mackie *et al.* 1989; Sprung 1993; Mackie & Schloesser 1996; Nichols 1996; McMahon 1996).[3] The spawning period is often prolonged, continuing in pulses through late summer or early fall. Eggs and sperm are released into the water where fertilization occurs, with a single spawning female releasing tens of thousands to millions of eggs (Mackie *et al.* 1989; Sprung 1993; Mackie & Schloesser 1996; Nichols 1996). The embryos develop into swimming larvae, called trochophores, in 6 to 96 hours after fertilization (Mackie *et al.* 1989; Sprung 1993; Ackerman *et al.* 1994).

After an initial nonfeeding or *lecithotrophic* phase, the larvae develop intestines and a feeding and swimming organ known as the velum, and begin a feeding or *planktotrophic* phase in 2-9 days after fertilization (Table 1). By this time they have also developed D-shaped (straight-hinged) shells about 70-100 μm long (Sprung 1993; Ackerman *et al.* 1994). Once the velum appears the larvae are called veligers, and they develop progressively through the D-shell stage, a veliconcha stage with a more rounded and ornamented shell (also called the umbonal stage), and a pediveliger stage with the initial development of a foot. After a week to a month or more of growth the veligers settle to the bottom, typically at shell lengths of around 200-240 μm (Mackie *et al.* 1989; Sprung 1993; Ackerman *et al.* 1994; Mackie & Schloesser 1996). In general, embryonic and larval development

[3] The seasonal pattern of gametogenesis and spawning can vary greatly in differe nt locations and dieffernt years. For example, a histological and biochemical analysis of zebra mussels from an Erie Canal site in 1992 found that gamete synthesis peake d in May, gametes matured in June and July, and spawning began in August (Wang *et al.* 1993). In the following year, when mussels overwintered in better condition, D NA concentrations increased more slowly through the spring and peaked later (Wa ng *et al.* 1994).

times are longer at lower temperatures and with lower food availability. Larval growth rates have been measured in the laboratory or estimated from field data to range from 1 to 24 μm per day, with lower growth rates suggesting a longer larval period (Mackie *et al*. 1989; Sprung 1993; Neumann *et al*. 1993; Ackerman *et al*. 1994; Mackie & Schloesser 1996; Nichols 1996). In addition, larvae that are produced in the fall may overwinter by delaying development for several months (Nichols 1996; McMahon 1996).

Table 1. Estimated planktonic periods for eggs, embryos and larvae

For some of the studies, the periods' initial and end points have been inferred from the context.

Phase	Temperature	Duration	Source
Spawning to fertilization	24° C 12° C	≤ 2.5 hr ≤ 5 hr	Sprung 1993 (delay in fertilization while retaining 50 % of initial success)
Fertilization to swimming larva	24° C 12° C	6 hr 20 hr	Sprung 1993; Sprung 1989, cited in Ackerman *et al.* 1994
	20-24° C	48-72 hr	Nichols unpubl., cited in Ackerman *et al.* 1994
	17-24° C	48-96 hr	Leitch & McLeod 1993, cited in Ackerman *et al.* 1994
Fertilization to D-shell	24° C 21° C 18° C 15° C 12° C	1.3 d 1.5 d 1.9 d 3.2 d 3.8 d	Sprung 1987, 1993; Sprung 1989, cited in Ackerman *et al.* 1994
	20-24° C	3-5 d	Nichols unpubl., cited in Ackerman *et al.* 1994
	17-24° C	7-9 d	Leitch & McLeod 1993, cited in Ackerman *et al.* 1994
Fertilization to veliconcha	?	8-10 d	Stoeckel & Garton 1993
Fertilization to settlement	22° C	21 d	Vanderploeg *et al.* 1994
	20-24° C	35 d	Nichols unpubl., cited in Ackerman *et al.* 1994
	20° C	18-37 d	Sprung 1989, cited in Ackerman *et al.* 1994
	?	90 d	Morton 1969a,b,c, cited in Ackerman *et al.* 1994
D-shell through veliconcha	≈ 16-24° C	23-27d	de Lafontaine & Cusson 1997 (based on period of occurrence in the Richelieu River)
D-shell to settlement	18-21° C	17 d	Borcherding & van Steveninck 1992 (estimated from growth rates in the Rhine River)
	≈ 21° C	14-21 d	Baldwin 1994
	21° C 14° C	30 d 100 d	Sprung 1993 (estimated from growth rates)
"Planktonic phase"	?	≈ 8 d	Korschelt 1892
	?	8 d	Katchanova 1961, cited in Sprung 1993
	?	usu. 8-10 d	Mackie *et al.* 1989, citing various authors
	?	10 d	Lewandowski 1982, cited in Garton & Haag 1993

?	8-12 d	Hillbricht-Ilkowska & Stanczykowska 1969, cited in Neumann *et al.* 1993
?	≥ 14 d	Neumann *et al.* 1993
?	5-16 d	Griffiths *et al.* 1991, citing Kornobis 1977, Stanczyko wska 1977 and Lewandowski 1982
?	12-16 d	Kirpichenko 1962, 1964, cited in Mackie *et al.* 1989 an d in Nichols 1996
?	21 d	Nichols 1993, cited in Nichols 1996
≈16-24° C	20-25 d	Cusson & de Lafontaine 1997 (in the Richelieu River)
?	5-26 d	Shevtsova 1968, cited in Sprung 1993
16-24° C 15-20° C	18 d 28 d	Neumann *et al.* 1993, based on data from Sprung 1987,1 989
20° C ?	18-33 d	Sprung 1989, cited in Sprung 1993
?	≈ 35 d	Walz 1973, 1975, 1978, cited in Sprung 1993, Ackerman *et al.* 1994 and Nichols 1996
?	3-90 d	Nichols 1993, citing Stanczykowska 1977, Sprung 1987 and Mackie *et al.* 1989
over winter	180 d	Nichols & Kovalak 1995, cited in Nichols 1996
over winter	240 d	Kirpichenko 1964, cited in Nichols 1996

Settling larvae attach by byssal threads to hard substrates such as rocks, shells or submersed plants, though they sometimes attach directly to sand grains (Mellina & Rasmussen 1994; Nichols 1996; Berkman *et al.* 1998). Upon settlement they are known as postveliger or plantigrade mussels, which metamorphose into juveniles by losing the velum and forming, enlarging and reorienting the body structures that are characteristic of adult mussels, including siphons, gills, a mouth, a larger foot, and a more rhomboidal shell (Ackerman *et al.* 1994; Nichols 1996). Zebra mussels reach sexual maturity at 1-2 years and shell lengths of 5-12 mm (Mackie *et al.* 1989; Smirnova & Vinogradov 1990; Mackie & Schloesser 1996; Nichols 1996). They live for 2-9 years, attaining maximum shell lengths of over 40 mm (Mackie *et al.* 1989; Smirnova & Vinogradov 1990; Mackie 1993; Mackie & Schloesser 1996).

Though primarily sedentary, zebra mussels, especially juveniles, may at times release their byssal threads and move to new attachment sites (Korschelt 1892; Martel 1993; Ackerman *et al.* 1994; Mackie *et al.* 1991; Mackie & Schloesser 1996). For example, zebra mussels have been reported to migrate between shallower water in the summer and deeper water in the winter (Korschelt 1892; Mackie *et al.* 1989; Mackie & Schloesser 1996). Juveniles and adults may move short distances by crawling (Korschelt 1892; Oldham 1930; Martel 1993; Ackerman *et al.* 1994), sometimes using byssal threads to assist their movement (Griffiths *et al.* 1991), and may be carried longer distances when attached to floating vegetation or debris (Mackie *et al.* 1991; O'Neill 1991; Martel 1993; Mackie & Schloesser 1996; Johnson & Carlton 1996; Ackerman *et al.* 1994, note that pediveligers may recruit preferentially to aquatic plants and later migrate to other substrates by transport on floating plant material). Small juveniles may also travel substantial distances by drifting in currents, remaining above the bottom with the aid of trailing threads acting as drag lines (Griffiths *et al.* 1991 and Carlton 1993 refer to "bysso-pelagic transport;" Martel 1993 and Ackerman *et al.* 1994 both note that the threads used for drifting are morphologically distinct from byssal threads), with threads in contact with the water surface from which the mussel hangs (Oldham 1930; Ackerman *et al.* 1994; Mackie & Schloesser 1996), by crawling on the underside of the air-water surface (Oldham 1930; Ackerman *et al.* 1994), or by simple resuspension (Martel 1993). Surprisingly large numbers of juveniles have been reported drifting in Lake Erie during periods of strong waves or storms (Martel 1993). If juvenile drift is initiated by the presence of strong currents, it could be an important form of transport in river systems. Floating by means of secreted bubbles has been observed in marine mussels, and may occur in zebra mussels (Ackerman *et al.* 1994). The possibility of transport by crayfish, turtles, birds, muskrats or other organisms has also been considered (reviewed by Carlton 1993; Mackie & Schloesser 1996; Johnson & Carlton 1996).

Review of Experimental Studies

We found thirteen studies that experimentally tested aspects of zebra mussels' survival, growth, development or reproduction in waters with different calcium concentrations. In some of these studies, zebra mussels' response to calcium concentrations varied with pH or total ion concentrations.

Sprung (1987) investigated the rearing success and larval condition of zebra mussels exposed to different ion concentrations from the eggs to 3-day-old larvae. Inducing adult mussels to spawn, he collected and counted the gametes and then inoculated them into flasks containing deionized water with a standard mix of salts, including sufficient calcium carbonate to produce a calcium concentration of 59 mg/l.[4] For six experimental treatments he used solutions that lacked either calcium carbonate or one of five other constituent salts ($MgSO_4$, NaCl, $KHCO_3$, $NaCO_3$, $MgCl_2$). For nine additional treatments he used calcium concentrations ranging from 12 to 106 mg/l. After three days, he counted the number of healthy and crippled larvae.

Sprung found that the solutions that lacked calcium produced no larvae. The solutions that lacked any of the five other salts produced a normal number of larvae, but with an increased proportion of crippled larvae. When exposed to different calcium concentrations, rearing success and larval condition were roughly constant for concentrations above about 40-60 mg/l. Below that level,

Figure 1. Larval production at different calcium levels

Larval production is the number of healthy larvae produced after 3 days, indexed to the number produced at calcium concentrations of 59 mg/l. Calculated from graphs in Fig. 3 of Sprung (1987).

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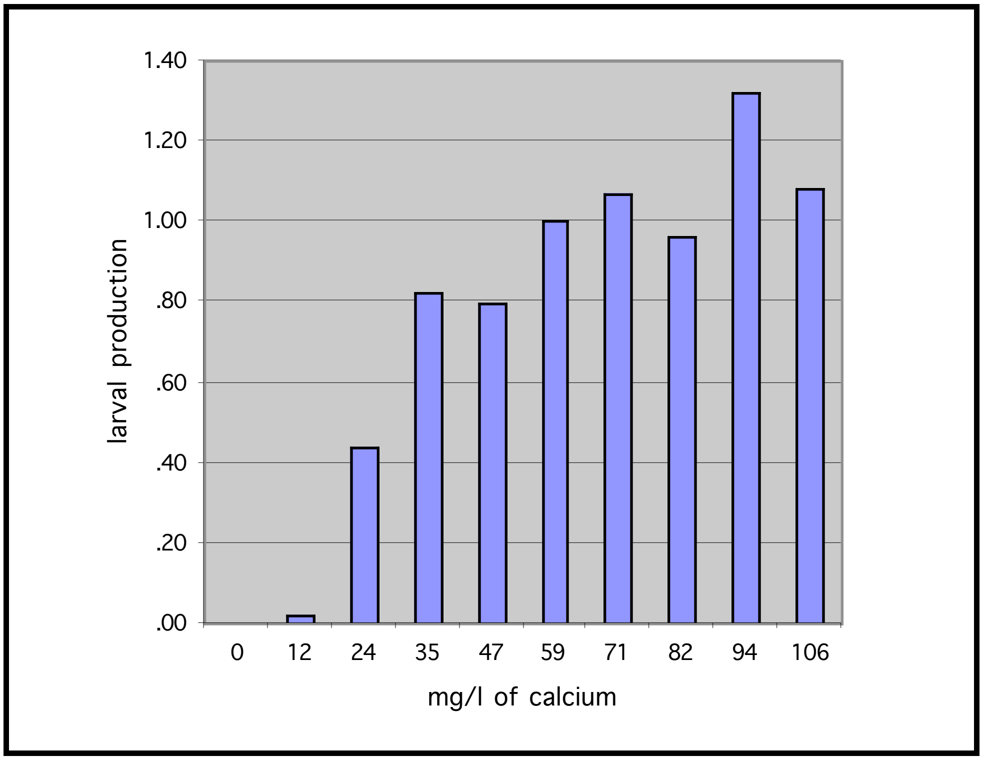
4 Nichols (1996) notes that since Sprung did not directly measure the amount of dis solved calcium in his experimental treatments, the reported concentrations may be i naccurate.



rearing success declined and the proportion of crippled larvae increased (Figure 1). At 12 mg/l virtually no larvae were produced, and about 90% of the few that were produced were crippled.

In two studies, Vinogradov *et al*. (1987, 1993) tested the ability of zebra mussels to maintain tissue ion balance in waters with different ionic compositions and concentrations. In the first study, they examined the calcium flux between freshwater bivalves and ambient water at calcium concentrations from near zero to about 22 mg/l. Of three bivalve species tested, zebra mussels were the most sensitive to low calcium levels, losing calcium when ambient concentrations were below 13-14 mg/l. In the second study, Vinogradov *et al*. (1993) tested the effect of acclimatization in very low salt waters (with calcium levels of 0.8-1.5 mg/l) on calcium metabolism. They found evidence of debilitation rather than acclimatization: unacclimatized zebra mussels lost calcium when calcium levels dropped below 14 mg/l, while acclimatized mussels lost calcium when ambient calcium dropped below 22 mg/l, and lost it at a faster rate. They also found that lowering the pH below about 7 increased the rate of calcium loss in waters with low calcium levels, and that the effect was greater in zebra mussels than in other freshwater bivalves.

Ram and Walker (1993) found that 70% of adult zebra mussels died within 14 days in deionized water, with small mussels tending to succumb quicker than large ones. However, since all zebra mussels survived when NaCl or $MgSO_4$ was added to the deionized water, they concluded that the lethal effect was due to general osmotic stress rather than the specific lack of calcium. Based on reported increases in blood calcium in a freshwater bivalve exposed to deionized water, they suggest that zebra mussels and other freshwater bivalves may be able to draw on reserves of calcium from shell or tissue to maintain osmolality when stressed, and that larger animals, having larger reserves, may fare better.

Dietz *et al*. (1994) similarly found that mussels died within five days in deionized water, but could survive over 51 days in water that contained no calcium but had minimal concentrations of NaCl, potassium and magnesium. They concluded that the mussels survived by mobilizing calcium from their shells in order to maintain necessary levels of calcium in their blood.

In one laboratory and one field study in 1992, Hincks and Mackie (1993, 1994) assessed the effect of different concentrations and combinations of calcium and alkalinity on survival, growth, gonad maturation and spawning. In the laboratory experiment, adult and juvenile zebra mussels from Lake Erie were placed in flow-through aquariums with 15 levels of calcium (0-35 mg/l), 15 levels of alkalinity (2.5-80 mg/l as $CaCO_3$), and 15 combinations. Preliminary analysis indicated that survival and growth in shell length increased with increasing calcium and alkalinity. Gonad maturation was normal at all alkalinity levels, but males did not release sperm unless the calcium concentration was above 15 mg/l. In the field experiment, juvenile mussels were placed in flow-through bio-boxes in three Ontario lakes with calcium concentrations of 7, 25 and 44 mg/l. Growth rates in the two higher calcium lakes were similar to rates observed in the Great Lakes but were only about 9-14% of the Great Lakes rate in the low calcium lake.

Hincks and Mackie (1997) tested adult survival, juvenile growth rates and veliger production against different concentrations of calcium, alkalinity, total hardness, chlorophyll and pH, by rearing adults and newly settled juveniles collected from Lake St. Clair in water from 16 Ontario Lakes (Table 2). Six of these lakes had mean calcium levels below 8.5 mg/l and mean pH of 8.4 or less. In these low calcium waters all adults died within 35 days, juvenile growth rates were near zero or negative, and no veligers were produced. The other ten lakes all had mean calcium levels of 20-48 mg/l and mean pH of 8.2-9.3. In these waters adult survival was 52-100%, juvenile growth rates ranged from 3 to 29 μm/day (low compared to rates measured in the field in Lake St. Clair of up to 125 μm/day), and very small numbers of veligers were produced, from 0 to 7 veligers from an initial population of 21 adults.

Table 2. Survival, growth and reproduction in water from 16 Ontario lakes (from Hincks & Mackie 1997)

	Mean calcium (mg/l)	Mean pH	% adult survival at 35 days	Mean juvenile growth rate (μm/day)	Production of veligers over 70 days
Dickie Lake	2.4	6.4	0	0	0
Lake of Bays	3.0	7.4	0	-12	0
St. Nora Lake	3.4	7.1	0	-7	0
Lake Muskoka	6.0	8.4	0	0	0
Beech Lake	7.8	8.0	0	-5	0
Big Clear Lake	8.3	8.1	0	-12	0
Balsam Lake	19.9	8.7	90	5	1
Buckhorn Lake	25.7	9.3	95	22	3
Devil Lake	26.7	8.6	81	24	1
Lake St. Clair	32.9	8.7	81	3	0
Big Rideau Lake	34.3	8.2	67	29	1
Upper Rideau Lake	35.4	8.7	81	13	2
Lake Erie	35.7	8.5	71	24	0
Lake Ontario	39.2	8.2	76	20	0
Lake Scugog	44.5	8.5	100	6	7
Lake Simcoe	47.6	8.4	52	9	2

Hincks and Mackie analyzed these data by fitting the responses to the environmental variables with multiple regressions. They concluded that adult mortality is best modelled by a logistic regression on calcium and pH, with mortality decreasing with increasing calcium for pH between 6.0 and 8.5, and, surprisingly, increasing with increasing calcium for higher pH. They found significant curvilinear relationships between juvenile growth rates and each of the buffer variables (calcium, alkalinity and total hardness), which were themselves correlated. The regression for calcium showed negative growth below 8.5 mg/l and maximum growth at 32 mg/l, with the growth rate declining at higher calcium levels. They found no significant relationship between the number of veligers produced and any of the environmental variables, although veligers were only produced in waters with 20 mg/l or more of calcium and pH of at least 8.2.

Balcer (1996a) collected small zebra mussels (≤20 mm long) from Lake Erie and the Mississippi River, and larger ones (25-30 mm long) from Duluth-Superior Harbor (at the western end of Lake Superior), and placed them in cages in Duluth-Superior Harbor for various periods during 1993-1996. All size classes survived well and grew during summers but there was high mortality during winters, especially for smaller mussels (80-95% mortality for 7-12 mm mussels, 54-97% mortality for 15-20 mm mussels, and 12-46% mortality for 25-30 mm mussels). The shells of many of the surviving and most of the dead mussels were thin and eroded, with gaping holes in many of the smaller mussels.

Balcer (1996b) collected zebra mussels from Lake Erie, the Mississippi River and Duluth-Superior Harbor in the spring of 1995 and reared them in the laboratory in water with 15, 30, 35, 45 and 60 mg/l of calcium, and in two treatments with calcium concentrations that were varied in the 15-35 mg/l range. Water temperatures were increased from 10 to 20°C over 11 weeks, and then varied between 20 and 23°C for 7 weeks. Survival was good in all treatments until temperatures reached 20°C; thereafter mortality rose and reached 80% by week 17. Mussels in all treatments released sperm and eggs after temperatures reached 21°C.

Baldwin *et al*. (1997; Baldwin, pers. comm. 1998) examined the ability of zebra mussels collected from the St. Lawrence River to survive and reproduce in water with low calcium levels from four uncolonized sites in northern New York (Table 3). Juvenile (5 mm shell length) and adult (15 mm shell length) mussels had comparable survivorship over 5 weeks in test waters with ≥4 mg/l of calcium, although mussels in 4 mg/l of calcium rapidly lost weight. Embryonic development up to the veliger stage was successful and comparable and veliger survival over 14 days was comparable in the waters with ≥22 mg/l of calcium, but unsuccessful in those with ≤4 mg/l.

Table 3. Survival and early development in waters from northern New York (from Baldwin *et al.* 1997 ; Baldwin, pers. comm. 1998)

Water source	Calcium lev el (mg/l)	Juvenile and adult sur vival after 35 d	Juvenile and adult grow th over 35 d	Development o f embryo to she lled veliger stage	Veliger survi val after 14 d
Raquette River	3	low	negative	failed	0 %
Upper St. Regis Lake	4	high	negative	failed	0 %
Upper Saranac Lake	4	high	negative	failed	0 %
Black Lake	22	high	positive	successful	≈60 %
St. Lawrence River	30	high	positive	successful	≈60 %

In a series of experiments, Nierzwicki-Bauer and her students investigated zebra mussel survival in tanks containing New York lake water with different calcium concentrations. Newly-settled and adult zebra mussels were collected from the Hudson River and reared in water from the Hudson River (with calcium concentrations of around 17 mg/l) and Lake George (with calcium declining from around 12-14 mg/l to 10-11 mg/l between weekly water changes). Mussels survived well in both waters for 19 weeks, but mussels reared in Hudson River water were 11% longer and had 25%-40% more dry tissue mass than those reared in Lake George water, with the latter also characterized by some dissolution of the umbonal region of the shell (Hansen *et al.* 1998; Hansen, pers. comm. 1998; Nierzwicki-Bauer, pers. comm. 2001). In other experiments, veligers collected from Lake Champlain and reared in Lake George water (with 11 mg/l of calcium and pH of 7.5) did not survive. They survived longer when either calcium or pH was raised to Lake Champlain levels (16.5 mg/l of calcium and pH of 7.8); and survived best, nearly as well as controls reared in Lake Champlain water, when both calcium and pH were raised to Lake Champlain levels (Nierzwicki-Bauer, pers. comm. 2001).

Lynn (pers. comm. 1998) tested the ability of zebra mussel eggs to complete first cleavage in waters with different calcium concentrations. He collected adult zebra mussels from hard waters, held them for weeks to months at 12-15 mg/l of calcium, and artificially spawned them. He found over 50% success rates for eggs held at dissolved calcium levels of 4-8 mg/l, with dramatically declining success rates below 4 mg/l. However, there was a high degree of variability, with success rates ranging to 10 to 90% between tests using the same group of animals held under the same conditions.

Table 4. Summary of experimental studies relative to possible calcium thresholds

The indicated calcium level is the minimum concentration needed to satisy the endpoint, as indicated by data or analyses in the cited sources.

Endpoint	Indicated calcium thre shold (mg/l)	Source
Fertilization/Embryonic Development		
Release of sperm	15	Hincks & Mackie 1994
Normal success in egg fertilization	between 4 and 22	Baldwin *et al.* 1998
≥50% mean success in completing first cleavage	4	Lynn, pers. comm. 1998
Larval Development		
Development of some larvae, 0-3 days	between 0 and 12	Sprung 1987
Significant numbers of healthy larvae, 0-3 days	between 12 and 24	Sprung 1987
Some veliger production	between 8 and 20	Hincks & Mackie 1997
Normal success in development from fertilization t o D-shell veliger	between 4 and 22	Baldwin *et al.* 1998
Normal success in development from D-shell velig er to juvenile	between 4 and 22	Baldwin *et al.* 1998
Veliger survival	between 11 and 16.5	Nierzwicki-Bauer, pers . comm. 2001
Juvenile Stage		
Normal juvenile (5 mm shell) survival for 35 days	between 3 and 4	Baldwin *et al.* 1998
Normal juvenile growth rate	between 7 and 24	Hincks & Mackie 1993
Juvenile growth (based directly on data)	between 8 and 20	Hincks & Mackie 1997
Juvenile growth (based on regression)	8.5	Hincks & Mackie 1997
Adult Stage		
Nonnegative calcium flux in unacclimatized adults	13-14	Vinogradov *et al.* 1987
Nonnegative calcium flux in unacclimatized adults	14	Vinogradov *et al.* 1993
Nonnegative calcium flux in acclimatized adults[1]	22	Vinogradov *et al.* 1993
Some adult (10-15 mm shell) survival for 35 days (based directly on data)	between 8 and 20	Hincks & Mackie 1997
Some adult (10-15 mm shell) survival for 35 days (based on regression)[2]	≈30 at pH≤7.4 0-25 at pH of 7.5-8.3	Hincks & Mackie 1997
Normal adult (15 mm length) survival for 35 days	between 3 and 4	Baldwin *et al.* 1998
Maintenance of tissue weight for 35 days	between 4 and 22	Baldwin *et al.* 1998

1 Acclimatized for 28 days in diluted artesian water with 0.8-1.5 mg/l of calcium.

2 Threshold calculated from multiple logistic regression model, for adult survival of ≤5%. By t he same model, calcium levels must be *below* 50 mg/l for adult survival at pH≥9.1.

Previously Reported Occurrences of Zebra Mussels in Low-Calcium Waters

We found published reports of zebra mussels in waters with calcium concentrations below 28 mg/l in five regions in North America: in Lake Superior, in the Hudson River downstream of Troy, in the St. Lawrence River downstream of Montreal, and in the Lake Champlain-Richelieu River system. For each of these populations we sought information regarding evidence of zebra mussel reproduction at the site, calcium levels at the site and upstream, and the presence of a potential upstream (or remote) source population established in higher calcium waters.

Lake Superior

Lake Superior is the westernmost of the Great Lakes, and drains from west to east into Lake Huron. Most of the Lake Superior basin is underlain by the low-calcium pre-Cambrian rock of the Canadian Shield (Beeton & Chandler 1963), and calcium concentrations in the open lake range from 12 to 15 mg/l (Beeton & Chandler 1963; Goldman & Horne 1983; STORET 1998; Balcer, 1996a). Calcium concentrations range from around 13 to 23 mg/l in Duluth-Superior Harbor at the

Table 5. Zebra mussel records in or near Duluth-Superior Harbor

Date	Record (References)
Nov 1989	20 adults found on buoys (Balcer 1994; Benson 1998)
spring 1990	2-3 cm-long mussels found on ship's rudder gearbox and in its seachest (ZM Update #2)
1990	3 veligers collected at 2 sites, and 19 post-veligers collected on substrate samplers (ZM Updates #4, #5, #6); 50 adults collected on buoys (Balcer 1994)
17 June 1991	one 2.3-cm mussel found attached to a native clam by an angler in the St. Louis River, 6 miles upriver from Lake Superior (ZM Update #9)
1991	veligers found in 4 of 33 harbor samples and 1 of 15 water intake samples, and post-veligers found on 5 of 33 substrate samplers (ZM Updates #10, #11); 151 adults collected on buoys (Balcer 1994)
1992	no veligers collected (Balcer 1994); 1 post-veliger found on 1 of 94 substrate samplers (ZM Update #15); 2 adults found on buoys (Balcer 1994)
1993	no veligers taken in 46 plankton tows, 1 post-veliger found on 1 of 110 substrate samplers in 1993 (ZM Update #19)
Nov 1993	low densities (<0.3/sq m) of mussels observed by divers on breakwalls and pilings, and moderate densities (≈50/sq m near surface to >130/sq m at 8 m depth) near an ore dock in an area that receives large amounts of ballast water from the lower lakes (ZM Update #19; Balcer 1994)
1994	weekly plankton samples and biweekly examination of substrate samplers found veligers on only 2 dates, in very small numbers (Balcer 1996a)
1995	weekly plankton samples and biweekly examination of substrate samplers found veligers on only 5 dates, in very small numbers (Balcer 1996a)
1993-1997	studies during 1993-1996 found 3 "colonies" in harbor in areas with "constant reintroductions via ballast water...[from] lower Great Lakes," but gonads not developing in adults (Balcer 1996a); otherwise, few veligers, post-veligers or adults found (ZM Update #22; Jensen, pers. comm. 2001; Balcer, pers. comm. 2001)
1998-2000	mussels increasingly common and widespread and apparently well established (Jensen, pers. comm. 2001; Balcer, pers. comm. 2001); mussels collected from the harbor in Nov. 1998 and kept in cages in the harbor had developing gonads in June 2000 and had apparently spawned by fall 2000 (Balcer, pers. comm. 2001)

western tip of the lake (Balcer 1996a), with the higher levels apparently due to an influx of calcium from the St. Louis River (Balcer, pers. comm. 2001; Jensen, pers. comm. 2001).

Zebra mussels were first found in the lake in Nov 1989 as adults on navigation buoys in Duluth-Superior Harbor (Balcer 1994). Small numbers of veligers and adult mussels were found in the harbor in the following years. Then, starting in 1998, mussels have become much more common and are apparently now well-established in the harbor (Table 5). This increase in zebra mussel abundance coincides with a warming period, though whether the increase is due to shorter winters, higher summer water temperatures, associated improvements in food resources (e.g. earlier or stronger phytoplankton blooms), or other factors is not clear. Calcium measurements are apparently not available for this period.

Large quantities of ballast water are discharged into Lake Superior each year, most of it originating from the lower Great Lakes. For example, in 1995 Lake Superior received about 2.1 million metric tons of ballast water, which is 62% of the total amount of ballast water discharged into the Great Lakes that year. Over a million metric tons were discharged into Duluth-Superior Harbor alone, of which over 81% came from the lower lakes (Aquatic Sciences 1996). Zebra mussels have also been found on the hulls and in the seachests of ships arriving in Lake Superior from the lower Great Lakes (ZM Update #2; ZM Map 03/10/1991). It thus seems possible that the mussels found in Duluth-Superior Harbor prior to 1998 resulted from the periodic arrival of veligers in ships' ballast or adult mussels that were scraped off or that detached from ships' hulls, rather than from local reproduction (Nichols, pers. comm. 1998; Balcer, pers. comm. 1998). Mussel populations present since 1998 may be in part supported by the ongoing transport of large numbers of mussels and veligers from the lower lakes.

There are a few zebra mussel records at other sites in Lake Superior (Table 6), but these apparently represent one-time occurrences rather than established populations (Nalepa, pers. comm. 1998; Padilla, pers. comm. 1998; Nichols, pers. comm. 2001; Jensen, pers. comm. 2001).

Table 6. Zebra mussel records elsewhere in Lake Superior

Site	Date	Record (References)
Thunder Bay, ON	1990	on a boat or barge hull (ZM Map 03/10/91; Jensen, pers. comm . 2001)
Sault Ste. Marie, ON	1990	collected by USACE (ZM Map 2/14/92; Jensen, pers. comm. 20 01)
Batchawana Bay, ON	1991	(ZM Map 10/15/92; Jensen, pers. comm. 2001)
Cape Gargantua, ON	1991	(O'Neill & Dextrase 1994; ZM Map 12/14/95; Jensen, pers. co mm. 2001)
Marquette, MI	1992	one 1-mm mussel found on substrate sampler at Presque Isle P ower Plant on Nov. 11, 1992 (ZM Update #15; Benson 1998)
Two Harbors, MN	1993	small cluster collected by USFWS or USGS in shipping chan nel near ore dock (Benson 1998; Jensen, pers. comm. 2001)
mouth of Ontonagan River, MI	1997	1 adult collected by USFWS or USGS (ZM Map 01/15/98; Be nson 1998; Jensen, pers. comm. 2001)
Chequamegon Bay, WI	1998	in the Fish Creek estuary (Jensen, pers. comm. 2001)

Hudson River

The Hudson River flows south from near Warrensburg, New York to enter the Atlantic Ocean at Manhattan Island, and the Mohawk River flows eastward into the Hudson River at Troy [at RKM 248[5]]. The Erie Canal links Lake Erie and Lake Ontario (through the Oswego Canal) to the upper Mohawk and the Hudson River. From April through June, the Mohawk River contributes one-quarter to one-half of the Hudson's flow at Troy. Below Troy the Hudson River is tidal with a mean tide range of about 1.4 m, but usually remains fresh downstream as far as Peekskill [RKM 75] (Stedfast 1982). Calcium concentrations range from 12 to 60 mg/l in the Mohawk River, 4-25 mg/l in the upper Hudson River, and 12-38 mg/l in the lower Hudson River below the mouth of the Mohawk River (Strayer *et al.* 1996; Mellina & Rasmussen 1994; STORET) (Table 7).

Table 7. Calcium records in the Hudson River downstream of Troy

Site	Dates	n	Mean (mg/l)	Range (mg/l)	Reference
Mohawk River	1985-97	300	34	12-60	STORET
Upper Hudson River	1983-97	285	10	4-25	STORET
Lower Hudson River:					
Troy to Newburgh	–	–	–	22-30	Strayer *et al.* 1996
Green Island	1982-94	45	23	12-30	STORET
Glenmont	1985-97	66	23	17-34	STORET
Catskill	1985	3	25	19-35	STORET
Catskill to New Hamburg	July 1992	11	24	22-26	Mellina & Rasmussen 1994
Poughkeepsie	1988-97	72	24	16-38	STORET

Zebra mussels were first found in the Hudson River in May 1991 near the town of Catskill [RKM 182], about 66 km downstream from Troy (Strayer *et al.* 1996). From August to the end of 1991, young-of-the-year mussels settled at numerous sites from Catskill to 80 km downstream at Newburgh [RKM 99], but only one mussel was found in the Hudson River more than 5 km upstream of Catskill prior to 1992 (Strayer & Powell 1992; Strayer *et al.* 1996). In 1992 zebra mussels were found downstream to Haverstraw [RKM 65], where increasing salinities apparently block any further downstream spread (Strayer *et al.* 1996). No zebra mussels have been collected in the Hudson River above Troy, where reported calcium concentrations are below 15 mg/l.

Based on the size of the population at Catskill, Strayer *et al.* (1996) estimated that it became established in 1989 or 1990. They interpreted this as indicating that zebra mussels were introduced to the Hudson River at Catskill by anthropogenic transport (such as mussels attached to the hulls of barges or trailered boats), rather than carried downstream as drifting larvae. They also concluded that the larvae that settled downstream of Catskill in the later part of 1991 were primarily from mussels near Catskill, while the larvae that settled upstream of Catskill after 1991 came from mussels in the Mohawk River.

We can examine the pattern of zebra mussel settlement in the Hudson in terms of distances and flow times downstream from possible source populations established in higher calcium waters upstream. The highest point on the Erie Canal is at Rome, where water enters from the Mohawk River, with water usually flowing toward the Great Lakes west of this point, and toward the Hudson

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5 "RKM 248" = 248 kilometers upriver from the mouth of the Hudson at the Battery in Manhattan.

River east of this point. However, flow directions and velocities may vary at least locally with the opening and emptying of locks, and the western portion of the canal at least as far east as Rochester is partially emptied in the winter and refilled with Great Lakes water in the spring, so that flows during refilling are from west to east (ZM Update #5; Sowinski, pers. comm. 2001). The normal, westerly flows in the western section are low, typically 0.01 to 0.1 m/s, while easterly flows in the eastern section may be much higher in the spring (Sowinski, pers. comm. 2001). Even at only 0.1 m/s the distance from Rome to Troy (Table 8) would be covered in 22 days, and presumably in much less time during high spring flows.

Table 8. Distances in the Erie Canal-Mohawk River-Hudson River system

Eastern end of Lake Erie to Rochester	129 km
Rochester to Rome	233 km
Rome to Troy	188 km
Troy to Catskill	66 km
Catskill to Haverstraw	117 km

For the lower Hudson River below Troy, we calculated residence times based on mean montly flows for 1989, 1990 and 1991, and on the long term means (1947-97) (Table 9; see Appendix 1 for an explanation of the calculations). Flows in the lower Hudson were generally above the long term means from May 1989 to March 1991. Residence times from Troy to Kingston, which is about 30 km below Catskill, ranged from 4 days (in May 1990) to 26 days (in July and September 1990) during 1989-90 when Strayer *et al*. (1996) estimated zebra mussels first became established at Catskill. Residence times from Troy to Clinton Point, which is about 15 km above Newburgh, ranged from 21 to 59 days during August-December 1991 when mussels first settled between Catskill and Newburgh. Residence times from Troy to Peekskill, which is about 10 km above the downstream range limit for zebra mussels at Haverstraw, range on average from 13 to 71 days.

Table 9. Residence times in the lower Hudson River

Residence time in days, based on mean monthly flows. See Appendix 1 for calculations.

From Troy to...	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
...Kingston [RKM 150]												
1989	21.3	16.2	12.1	6.1	5.4	7.9	18.3	21.4	16.6	10.1	6.8	13.8
1990	10.8	5.9	4.2	4.6	4.2	16.6	26.4	16.8	26.1	8.6	6.3	6.0
1991	8.1	8.3	6.0	6.3	11.9	28.4	36.4	32.9	29.9	19.2	13.7	11.4
1947-97	10.8	10.4	6.6	4.8	7.7	14.6	21.8	25.2	22.7	16.2	11.2	9.7
...Clinton Point [RKM 113]												
1989	38.4	29.2	21.9	11.0	9.8	14.3	33.1	38.5	29.9	18.2	12.3	24.9
1990	19.5	10.6	7.5	8.4	7.5	29.9	47.5	30.2	47.1	15.5	11.4	10.8
1991	14.6	15.0	10.7	11.4	21.5	51.2	65.5	59.3	53.8	34.6	24.8	20.5
1947-97	19.5	18.7	11.9	8.6	13.8	26.3	39.2	45.3	40.8	29.3	20.1	17.5
...Peekskill [RKM 75]												
1989	60.0	45.6	34.2	17.2	15.2	22.3	51.6	60.1	46.7	28.5	19.3	38.9
1990	30.5	16.6	11.7	13.0	11.8	46.6	74.2	47.1	73.5	24.3	17.8	16.9
1991	22.7	23.5	16.7	17.8	33.6	80.0	102.3	92.6	84.0	54.0	38.7	32.1
1947-97	30.4	29.2	18.6	13.4	21.6	41.0	61.2	70.8	63.8	45.7	31.4	27.3

Zebra mussel larvae are planktonic for up to a month or more (Table 1), and juvenile zebra mussels can detach and drift in the planton for unknown periods, Thus, if a zebra mussel population were established in the Erie Canal or Mohawk River anywhere east of Rome by 1989 or 1990, then larvae or juveniles drifitng downstream could have produced the initial settlement of zebra mussels at Catskill; and a population in the Erie Canal or Mohawk River could account for zebra mussel's subsequent presence in the Hudson River from Troy to Haverstraw. There are records of zebra mussels established in eastern Lake Erie by the summer of 1989, in the Erie Canal from the Niagara River to east of Rochester by the summer of 1990 (ZM Updates #4, #5; Griffiths *et al*. 1991), and in the lower Mohawk River to its confluence with the Hudson by February 1991 (Griffiths *et al*. 1993; O'Neill & Dextrase 1994).

However, taken all-in-all, the patterns of zebra mussel distribution in the lower Hudson River strongly suggest that substantial reproduction of zebra mussels has occurred within the river itself. These patterns include the zebra mussel's estimated initial appearance at Catskill prior to any record of zebra mussels east of Rome, and the common occurrence of veligers in the river in the summer and early fall (Strayer *et al*. 1996) when relatively high temperatures (typically 17-25° C [Limburg 1996]) would make development times short and low flows would make travel times from upstream populations long.

St. Lawrence River

The St. Lawrence River drains the eastern end of Lake Ontario (the easternmost of the Great Lakes) and flows northeastward along the New York-Ontario border and across Quebec. The Ottawa River flows east-southeast along the Ontario-Quebec border and enters the St. Lawrence just upstream of Montreal. Above this junction, the St. Lawrence River carries relatively high calcium water from Lake Ontario. For 180 km below the junction, low calcium water from the Ottawa River flows along the north shore of the St. Lawrence, while higher calcium Lake Ontario water flows along the south shore (Mellina & Rasmussen 1994).

Zebra mussels were found in eastern Lake Ontario and the upper St. Lawrence River to above Montreal in 1990 (O'Neill & Dextrase 1994). In 1991-92, Mellina & Rasmussen (1994) collected zebra mussels downstream of Montreal as far as Ile d'Orléans near Quebec. They found mussels at all collecting sites on the south shore of the river, where calcium levels ranged from 16 to 38 mg/l, and collected no zebra mussels at 11 north shore sites, with calcium levels ranging from 8 to 14 mg/l. They concluded that 15 mg/l is the calcium threshold for zebra mussels.

Ile d'Orléans is about 250 km below Montreal and about 500 km downstream from eastern Lake Ontario. Since the average water velocity in the river is 1-5 m/s (Rasmussen, pers. comm. 1998), or 86-430 km/day, zebra mussel populations in either Lake Ontario or the St. Lawrence River above Montreal could be the source of all larvae settling at sites below Montreal.[6] There has been no investigation of whether reproduction is occurring at the St. Lawrence River sites with calcium levels as low as 16 mg/l (Rasmussen, pers. comm. 1998). Thus, the discovery of zebra mussels at

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6 De Lafontaine *et al*. 1995, cited in De Lafontaine & Cusson 1997, apparently reach ed this same conclusion. On the other hand, Lapierre *et al*. (1993) and Lapierre and F ontaine (1993) argued that their finding a higher proportion of recruitment onto buo ys consisting of juveniles (<6 mm long) rather than "translocators" (>10 mm long) at more downstream buoys, and their finding higher larval densities downstream, indi cated that zebra mussels were reproducing locally (i.e. near Quebec City) in the St. L awrence River.

these sites only indicates that settlement and adult growth are possible at these calcium levels, not that reproduction or embryonic and larval development are.

Furthermore, the absence of zebra mussels from the low calcium, north shore sites may not even be evidence of a calcium threshold for settlement and adult growth. Since water from the Ottawa River dominates the north shore flow and there are no known zebra mussel populations in the Ottawa River, there may be few or no zebra mussel larvae arriving at the north shore sites.

Furthermore, the absence of zebra mussels from the low calcium, north shore sites may not even be evidence of a calcium threshold for settlement and adult growth. Since water from the Ottawa River dominates the north shore flow and there are no known zebra mussel populations in the Ottawa River, there are likely far fewer zebra mussel larvae delivered to north shore than to south shore sites, which alone may explain the collection of zebra mussels on the south shore but not the north shore.

Lake Champlain and the Richelieu River

Lake Champlain is a long narrow lake oriented north-south along the border between New York and Vermont. Much of the lake lies on a narrow belt of Cambrian and Ordovician limestones (Brooks & Deevey 1963). Water in the lake drains northward into the Richelieu River, which flows into the St. Lawrence River at Quebec. Calcium concentrations generally decline northwards, from average values of 25-31 mg/l (with a range of 13-60 mg/l) at the south end of the lake, to 14-20 mg/l (range of 8-47 mg/l) in the central and northern parts, and 16-18 mg/l in the Richelieu River (Vermont DEC 1996, 1997, 1998; De Lafontaine & Cusson 1997).

The first zebra mussels were found in the lake in 1993 at its extreme southern end (Eliopoulos & Stangel 1997). The mussels may have been introduced by boat traffic from the Great Lakes and Erie Canal, or from the lower Hudson River (including commercial tour boats from New York City), reaching the lake through the Champlain Canal, or by boats trailered overland. The mussels spread northward through the lake and by the summer of 1996 veligers were found in the northeast arm of the lake and adults or settled juveniles had been found throughout the rest of the lake. Adults or settled juveniles had been found in many parts of the northeast arm by 1999 (Eliopoulos & Stangel 1997, 1998, 1999, 2000). Zebra mussel adults and veligers are generally most abundant in the southern end of the lake, are common in many parts of the lake where calcium concentrations are at least 18 mg/l, and have been found at two sites with median calcium concentrations of 13-14 mg/l in the northeast arm (Hauser, pers. comm. 1997; Vermont DEC 1998; Eliopoulos & Stangel 1997, 1998, 1999, 2000). In the Richelieu River, zebra mussels were first observed as larvae in June 1996 (De Lafontaine & Cusson 1997), and as juveniles and adults in 1997, primarily in the upper (southern) part of the river (Cusson & De Lafontaine 1998).

The general flow of water in the lake is from south to north, but flow velocities and retention times are not well understood, and there may be considerable temporal and spatial variation in the strength and direction of currents (Hauser, pers. comm. 2001). Larval production in the southern part of the lake is high (Eliopoulos & Stangel 1997, 1998, 1999, 2000; Hauser, pers. comm. 2001), and some observations suggest that larvae may be carried northward in pulses from a southern source. For example, in 1994 increasing veliger densities were observed in the southern portion of the lake in early August, followed by the first detection of veligers in the northern part of the lake in early September (Hauser 1994). In subsequent years when an extensive monitoring program was in place, veligers were first detected in the southern end of the lake around mid-May, in the central part of the lake 1-2 weeks later, and in the northernmost part of the lake another 2 weeks after that (Eliopoulos & Stangel 1998, 1999, 2000). On the other hand, the later initial collection of veligers in the northern part of the lake may simply be due to later warming of the water in the spring, or it may in part be an artifact of the sampling schedule (Eliopoulos, pers. comm. 2001).

From the north end of the lake, water flows down the Richelieu River to St. Ours, a distance of 110 km, in 8-25 days during the summer (based on river flows of 100-300 m^3/s and estimated water velocities of 0.05-0.15 m/s [De Lafontaine & Cusson 1997]). In 1996, veligers were first observed in the river at Fort Lennox (25 km downstream from Lake Champlain) in late June and at St. Ours (85 km further downstream) 10 days later. Maximum densities of 8,000/m^3 were reached at the upstream river site in mid-July, with maximum densities of 140/m^3 at the downstream site three days later (De Lafontaine & Cusson 1997).

Since zebra mussel larvae drift in the plankton for up to a month or more (Table 1), it may be that the mussels found in low calcium waters in northern Lake Champlain and the Richelieu River are recruited from reproducing populations in higher calcium waters at the southern end of the lake. The fact that a high proportion of larvae collected at the upstream site in the Richelieu River were in advanced stages of development suggests that they were not spawned locally (De Lafontaine & Cusson 1997). An overall decline in veliger densities by an order of magnitude from southern Lake Champlain to northern Lake Champlain, and by another order of magnitude to the northern part of the Richelieu River, is consistent with this scenario. However, Eliopoulos & Stangel (1998) argue that peak veliger densities at a site in the northern lake site that are higher than densities at central lake sites may indicate local reproduction in the northern lake.

Recruitment of larvae to northern sites could also be augmented by drifting juveniles (Oldham 1930; Martel 1993) or adults attached to boat hulls. Adults on boat hulls have been frequently observed in the lake (Hauser 1994), and during the summer many recreational boats travel from the lake down the Richelieu River (De Lafontaine & Cusson 1997).

Eliopoulos & Stangel (1999) found no significant relationship between ambient calcium concentrations in different parts of Lake Champlain and the zebra mussels' dry weight to length ratios, or between calcium concentrations and the ratio of the density of settled juveniles to the density of veligers; and found that the calcium content of zebra mussel shells was significant lower in areas with higher calcium concentrations. They interpret these findings as indicating that low calcium levels in the lake are not harmful to zebra mussels, but warn that there were few data from low calcium areas, and that some of the results could have been affected by the mussels' reproductive state which might vary with ambient calcium levels.

Zebra Mussel Occurrences in Inland, Low-Calcium Waters

The U.S. Geological Survey maintains a database of zebra mussel occurrences reported in North America. Amy Benson at the Survey's Florida Caribbean Science Center assembled records for all inland water bodies where zebra mussels had been reported, excluding the Great Lakes, the Mississippi River system, and the rivers and lakes discussed in the preceding sections. We extended and updated this list with additional information on zebra mussel records in Ontario, New York, Vermont, Connecticut and Minnesota (Table 10).

From STORET, the U.S. Environmental Protection Agency's clearinghouse of water quality data, we obtained all reported calcium data from 1980 until the present for surface waters in the states with reported zebra mussel occurrences in inland waters (listed in Table 10). These records contained the name and location of the water body, the sampling agency, the number of sampling events and the mean, high and low readings reported as total calcium, dissolved calcium or calcium carbonate. Few sites reported more than 10 sampling events. We extracted calcium data for water bodies with zebra mussel occurrences, supplemented with data from state agencies and other sources. Alan Dextrase of the Ontario Ministry of Natural Resources provided data on water bodies in Ontario.

Table 10. Summary of inland water bodies with zebra mussel occurrences

	Water bodies with reported occur rences	Number of these with calcium data	Number with mean calcium of <28 mg/l
Michigan	70	34	2
Ontario	37	17	6
Indiana	21	20	1
New York	17	14	1
Wisconsin	7	5	0
Ohio	7	5	0
Vermont	3	3	2
Connecticut	2	2	1
Illinois	1	0	0
Minnesota	1	0	0
Total	166	100	13

We obtained calcium data for 100 of the 166 inland water bodies with reported occurrences.[7] Thirteen lakes in Michigan, Ontario, Indiana, New York, Vermont and Connecticut reported mean calcium levels below 28 mg/l (Table 11). Where calcium data was not available, we assessed the likelihood of low calcium levels by checking the concentrations reported for other water bodies in the region and by checking geologic maps and descriptions for rock types that are notably rich or poor sources of calcium.

Connecticut

Most of Connecticut's waters are low in calcium, being underlain by Paleozoic igneous and metamorphic rocks that produce soft waters throughout most of New England (Brooks & Deevey 1963). The highest calcium concentrations are found in lakes and ponds in the northwest corner of the state (Jokinen 1983; Murray *et al.* 1993), on the southern end of a narrow belt of Paleozoic limestone that reaches north to Lake Champlain (Brooks & Deevey 1963). Moderate calcium concentrations have also been measured in the Housatonic River which drains this area, and in a few water bodies in the southern central portion of the state (Jokinen 1983; Murray *et al.* 1993), possibly related to a bed of Mesozoic rock running down the center of the state (Brooks & Deevey 1963).

Adult zebra mussels have been found only in East Twin Lake in the northwest corner of the state, where they were first observed in the summer of 1998 (Balcom pers. comm., 1998). The calcium concentration in East Twin Lake was measured at 35 mg/l (Jokinen 1983) and 24 mg/l (Murray *et al.* 1993, measured in the summer of 1992), which are nearly the highest reported for the state (the second highest of 180 statewide measurements by Jokinen 1983, and the second highest of 24 statewide measurements made by Murray and reported in Murray *et al.* 1993 (the value for Wononpakook Lake apparently being an error [Balcom, pers. comm. 2000]). East Twin Lake feeds directly into West Twin Lake, with 21 mg/l of calcium, where veligers were collected in 1999 or 2000 (Balcom pers. comm., 2001).

Illinois

We were unable to find calcium data for Lincoln Lake, the only inland water body in Illinois on the occurrence list. However, of the hundreds of Illinois sites reported in STORET, virtually all had

[7] In a few cases we used calcium data from connected or adjacent waters.

over 30 mg/l of calcium. Lincoln Lake is about 80 km southwest of Lake Michigan and within 10 km of the Illinois River, which averages about 60 mg/l calcium and is nearly always over 30 mg/l calcium.

Table 11. Inland zebra mussel occurrences in less than 28 mg/l of calcium

	Records and life st ages collected	Mean reported calcium mg/l	n	date	Comments (Data sources)
Devil Lake, ON	1995: veligers & ad ults	27	15	91	on Rideau Waterway (Hincks & Mackie 1997; Dextrase, pers . comm. 1998)
Buckhorn Lake, ON	1996	26	15	91	on Trent-Severn Waterway (Hincks & Mackie 1997; Dextr ase, pers. comm. 1998)
Dogwood Lake, IN	1998: veligers	26	4	91,96	(Indiana DNR 1998; Indiana CLP 1998)
Lake Opinicon, ON	1991: veligers & ad ults	25	?	?	on Rideau Waterway (Dextra se, pers. comm. 1998)
West Twin Lake, CT	1999: veligers	21	1	83	connected to East Twin lake (Murray *et al.* 1993; Balcom, pe rs. comm. 2001)
Houghton Lake, MI	1993: veligers	20	1	88	(Benson 1998; STORET 1998)
Balsam Lake, ON	1991: veligers & ad ults	20 25	15 ?	91 92	on Trent-Severn Waterway (Hincks & Mackie 1993, 1997; Dextrase, pers. comm. 1998)
Lake St. Helen, MI	1994: adults	18	1	88	(Benson 1998; STORET 1998)
Lake Bomoseen, VT	1999: adults & veli gers 2000: adults only	18	2	96,98	(Eliopoulos & Stangel 2000; H auser, pers. comm. 2001)
Lake George, NY	1996: few veligers at north end 2000: adults at sout h end	11	?	?	(Hansen *et al.* 1998) adults fou nd in area with elevated calci um (Nierzwicki-Bauer, pers. c omm. 2001)
Crotch Lake, ON	1995: veligers	11	?	?	(Dextrase, pers. comm. 1998)
Lake Muskoka, ON	1991: veligers	6 7	15 ?	91 92	(Hincks & Mackie 1993, 1997; Dextrase, pers. comm. 1998)
Lake Dunmore, VT	1999: veligers 2000: no adults or v eligers	4	4	96-00	(Eliopoulos & Stangel 2000; H auser, pers. comm. 2001)

Indiana

Zebra mussels have been reported in 20 Indiana lakes and the St. Joseph River. We obtained calcium carbonate data for all of the lakes, and in 19 of them the corresponding calcium concentrations range from 32 to 89 mg/l in the epilimnion and 45 to 100 mg/l in the hypolimnion.

In Dogwood Lake in southwestern Indiana, where veligers were collected in 1998, the corresponding mean calcium concentrations are 21 mg/l in the epilimnion and 31 mg/l in the hypolimnion (each based on 2 samples), with an overall mean of 26 mg/l. The St. Joseph River system has been described as a high-alkalinity system (Horvath *et al.* 1994).

Michigan

The surface waters of Michigan's lower peninsula and the eastern half of its upper peninsula are generally hard, reflecting the underlying geology dominated by calcium-rich organic, clay and glaciofluvial deposits. The western half of the upper peninsula is dominated by tills and bedrocks that produce lower-calcium surface waters (Kincarn, pers. comm. 1998; Farrand 1982). None of the 70 Michigan lakes on the zebra mussel occurrence list are located in this calcium-poor region.

We obtained calcium data for 34 of the lakes, of which 32 had mean reported calcium concentrations of 28 mg/l or higher. Houghton Lake and Lake St. Helen each have a single reported calcium measurement from 1988, of 20 and 18 mg/l respectively.

Minnesota

Most lakes in central and southern Minnesota are hardwater lakes underlain by calcareous glacial till, while many lakes in northeastern Minnesota are formed on non-calcareous bedrock and have lower calcium concentrations (Minnesota DNR 2001). Minnesota's only inland site with zebra mussels is Zumbro Lake in the southern part of the state, where two size classes of zebra mussels were found in 2000. We were unable to find calcium data for Zumbro Lake, but its mean alkalinity of 221 mg/l as $CaCO_3$ makes it a hardwater lake.

New York

Outside of the Great Lakes, the Erie Canal system, the Mohawk River, lower Hudson River and Lake Champlain (discussed above), zebra mussels have been reported in New York from 16 lakes and the Susquehanna River. Calcium levels in New York waters vary between geologic regions, with concentrations typically around 400 mg/l in the Limestone Belt region stretching east-to-west through the middle of the state, characterized by limestone bedrock and glacial till derived from limestone and alkaline shales; about 30-50 mg/l in the Lake Plain region to the north of the Limestone Belt and around lakes Erie, Ontario and Champlain, characterized by deposits of calcareous silts and clays derived from Paleozoic limestones and shales; about 30-50 mg/l in the Northern Appalachian Plateau in the southern part of the state, characterized by Paleozoic sandstones, shales and some limestone; and less than 5 mg/l in the Adirondack Mountain region, characterized by pre-Cambrian granites, schists and gneisses (Berg 1963).

We found calcium data for 14 of the 17 inland water bodies with zebra mussel records. All had mean calcium concentrations of 28 mg/l or higher except Lake George, which has a mean calcium concentration of 11 mg/l (Hansen *et al.* 1998). In 1995-1999 the lake was monitored for zebra mussel veligers, with a few veliger shells (probably <5 total) but no live veligers collected in 1995 and 1997 near the northern end of the lake. Then, starting in December 1999, over 20,000 adult mussels, but no veligers or newly settled juveniles, were collected in a 1,500 m^2 area at the southern end of the lake, where calcium concentrations were measured at 11-13 mg/l. A culvert emptying into the area carries groundwater and stormwater runoff with 40-50 mg/l of calcium, and a concrete boardwalk was constructed along the shore in 1998. The presence of large numbers of adult mussels in this small area might be explained if (1) adult mussels in spawning condition were brought into the lake attached to the equipment used to construct the boardwalk, or (2) there was a temporary, local increase in calcium levels sufficient to allow a successful spawn, caused by calcium inputs related to the boardwalk construction, increased calcium inputs from the culvert, or impoundment of the culvert's high calcium water behind a silt curtain deployed during construction (Nierzwicki-Bauer, pers. comm. 2001).

Ohio

The bedrock of northwestern Ohio is dominated by limestone, and the rest of the state by sandstone. It is a generally hard water state, with virtually all of the hundreds of sites reported in STORET having mean reported calcium concentrations of over 40 mg/l.

We found calcium data for four of the seven sites on the occurrence list, all of which have mean reported calcium levels of 39 mg\l or higher. For the three remaining sites, nearby waters had mean reported calcium levels of 66 mg/l or higher. Two of these sites are limestone quarries and are therefore likely to be high in calcium (Snyder, pers. comm. 1998).

Ontario

Outside of the Great Lakes, zebra mussels have been reported in Ontario in 33 lakes and four rivers. We obtained calcium data for 17 of the lakes (Hincks & Mackie 1997; Dextrase, pers. comm. 1998). In four lakes the mean reported calcium concentrations were between 20 and 28 mg/l, and in two lakes they were under 15 mg/l.

All 37 water bodies with zebra mussel records are in a triangular region in southeastern Ontario south of the Ottawa River, north of Lake Ontario, and east of Georgian Bay on Lake Huron. Several are on the Rideau Waterway, which connects Lake Ontario at Kingston to the Ottawa River at Ottawa. The waterway is 202 km long and takes 3-5 days to transit (Rideau Region Website). Most of the Rideau lakes region is underlain by Precambrian limestone, gneiss, quartzite, granulite, migmatite and granitic plutons. The limestone (really a coarsely crystalline calcite marble) is softer and has been more deeply eroded than the other rocks, and forms the basins for most of the large lakes in the region (Rideau Region Website). Mean reported calcium concentrations for the water bodies with zebra mussel records range from 25 to 34 mg/l (Hincks and Mackie 1997; Dextrase, pers. comm. 1998). Another group of records are from water bodies on the Trent-Severn Waterway, which connects Lake Ontario to Georgian Bay. The Trent-Severn Waterway is 386 km long and takes about a week to transit (Friends of the Trent-Severn Waterway Website). These water bodies have mean reported calcium concentrations ranging from 20 mg/l in Balsam Lake (the highest point of the waterway) to 48 mg/l (Hincks and Mackie 1997; Dextrase, pers. comm. 1998). In either of the Rideau or Trent-Severn waterways the transport of adults, including adults in spawning condition, on the hulls of vessels from Lake Ontario could confound reports of "established" populations.

Veligers but no adults have been reported in Crotch Lake, with a calcium concentration of 11 mg/l, located west of the Rideau Waterway. Veligers but no adults have also been reported in Lake Muskoka, with 6 mg/l, which drains into Georgian Bay, and in lakes Joseph and Rosseau which are connected to Lake Muskoka. Lake Muskoka and the two connected lakes are visited by large numbers of boats arriving overland from the Great Lakes (Dextrase, pers. comm. 2001). As reported in Table 2, Hincks and Mackie (1997) attempted to rear zebra mussels in water taken from several Ontario lakes including four of those with zebra mussel records and mean calcium concentrations under 28 mg/l. In Lake Muskoka water, all adult mussels died within 35 days. In water from Balsam, Buckhorn and Devil lakes, with mean calcium concentrations between 20 and 27 mg/l, most adults survived and a small number of veligers were produced over 70 days.

Vermont

Outside of Lake Champlain, adults and veligers were collected in Lake Bomoseen in the summer of 1999 and adults but no veligers in 2000. Veligers were collected in lakes Hortonia and Dunmore in

1999, but none were found in 2000 (Eliopoulos & Stangel 2000; Hauser, pers. comm. 2001). These three lakes are within 25 km of the south end of Lake Champlain, and lie on or near a narrow belt of Cambrian and Ordovician limestones that runs along the western border of New England (Brooks & Deevey 1963). The mean reported calcium concentrations are 32 mg/l for Lake Hortonia, 18 mg/l for Lake Bomoseen and 4 mg/l for Lake Dunmore (Vermont DEC 2001).

Wisconsin

Southeastern and western Wisconsin is dominated by calcium-rich carbonate rock (calcareous drift and underlying dolomites) and sandstone, and the lakes in these regions are generally classified as hard water. In contrast, the northeastern part of the state is covered by noncalcareous outwash deposits, with bedrock of pre-Cambrian granites, schists and quartzites, and the waters are soft with calcium concentrations typically under 10 mg/l (Frey 1963; Koutnik & Padilla 1994). Zebra mussels have been reported from seven lakes or ponds, all of them located in the southeastern, higher-calcium part of the state. We found calcium data for five of these, all of which have mean reported calcium concentrations of 36 mg/l or higher.

Review of Available Evidence Regarding Zebra Mussels' Calcium Threshold

The available studies and data do not allow an unambiguous determination of the calcium threshold needed for the establishment of zebra mussel populations. In part this is due to inherent complexities in the dynamics of establishment and the mussel's physiological response to its chemical environment, and to the variation in calcium concentrations within water bodies with depth, location, season and year. Nevertheless, it is clear from numerous studies that low calcium concentrations may prevent the establishment, reproduction, development, growth or survival of zebra mussels (e. g. Sprung 1987; Vinogradov *et al*. 1987; Ramcharan *et al*. 1992; Hincks & Mackie 1997; Baldwin *et al*. 1997). A few studies also indicate that zebra mussels' calcium requirements may vary with pH, with the calcium threshold increasing with decreasing pH (Ramcharan *et al*. 1992; Vinogradov *et al*. 1993; Nierzwicki-Bauer, pers. comm. 2001). The specific evidence from experiments and field distributions includes the following:

Laboratory experiments on reproduction and larval development

Hincks and Mackie (1994) reported that males did not release sperm unless calcium concentrations were above 15 mg/l. Lynn (pers. comm. 1998) reported over 50% success in fertilization and attaining first cleavage of eggs in water with 4-8 mg/l of calcium, with declining success below 4 mg/l, but with large variation within tests. Sprung (1987) found that eggs hatched healthy larvae at about 40% of the normal rate at 24 mg/l of calcium, and virtually no healthy larvae at 12 mg/l and below. Baldwin *et al*. (1997) found that rates of successful embryonic and larval development were similar in waters with 22 and 30 mg/l, but reported no success in waters with 4 mg/l and below. Hincks and Mackie (1997) found that veliger production was comparable to production in apparently suitable waters when adults were held experimentally in water at 33-48 mg/l of calcium, low at 20-27 mg/l of calcium, and zero at 8 mg/l of calcium or less. Nierzwicki-Bauer (pers. comm. 2001) found that veligers survived in water with 16.5 mg/l of calcium and appropriate pH, but not at 11 mg/l of calcium.

Laboratory experiments on juvenile and adult growth and survival

Baldwin *et al*. (1997) found high juvenile and adult mortality in water with 3 mg/l, and low mortality but substantial weight loss and poor condition in 4 mg/l. Vinogradov *et al*. (1987) found that adults lost calcium in water with less than 14 mg/l of calcium. Hansen (1998) found good survival but lower weight and some loss of shell material in newly-settled and adult mussels held in 12-14 mg/l compared to about 20 mg/l of calcium. Ram and Walker (1993) and Dietz *et al*. (1994) found that adults could survive in water without calcium for at least 14 days and at least 51 days, respectively, though each concluded that the mussels mobilized calcium from their shells or tissue to maintain blood calcium levels. Hincks and Mackie (1993) reported normal (compared to Great Lakes populations) juvenile growth rates in water with 25 or 44 mg/l, but growth rates about one-tenth of normal in water with 7 mg/l. Hincks and Mackie (1997) reported zero or negative juvenile growth rates and 100% adult mortality in waters with 8 mg/l or less, and low juvenile growth rates (compared to rates measured in the field) and high adult survival (81-95%) in waters with 20-27 mg/l.

Evidence from zebra mussel distributions

As discussed above, the zebra mussel populations reported in waters with calcium levels below 25-28 mg/l in the St. Lawrence River, Lake Champlain and the Richelieu River could be result from recruitment of veligers carried downstream from populations established in higher calcium waters upstream, possibly augmented by downstream transport of drifting juveniles, rather than *in situ* reproduction. Although populations in the lower Hudson River are at least in part due to recruitment of larvae or drifting juveniles from upstream populations in the Mohawk River or Erie Canal, there is good evidence that substantial reproduction has occurred within the lower Hudson in at least some years. Mean reported calcium concentrations in the lower Hudson are 23-24 mg/l, with a range of 12-38 mg/l (Table 7). Zebra mussels were regularly though sparsely collected since 1989 in Duluth-Superior Harbor at the western end of Lake Superior, which has calcium concentrations that ranged from 13 to 23 mg/l in 1994-95. The relatively small numbers collected through 1997 could possibly have resulted from the regular transport of large numbers of veligers (in ballast water) or adults (as hull foulers) by ships from the lower Great Lakes. Since 1998 zebra mussels have become much more abundant and are probably established in the harbor, but we have no calcium data for this period. The few records of zebra mussels at other sites in Lake Superior (Table 6), which has calcium concentrations of 12-15 mg/l in open lake waters, apparently do not represent established populations.

In an inland water body that is not downcurrent of other zebra mussel populations, we would normally expect the collection of veligers to indicate the presence of reproducing mussels in that water body (Marangelo & Johnson 1993), since the relatively small number of individual veligers that could be carried in by human transport (such as in the bait wells of boats) would be unlikely to be subsequently collected by researchers. On the other hand, the initial collections of veligers in inland Michigan lakes recorded very low densities, with many appearing to be "either dead or in very poor condition" (Marangelo & Johnson 1993); in some inland waters only veliger shells were found (Marangelo, Carlton & Johnson 1994); and in the Susquehanna River veligers were collected for a few years but substantial later sampling found no adults (Harman 1996, Oleson 1998). These data suggest that even in water bodies where veligers have been collected (probably indicating that reproduction is at least occasionally successful), conditions may not be appropriate for sustaining a population. A further complication is that in some cases ostracods can be easily mistaken for zebra mussel veligers, even by experts; and where reports are based on only one or a few veligers, the possibility of cross-contamination from other sampling sites must be considered (Johnson, pers. comm. 2001).

In our review we found 13 inland lakes with records of zebra mussels that also had mean reported calcium concentrations below 28 mg/l, with eleven of them reporting veligers (Table 11). Seven of these lakes have mean calcium concentrations between 20 and 28 mg/l. Of these, four are connected to the Great Lakes by canals, and one is directly downstream of another lake with a zebra mussel population and higher reported calcium concentrations. Lake St. Helen in Michigan and Lake Bomoseen in Vermont both have mean reported calcium of 18 mg/l. The mean calcium concentration in Lake George, New York is 11 mg/l (Hansen *et al*. 1998), but less than 5 veligers were reported from the lake in 1995-97, with none seen since then despite significant sampling effort; and the adult mussels found in 1999-2001 appear to be growing in an area with enhanced calcium levels, and do not appear to be reproducing (Nierzwicki-Bauer, pers. comm. 2001). Veligers were reported from Crotch Lake and Lake Muskoka in Ontario and Lake Dunmore in Vermont, with mean reported calcium concentrations of 11, 6-7 and 4 mg/l respectively, and in two lakes connected to Lake Muskoka which may have similar low calcium concentrations (Dextrase, pers. comm. 1998). For several of these lakes, the reported mean calcium is based on only a few (1-4) measurements.

Table 12. Summary of evidence regarding zebra mussels' calcium threshold

Calcium level	Evidence
>28 mg/l	Many abundant, reproducing populations are established at these calcium levels. In two studies of large numbers of European lakes, zebra mussels were only found in lake s with more than 25 or 28 mg/l of calcium.
20-28 mg/l	Experiments indicate good adult survival; and embryonic, larval and juvenile devel opment and growth rates comparable to those in higher calcium waters. Zebra musse l adults have apparently been established in Duluth-Superior Harbor since 1998, w here calcium was measured at 13-23 mg/l in 1994-95, and mussels kept in cages in the harbor since 1968 had normal gonad development. The population in the harbor may be in part supported by regular inputs of veligers or adults via ships from the lower Great Lakes. Large populations are present and reproduction has apparently occurre d in the lower Hudson River where mean calcium concentrations are 23-25 mg/l, alth ough calcium concentrations from 12-38 have been recorded and the concentrations at the sites and times where reproduction occurred are not known; and the large popula tions could be due in part to recruitment of larvae or juveniles from upstream. Zebra mussel veligers or adults have been reported from seven inland lakes with mean calc ium levels of 20-27 mg/l; for at least a few of these the records are probably due to v eligers drifting in from upstream or individuals introduced via boats.
15-20 mg/l	There is little experimental evidence or field data regarding threshold limits to zeb ra mussel reproduction or establishment within this calcium range. Zebra mussels we re reported from two inland lakes with mean reported calcium of 18 mg/l, based on v ery few measurements.
<15 mg/l	Some experiments found good adult survival down to 0 or 4 mg/l, while another repo rted no survival at 8 mg/l. Two studies reported loss of calcium or shell at ≤14 mg/l; and survival at low calcium levels may in part be at the cost of mobilizing calcium fr om shell or tissues. Weight loss in juveniles or adults was reported in waters up to 8 mg/l, and depressed growth rates in waters of 12-14 mg/l. One experiment found 50% success in fertilization and first cleavage at 4-8 mg/l, but other experiments found no release of sperm and poor or no larval production at concentrations up to 15 mg/l. Zeb ra mussels have been reported in the northeast arm of Lake Champlain at sites with 13-14 mg/l, and in four inland lakes with mean reported calcium of 4-11 mg/l, but it is not clear that these are established populations or if the reported calcium measur ements reflect typical concentrations at these sites.

In summary, it is clear that abundant reproducing populations can become established at calcium concentrations of about 28 mg/l and above. There are a few examples of apparently independent zebra mussel populations (i. e. reproducing in place and not entirely due to recruitment from upstream) in inland waters with mean calcium concentrations of 20-28 mg/l. There is a paucity of experimental or distribution data in the 15-20 mg/l range. Experimental data suggest that populations cannot be sustained where calcium levels are below 15 mg/l, although there a few reports of zebra mussel veligers from inland lakes with calcium measurements in this range, and adults in Lake George in a small area with possibly higher calcium than the levels usually reported for the lake.

A more precise assessment of zebra mussels' calcium threshold would be possible through:

- further laboratory or field experimental studies of zebra mussels' responses to low ambient calcium concentrations during reproductive and early larval development stages, or other experiments to investigate the effect of different ambient calcium concentrations on zebra mussel life stages;
- re-examination of zebra mussel records, particularly those based on collection of veligers at low-calcium sites;
- more thorough population sampling and physiological/histological examinations to determine whether zebra mussels reported from low calcium waters are in fact established and reproducing; and
- obtaining more complete data on the temporal/spatial range and variation in calcium concentrations in those apparently low calcium waters where zebra mussels have been reported.

Implications for Estimates of Potential Distribution

We identified 12 studies that estimate the potential ranges of zebra mussels in various parts of North America (Table 13). To assess the importance of the value used for calcium threshold in these studies, we re-analyzed the studies that provided adequate data using a range of calcium thresholds.

Strayer (1991) analyzed the distribution of zebra mussels in Europe relative to climate variables, and estimated and mapped their minimum potential range in North America based on mean annual air temperatures (between 0 and 18 °C) and extreme monthly mean air temperatures (extreme high and low between $^{-}15$ and 27 °C). The estimated range includes most of southern Canada and the continental United States, except Alaska and parts of the southeast and southwest, although Strayer noted that in parts of this range calcium levels may be too low to support zebra mussels.

Neary and Leach (1992) mapped zebra mussels' potential range in Ontario using as criteria the calcium concentrations (using measured concentrations or estimates made from soil and bedrock classifications) and pH needed for larval survival based on Sprung (1987) (Table 14), and combined these with information on boat access to map the probability of invasion. Tammi *et al.* (1995) applied the calcium and pH criteria from Neary and Leach (1992) to Rhode Island waters (Table 14) and estimated the invasion potential to be unlikely at 93% of sites, possible at 7% of sites and probable at none.[8]

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8 Tammi *et al.* (1995) stated that 52 lakes and ponds and five rivers were assessed, but only reported ratings for 51 lakes and ponds. The figures here and in the re-anal ysis below are based on the sites for which they reported ratings.

Table 13. Analyses of potential zebra mussel distribution

Region analyzed	Analysis
North America (Strayer 1991)	Developed minimum estimates of potential range based on (a) mean ann ual air temperature and (b) extreme monthly mean air temperature.
Ontario (Neary & Leach 1992)	Estimated likelihood of larval survival based on calcium and pH (Table 14).
Rhode Island (Tammi *et al.* 1995)	Estimated invasion potential based on calcium and pH (Table 14).
Connecticut (Murray *et al.* 1993)	Estimated likelihood of invasion success based on calcium (Table 15).
Virginia (Baker *et al.* 1993, Baker & Baker 1993)	Estimated susceptibility to invasion based on calcium and pH.
North and South Carolina (Duke Power 1995)	Estimated potential for infestation based on calcium, pH, turbidity and *Corbicula* abundance.
North Carolina (Doll 1997)	Estimated suitability with regard to calcium, pH, temperature, dissolve d oxygen and salinity (Table 16).
California (Cohen & Wein stein 1998a, b)	Estimated colonization potential based on calcium, pH, temperature, dis solved oxygen and salinity (Table 17a, b).
Manitoba (Sorba & Williamson 1997)	Estimated colonization potential based on calcium, total hardness, pH, t emperature, dissolved oxygen, conductivity and turbidity (Table 18).
United States (Ashby *et al.* 1998)	Estimated potential for infestation based on alkalinity, pH, temperatur e and dissolved oxygen.
Wisconsin (Koutnik & Padilla 1994)	Used 3 models to estimate presence/absence, categorical density (low or high) and numerical density as a function of calcium, pH, nitrate and ph osphate.
Florida (Hayward & Estevez 1997)	Estimated habitat suitability based on calcium, pH, temperature, dissol ved oxygen, salinity, turbidity and sediment size.

Table 14. Criteria for Neary & Leach (1992) analysis of Ontario and Tammi *et a l.* (1993) analysis of Rhode Island

		– – – –	Larval survival	– – – –
	Units	Unlikely	Possible	Probable
calcium	mg/l	any	12-20	>20
pH		<7.4	≥7.4	≥7.4

Murray *et al*. (1993) used calcium criteria similar to those in Neary and Leach (1992) to classify lakes, ponds and rivers in Connecticut (Table 15), and found successful invasion to be possible at 19% of sites and probable at 8% of sites.[9] Baker *et al*. (1993), using different critical levels for pH

9

These figures and those in the re-analysis below are based on the calcium data in the appendices in Murray *et al.* (1993), except that the value for Wononpakook Lake was assumed to be 18 mg/l (as given in Table 4) rather than 38 mg/l (the value in t and calcium (based on maximum reported monthly means for May-September), characterized 24% of selected Virginia lakes and river systems as having low susceptibility to invasion, 28% as having moderate susceptibility, and 43% as having high susceptibility.[10] They defined low susceptibility as conditions where zebra mussels are unlikely to reproduced successfully; and moderate and high susceptibility as indicating the likelihood of attaining dense, pest-level populations.

Table 15. Criteria for Murray *et al.* (1993) analysis of Connecticut

		– – – – Invasion Success – – – –		
	Units	Unlikely	Possible	Probable
calcium	mg/l	<12	12-20	>20

Duke Power (1995) used calcium concentration, pH, turbidity (secchi disk depth) and the abundance of another exotic clam, *Corbicula fluminea*, to assess the zebra mussel infestation potential at 16 water bodies in its service area in North and South Carolina, finding infestation unlikely at 19% of sites, possible at 44% of sites and probable at 37% of sites. Doll (1997) estimated the suitability of North Carolina waters for zebra mussel colonization in terms of calcium concentration, pH, temperature (based on mean summer temperature), dissolved oxygen concentration and salinity (Table 16); but did not combine the individual rankings for these five factors into an overall ranking for each site.

Table 16. Criteria for Doll (1997) analysis of North Carolina

		Suitability with regard to each variable		
	Units	Unlikely	Maybe	Definite
calcium	mg/l	<9	9-15	>15
pH		<6.8 or >9.5	6.8-7.4 or 8.7-9.5	7.4-8.7
temperature	°C	<15 or >32	31-32	15-31
dissolved oxygen	mg/l	<4	4-8	>8
salinity	mg/l	>10	5-10	<5

Temperature criteria based on average summer temperature.

Cohen and Weinstein (1998a, b) used data on the same five factors used by Doll (1997) to assess California waters (employing mean April-September data), but combined them to develop overall rankings of low-or-no colonization potential at 54% of sites, moderate potential at 2% of sites and high potential at 44% of sites (Tables 17a and b). Sorba and Williamson (1997) used seven factors—calcium, total hardness (as $CaCO_3$), pH, temperature (mean June-September data), dissolved oxygen, conductivity and turbidity (secchi disk depth)—to assess Manitoba waters (Table 18). They estimated overall rankings based on the lowest potential for any single factor, finding

he appendices), the latter apparently being in error (Balcom, pers. comm. 2000). The discussion and tables in the body of Murray *et al.* (1993) suggest lower percentages, about 12% possible and 5% probable.

10 Although the text in Baker *et al.* (1993) and Baker & Baker (1995) ranks the Matta poni/Pamunkey river system as moderate in susceptibility, Table 1 in Baker *et al.* (1 993) and the calcium values given in that paper indicate a ranking of low susceptibilit y, which we used here and in the re-analysis below.

very low colonization potential at 34% of sites, low potential at 19%, moderate potential at 22% and high potential at 25%. Ashby *et al*. (1998) evaluated the potential for zebra mussel infestation at 453 U.S. Army Corps of Engineers projects across the U.S., based on temperature, pH, dissolved oxygen and alkalinity, and concluded that more than half the sites have suitable water quality for zebra mussels.

Table 17a. Criteria for Cohen & Weinstein (1998a, b) analysis of California: indiv idual factor ranking

		– – – Colonization potential – – –		
	Units	Low-to-no	Moderate	High
calcium	mg/l	<15	15-25	>25
pH		<7.3 or >9.0	7.3-7.5 or 8.7-9.0	7.5-8.7
temperature:				
mean summer	°C	–	0-15	15-31
maximum	°C	<10 or >31	10-31	10-31
dissolved oxygen	mg/l	<4	4-8	>8
salinity	mg/l	>10	5-10	<5

Table 17b. Criteria for Cohen & Weinstein (1998a, b) analysis of California: overall ranking

<table>
<tr><th colspan="5">Individual factor ranking</th><th rowspan="2">Overall ran king</th></tr>
<tr><th>Calcium</th><th>pH</th><th>Temperature</th><th>Dissolved oxy gen</th><th>Salinity</th></tr>
<tr><td colspan="2">at least one ranked as High and neither ranked as Low-to-no</td><td colspan="3">each factor ranked as High or Moderate</td><td>High</td></tr>
<tr><td colspan="2">both factors ranked as Moderate</td><td colspan="3">each factor ranked as High or Moderate</td><td>Moderate</td></tr>
<tr><td colspan="5">at least one factor ranked as Low-to-no</td><td>Low-to-no</td></tr>
</table>

Table 18. Criteria for Sorba & Williamson (1997) analysis of Manitoba

The colonization potential for a site is equal to the lowest potential for an individual variable.

		– Colonization potential with regard to each variable –			
	Units	Very Low	Low	Moderate	High
calcium	mg/l	<9	9-20	20-25	≥25
total hardness	mg/l	<25	25-45	45-90	≥90
pH		<6.5	6.5-7.2	7.2-7.5 or 8.7-9.0	7.5-8.7
temperature	°C	<10 or >30	10-20 or 28-30	16-18 or 25-28	18-25
dissolved oxygen	mg/l	<4	4-6	6-8	≥8
conductivity	μS/cm	<22	22-36	37-82	≥83
secchi depth	cm	<10 or >250	10-20 or 200-250	20-40	40-200

Temperature criteria based on June-September average.

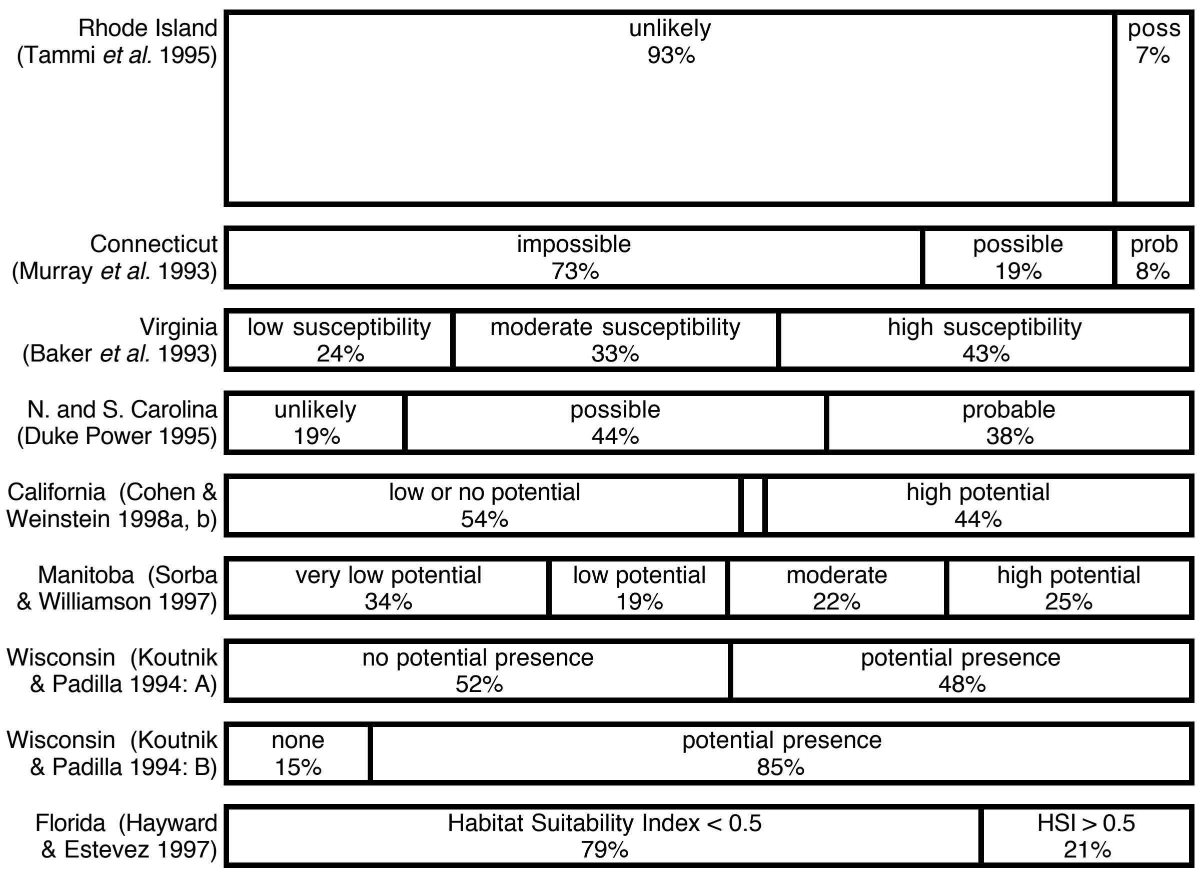
Koutnik and Padilla (1994) estimated potential distribution and abundance in Wisconsin lakes using three models that Ramcharan *et al*. (1992) derived from European lake data. The occurrence model (derived from a discriminant function analysis using pH and calcium concentration) predicts potential presence in 48% of lakes. The density (categorical density) model (derived from a discriminant function analysis using pH and calcium, nitrate and phosphate concentrations) and the abundance (numerical density) model (derived from a multiple regression using pH and nitrate and phosphate concentrations) predict potential presence in 84-85% of lakes.

Using information in the literature, Hayward and Estevez (1997) constructed habitat suitability index (HSI) curves ranging from 0.0 (perfectly unsuitable or lethal) to 1.0 (perfectly suitable or optimal) for each of seven factors: calcium, pH, dissolved oxygen, temperature, salinity, turbidity (secchi disc depth) and sediment size (phi). From these curves they calculated composite HSI values from STORET data for 9,028 sites in Florida. Twenty-one percent had HSI values above 0.5, 3% had values above 0.8, and 2% had values above 0.9.

The results from several of these assessments are summarized in Figure 2.

Figure 2. Assessments of zebra mussel colonization potential by different studies

See text for explanation. A and B of Koutnik and Padilla (1994) refer to different models.



To assess how our understanding of zebra mussels' calcium threshold affects our estimate of susceptibility to invasion, we re-analyzed the ranges estimated by eight of these studies where suitable data were available, assuming minimum calcium thresholds of 15, 20, 25 or 28 mg/l. The results are presented in terms of the number of sites deemed suitable for colonization in Table 19, in terms of the percentages of the number of sites analyzed that are deemed suitable for colonization in Figure 3.

- The Ontario study (Neary & Leach 1992) used 12 mg/l as the calcium threshold between "unlikely" and "possible" colonization potential based on larval survival, where pH levels were judged to be appropriate. The data provided in this paper are not sufficient to allow direct re-analysis of this study. However, the paper does summarize calcium data for 6,147 Ontario lakes, and if an estimate is made based on calcium concentrations alone, then 22% of these (1,339 lakes) are rated as possible colonization sites if the calcium threshold is assumed to be 12 mg/l, while only 12% are rated as possible if the threshold is assumed to be 20 mg/l.

- The Rhode Island study (Tammi *et al.* 1995) also used 12 mg/l as the calcium threshold between "unlikely" and "possible" colonization potential, and estimated that colonization is possible at 7% of the sites analyzed. However, none of the sites are rated as possible if the calcium threshold used is 20 mg/l or higher.

- The Connecticut study (Murray *et al.* 1993) also used 12 mg/l as the calcium threshold between "unlikely" and "possible" colonization potential, and estimated that colonization is possible at 27% of the sites analyzed. Re-analyzing with higher calcium thresholds produces lower estimates of 19% of sites possible at a threshold of 15 mg/l, 8% possible at 20 mg/l, and only 2% possible at 28 mg/l.

- The Virginia study (Baker *et al.* 1993; Baker & Baker 1993) similarly used 12 mg/l as the calcium threshold between low or no susceptibility to invasion and moderate susceptibility, and found 71% of the lakes and river systems analyzed to be of moderate or greater susceptibility. Re-analyzing with higher calcium thresholds produces estimates of 57% of sites having at least moderate susceptibility at a threshold of 15 mg/l, 38% of sites at 20 mg/l, and 14% of sites at 28 mg/l.

Table 19. Potential distributions re-analyzed at various calcium thresholds

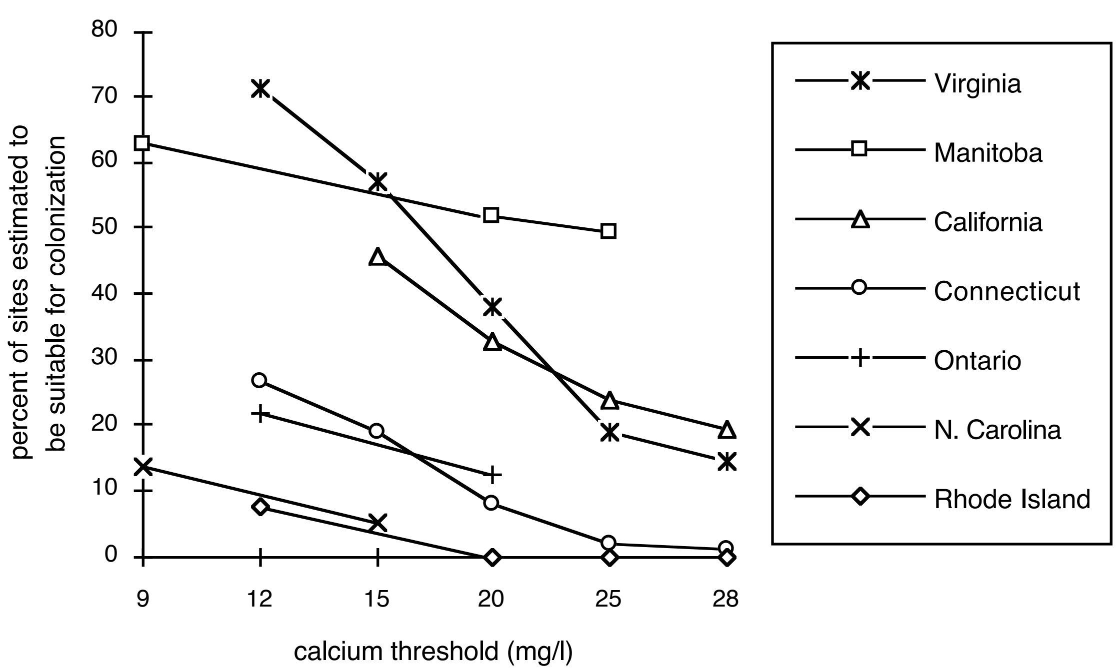
Region	Number of sites analyzed	Number of sites suitable for colonization based on calcium threshold of: 9 mg/l	12 mg/l	15 mg/l	20 mg/l	25 mg/l	28 mg/l
Ontario	6,147	–	**1,339**	–	758	–	–
Rhode Island	52	–	**4**	–	0	0	0
Connecticut	211	–	**56**	40	17	4	2
Virginia	21	–	**15**	12	8	4	3
N. & S. Carolina	16	0	0	0	0	0	0
N. Carolina	336	**46**	–	17	–	–	–
California	159	–	–	**73**	52	38	31
Manitoba	525	**330**	–	–	273	259	–

Numbers in bold indicate the calcium threshold used in the initial study.

- It's unclear what calcium threshold was used in the Duke Power (1995) study of 16 sites in North and South Carolina, which found the infestation potential to be possible or greater at 81% of sites. However, none of the calcium concentrations given for these sites is above 6 mg/l, and so none would be considered susceptible to infestation based on any of the calcium thresholds considered here.

- The North Carolina study (Doll 1997) rated the colonization potential for 340 sites independently for each of five environmental factors including calcium concentration, rating the potential as "unlikely" (also described as "unsuitable"), "maybe" ("borderline") or "definite" ("highly suitable"), using 9 mg/l as the threshold between ratings of "unlikely" and "maybe" for calcium The study did not combine the individual factor ratings into an overall rating for each site, but we can do so for the purpose of this re-analysis by setting the overall rating for a site equal to the lowest rating for any single factor at that site. In all, there are adequate data to analyze 336 sites,[11] of which 46 or 14% have an overall potential colonization rating of "maybe" or better at a calcium threshold of 9 mg/l, while only 5% have a rating of "maybe" or better at a threshold of 15 mg/l.

Figure 3. Estimates of colonization potential based on different calcium thresholds



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11 Twenty sites had no calcium data, but 16 of these were rated overall as "unlikely" on the basis of salinity or pH and thus could be included in the re-analysis (i.e. havin g calcium data for them would not change their overall rating). Four sites were exclu ded from the re-analysis. Six sites lacked temperature data, but since temperature w as rated "definite" at all 334 other sites, we assumed that it would rate at least a "may be" at those six sites.

- The California study (Cohen & Weinstein 1998a, b) used 15 mg/l as the calcium threshold between "low-to-no" and "moderate" colonization potential in rating 160 sites. There are adequate data to re-analyze 159 sites,[12] with 46% rating as moderate colonization potential at a calcium threshold of 15 mg/l, 33% at a threshold of 20 mg/l, and 19 % at a threshold of 28 mg/l.

- The Manitoba study (Sorba & Williamson 1997) estimated the colonization potential for 579 sites on 164 watercourses, using 9 mg/l as the calcium threshold between "very low" and "low" colonization potential. If we take this as the boundary between colonization being not possible and possible, there are adequate data provided for 525 sites to re-analyze using thresholds of 20 and 25 mg/l.[13] On this basis, colonization is estimated to be possible at 63% of sites using a calcium threshold of 9 mg/l, at 52% of sites using a threshold of 20 mg/l, and at 49% of sites using a threshold of 25 mg/l.

Conclusions and Recommendations

Contrary to views frequently published in peer-reviewed scientific journals and in the gray literature, our review herein found little evidence that zebra mussels can become established (i.e. persistently complete their full life cycle) at ambient calcium concentrations below about 20 mg/l. While some experiments have shown good adult survival for weeks to months even in calcium-free water, negative effects detected below around 15 mg/l include decreased growth rates, loss of shell material, loss of calcium from tissues, poor larval production and non-release of sperm. Zebra mussels have been reported from a small number of inland lakes with mean reported calcium concentrations below 20 mg/l, but these should be investigated further to determine if zebra mussels are truly established in these water bodies and, if so, to determine if calcium concentrations are consistently below 20 mg/l at the sites where the mussels are established. At other sites that have been reported as locations with both zebra mussel populations and ambient calcium concentrations below 20 mg/l (St. Lawrence River, Lake Champlain, Richelieu River), it seems possible that the populations are actually the result of ongoing recruitment of larvae or juveniles drifting down from upstream populations established in higher calcium waters.

There is considerably better evidence that zebra mussels can become established in ambient calcium concentrations below 28 mg/l to as low as 20 mg/l. Various experiments have demonstrated good embryonic, larval and juvenile development and growth at concentrations down to 20 mg/l, although reproductive stages and some developmental stages may yet await adequate testing. There do appear to be established populations with *in situ* reproduction and development in Duluth-Superior Harbor and the lower Hudson River, where calcium concentrations are generally in this range. However, further investigation is needed at these sites to determine the calcium concentrations at the particular times and places where zebra mussels pass critical reproductive and developmental stages. In both these areas, our understanding of where populations are truly established may be confounded by the arrival of large numbers of exogenous mussels—carried to Duluth-Superior Harbor by ships from the lower Great Lakes, or drifting into the Hudson River from upstream spources. Zebra

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12 Six sites had no calcium data, but five of these were rated overall as "low-to-no " c olonization potential on the basis of other factors, and thus could be included in the r e-analysis. One site was excluded from the re-analysis.

13 Calcium data were provided for 516 sites; another nine sites were rated "very lo w" based on other factors (so that having calcium data for them would not change t heir overall rating), and could be included in the re-analysis.

mussels have also been reported from a few inland lakes with mean reported calcium concentrations between 20 and 28 mg/l, and these should be investigated further to assess both establishment and the actual calcium concentrations at the locations and seasons that reproduction and development occur.

Our re-analysis of several studies of zebra mussel's colonization potential in North America shows that using different estimates of zebra mussel's calcium threshold, within the range from 9 to 28 mg/l, results in very different estimates of the vulnerability of different regions. For example, if the true calcium threshold is 20 mg/l rather than whichever value was used for the initial studies, then the Manitoba study overestimated the number of vulnerable sites by 21%, the California study overstimated by 39%, the Ontario study overestimated by approximately 77%, the Virginia study by 88%, the North Carolina study by approximately 180%, and the Connecticut study by 229%. None of the sites analyzed in the Rhode Island study would be vulnerable although the study had estimated that 8% were, nor would any of the sites analyzed in the Duke Power (North and South Carolina) study be vulnerable although the study had estimated that 81% were. If the true calcium threshold is lower than 20 mg/l, then the degree of overestimation by these studies is even greater.

It thus appears that North America waters may be substantially less vulnerable to zebra mussel colonization than has been indicated by most of the risk analyses done to date. Further studies of zebra mussel's calcium requirements in order to better define the threshold for population establishment would therefore appear to be of significant urgency. In particular we recommend support for the following areas of research:

- Laboratory investigations of zebra mussel's calcium requirements, focusing on ambient calcium levels needed during gonad development, reproduction, and embyronic and early larval development; including research into how calcium requirements vary with other environmental variables such as pH, temperature and the concentrations of other ions.

- Field investigations of whether apparent zebra mussel populations reported at low calcium sites are completing their full life cycle at these sites (focusing again on gonad development, reproduction, and embyronic and early larval development), along with measurements of ambient calcium concentrations at these sites.

- More thorough measurements of ambient calcium concentrations over time and space in waters of concern.

- Investigation of the claim made by some researchers that calcium concentrations are elevated in the vicinity of large concrete structures.

Such investigations could substantially improve risk analyses for yet-uncolonized regions, and improve the targeting and greatly reduce the costs of monitoring, outreach and education efforts. Near-term investments in research to answer questions about zebra mussel's calcium requirements thus seem likely to save money over the medium- to long-term.

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Appendix 1. Calculating Residence Times in the Lower Hudson River

(1) Estimating Reach Volumes

Published reach volumes for the Hudson River are shown in Table 1. The volumes for three successive reaches (Troy to Kingston, Kingston to Clinton Point, and Clinton Point to Peekskill), calculated from the volumes in Table 1, are shown in Table 2.

Table 1. Published Reach Volumes

Reach	Volume (cu m)	Volume (cu ft)	Source
Troy-Kingston	408,176,559	14,367,814,877	Limburg1996: 796
Troy-Clinton Point		29,000,000,000	Busby & Darmer 1970: 812
Kingston-Peekskill	952,334,777	33,522,184,150	Limburg1996: 796

Table 2. Volumes for 3 Successive Reaches

Reach	RKM	Volume (cu ft)
1 Troy-Kingston	150-248	14,367,814,877
2 Kingston-Clinton Pt	113-150	14,632,185,123
3 Clinton Pt-Peekskill	75-113	18,889,999,027

(2) Estimating the Ratio of Reach Inflows to Green Island Flows

The flow at Green Island (GI) in Troy is the combined flow from the Upper Hudson Region (Upper Hudson, Sacandaga and Hudson-Hoosic watersheds) and the Mohawk Region (Mohawk and Schoharie watersheds). The Middle Hudson watershed (MH) lies above Kingston, the Wallkill-Roundout watershed (WR) enters at Kingston, and the Hudson-Wappinger watershed (HW) is estimated to lie ≈41% above Clinton Point and entirely above Peekskill (Swaney *et al.* 1996, Fig. 1 [p. 836]). The inflows from MH, WR and HW as a fraction of GI inflow, based on the estimated 3-yr average annual flows in Swaney *et al.* 1996, Table 3 (p. 839), are as follows:

Table 3. Ratio of Watershed Outflows to Green Island Flows

	CIA	FIA	OA	USGS	mean
MH/GI	.273	.270	.266	–	.270
WR/GI	.138	.161	.159	.137	.149
HW/GI	.106	.119	.118	–	.114

where CIA = Coarse-Scale-Input-Average (an estimate made by averaging daily weather data over the regions, then using these averages to drive the model); FIA = Fine-Scale-Input-Average (same as CIA, except weather data is averaged over the watersheds); OA = Output-Average (daily weather data is used to drive the model, then results are averaged); USGS = U.S. Geological Survey measurements (see Swaney *et al.* 1996 for further explanation). The mean fractions from Table 3 were then used to estimate MH, GR and HW inflows, so that:

The inflow to Reach 1 is estimated as GI + (0.5 x MH) = 1.135 x GI.
The inflow to Reach 2 is estimated as GI + MH + WR + (0.5 x 0.41 x HW) = 1.442 x GI.
The inflow to Reach 3 is estimated as GI + MH + WR + (0.5 x HW) = 1.476 x GI.

(3) Calculating Reach Inflows and Residence Times

These equations in section (2) were used to calculate the inflows to each on a monthly basis, based on mean monthly Green Island flows for 1989, 1990, 1991, and for the period of record from 1947-97 (USGS streamflow data) (Tables 5a, 6a, 7a and 8a). The reach volumes from Table 2 were then divided by the monthly inflows to each reach to estimate the residence times for each of the three successive reaches (Tables 5b, 6b, 7b and 8b), and these were summed to estimate the residence times from Green Island at Troy to the downstream point of each reach (Tables 5c, 6c, 7c and 8c).

Table 4. Mean Monthly Inflow (cfs) at Green Island for 1989, 1990, 1991 and 1947-97
Data from U.S. Geological Survey, Surface Water Data for New York: Monthly Streamflow Statistics [http://water.usgs.gov/ny/nwis/monthly]

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1989	6,870	9,030	12,060	23,890	27,060	18,490	7,985	6,853	8,831	14,470	21,390	10,600
1990	13,530	24,850	35,110	31,590	34,980	8,839	5,553	8,746	5,604	16,990	23,100	24,330
1991	18,130	17,560	24,620	23,200	12,270	5,154	4,027	4,448	4,908	7,629	10,660	12,850
1946-97	13,540	14,110	22,100	30,790	19,090	10,050	6,733	5,823	6,462	9,020	13,130	15,070

Table 5a. Mean Monthly Inflow by Reach (cfs), based on 1989 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	7,797	10,249	13,688	27,115	30,713	20,986	9,063	7,778	10,023	16,423	24,278	12,031
Reach 2	9,907	13,021	17,391	34,449	39,021	26,663	11,514	9,882	12,734	20,866	30,844	15,285
Reach 3	10,140	13,328	17,801	35,262	39,941	27,291	11,786	10,115	13,035	21,358	31,572	15,646

Table 5b. Residence Times by Reach (days), based on 1989 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	21.3	16.2	12.1	6.1	5.4	7.9	18.3	21.4	16.6	10.1	6.8	13.8
Reach 2	17.1	13.0	9.7	4.9	4.3	6.4	14.7	17.1	13.3	8.1	5.5	11.1
Reach 3	21.6	16.4	12.3	6.2	5.5	8.0	18.6	21.6	16.8	10.2	6.9	14.0

Table 5c. Residence Times (days), based on 1989 Flows, from Troy to:

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Kingston	21.3	16.2	12.1	6.1	5.4	7.9	18.3	21.4	16.6	10.1	6.8	13.8
Clinton Point	38.4	29.2	21.9	11.0	9.8	14.3	33.1	38.5	29.9	18.2	12.3	24.9
Peekskill	60.0	45.6	34.2	17.2	15.2	22.3	51.6	60.1	46.7	28.5	19.3	38.9

Table 6a. Mean Monthly Inflow by Reach (cfs), based on 1990 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	15,357	28,205	39,850	35,855	39,702	10,032	6,303	9,927	6,361	19,284	26,219	27,615
Reach 2	19,510	35,834	50,629	45,553	50,441	12,746	8,007	12,612	8,081	24,500	33,310	35,084
Reach 3	19,970	36,679	51,822	46,627	51,630	13,046	8,196	12,909	8,272	25,077	34,096	35,911

Table 6b. Residence Times by Reach (days), based on 1990 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	10.8	5.9	4.2	4.6	4.2	16.6	26.4	16.8	26.1	8.6	6.3	6.0
Reach 2	8.7	4.7	3.3	3.7	3.4	13.3	21.1	13.4	21.0	6.9	5.1	4.8
Reach 3	10.9	6.0	4.2	4.7	4.2	16.8	26.7	16.9	26.4	8.7	6.4	6.1

Table 6c. Residence Times (days), based on 1990 Flows, from Troy to:

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Kingston	10.8	5.9	4.2	4.6	4.2	16.6	26.4	16.8	26.1	8.6	6.3	6.0
Clinton Point	19.5	10.6	7.5	8.4	7.5	29.9	47.5	30.2	47.1	15.5	11.4	10.8
Peekskill	30.5	16.6	11.7	13.0	11.8	46.6	74.2	47.1	73.5	24.3	17.8	16.9

Table 7a. Mean Monthly Inflow by Reach (cfs), based on 1991 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	20,578	19,931	27,944	26,332	13,926	5,850	4,571	5,048	5,571	8,659	12,099	14,585
Reach 2	26,143	25,322	35,502	33,454	17,693	7,432	5,807	6,414	7,077	11,001	15,372	18,530
Reach 3	26,760	25,919	36,339	34,243	18,111	7,607	5,944	6,565	7,244	11,260	15,734	18,967

Table 7b. Residence Times by Reach (days), based on 1991 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	8.1	8.3	6.0	6.3	11.9	28.4	36.4	32.9	29.9	19.2	13.7	11.4
Reach 2	6.5	6.7	4.8	5.1	9.6	22.8	29.2	26.4	23.9	15.4	11.0	9.1
Reach 3	8.2	8.4	6.0	6.4	12.1	28.7	36.8	33.3	30.2	19.4	13.9	11.5

Table 7c. Residence Times (days), based on 1991 Flows, from Troy to:

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Kingston	8.1	8.3	6.0	6.3	11.9	28.4	36.4	32.9	29.9	19.2	13.7	11.4
Clinton Point	14.6	15.0	10.7	11.4	21.5	51.2	65.5	59.3	53.8	34.6	24.8	20.5
Peekskill	22.7	23.5	16.7	17.8	33.6	80.0	102.3	92.6	84.0	54.0	38.7	32.1

Table 8a. Mean Monthly Inflow by Reach (cfs), based on 1947-97 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	15,368	16,015	25,084	34,947	21,667	11,407	7,642	6,609	7,334	10,238	14,903	17,104
Reach 2	19,525	20,347	31,868	44,399	27,528	14,492	9,709	8,397	9,318	13,007	18,933	21,731
Reach 3	19,985	20,826	32,620	45,446	28,177	14,834	9,938	8,595	9,538	13,314	19,380	22,243

Table 8b. Residence Times by Reach (days), based on 1947-97 Flows

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reach 1	10.8	10.4	6.6	4.8	7.7	14.6	21.8	25.2	22.7	16.2	11.2	9.7
Reach 2	8.7	8.3	5.3	3.8	6.2	11.7	17.4	20.2	18.2	13.0	8.9	7.8
Reach 3	10.9	10.5	6.7	4.8	7.8	14.7	22.0	25.4	22.9	16.4	11.3	9.8

Table 8c. Residence Times (days), based on 1947-97 Flows, from Troy to:

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Kingston	10.8	10.4	6.6	4.8	7.7	14.6	21.8	25.2	22.7	16.2	11.2	9.7
Clinton Point	19.5	18.7	11.9	8.6	13.8	26.3	39.2	45.3	40.8	29.3	20.1	17.5
Peekskill	30.4	29.2	18.6	13.4	21.6	41.0	61.2	70.8	63.8	45.7	31.4	27.3