

## The effects of source and destination on growth and metal uptake in freshwater clams reciprocally transplanted among south central Ontario lakes

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The relative contributions of genotype and environment to growth and metal uptake in freshwater unionid clams (*Elliptio complanata*) were evaluated using a reciprocal transplant experiment. In August 1985, comparable sites were selected in three south central Ontario lakes with alkalinities of 22, 153, and 238  $\mu\text{equiv} \cdot \text{L}^{-1}$ . Shell length, height, and width varied in a manner that could not be related to lake alkalinities. There were differences among the clam populations in allelic frequencies (at the *Pgm* and *Lap-2* loci). Clams were marked, measured, and reciprocally transplanted among the three lakes. In August 1986, marked individuals were recovered, remeasured, and analysed for levels of Cu, Zn, Mn, and Cd in soft tissues. The transplant source had a strong influence on clam growth during the post-transplant year. This source effect may result from genetic differences among the populations. Tissue metal concentrations at the end of the post-transplant year were a function of both source and destination. The use of freshwater clams as transplant biomonitors must be reassessed since there is a strong source component to growth and metal uptake. In transplant experiments a common source (a particular site within a particular lake) should be used, and post-transplant periods of more than 1 year may be necessary for the influence of the destination environment to dominate the influence of the source environment.

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La contribution relative du génotype et celle du milieu à la croissance et à l'absorption des métaux ont été évaluées chez la moule d'eau douce *Elliptio complanata* au cours d'une expérience de transferts réciproques. En août 1985, des sites comparables ont été choisis en trois lacs du centre sud de l'Ontario à alcalinités de 22, 153 et 238  $\mu\text{equiv} \cdot \text{L}^{-1}$ . Il n'y avait pas de corrélation entre les variations de longueur, de hauteur ou de largeur de la coquille et l'alcalinité des lacs. Les populations différaient quant à la fréquence de leurs allèles (aux locus *Pgm* et *Lap-2*). Des individus ont été marqués, mesurés et transférés à l'un des deux autres lacs. En août 1986, les individus marqués ont été récupérés, mesurés de nouveau et soumis à une analyse des concentrations du Cu, de Zn, de Mn et de Cd dans leurs tissus mous. Le lac d'origine d'un individu avait une forte influence sur sa croissance au cours de l'année qui suivait le transfert. Cet effet de l'origine résulte peut-être des différences génétiques entre les populations. Les concentrations des métaux à la fin de l'année dans un autre lac dépendaient à la fois du lac d'origine et du lac d'emprunt. L'utilisation de moules d'eau douce comme bioindicateurs de milieux d'emprunt doit être repensée, puisqu'il y a une forte influence du milieu d'origine sur la croissance et l'absorption des métaux. Dans les expériences de transfert, il faudrait utiliser des animaux d'origine commune (un point particulier dans un lac donné) et il se peut que des périodes post-transfert de plus de 1 an soient nécessaires pour que l'influence du milieu d'emprunt surpasse l'influence de milieu d'origine.

[Traduit par la revue]

### Introduction

Biological monitoring of aquatic habitats is important for evaluating ecosystem health. Bivalve molluscs can be useful for monitoring patterns of natural and anthropogenically induced environmental variation (Green et al. 1985). Over the past 15 years, tissues and shells from marine bivalves have been used in trace contaminant monitoring (Hugget et al. 1973; Bryan and Uysal 1978; Romeril 1979; Strong and Luoma 1981; Koide et al. 1982; Ritz et al. 1982; Roesijadi et al. 1984; Johnson and Lack 1985; Cain and Luoma 1985, 1986), while size and growth patterns have been used to evaluate environmental changes in salinity, total organic carbon, and suspended material (Essink and Bos 1985).

Tissues and shells of freshwater bivalves have also been used in trace contaminant monitoring (Foster and Bates 1978; Adams et al. 1981; Imlay 1982; Pugsley et al. 1985; Kauss and Hamdy 1985; Dermott and Lum 1986; Creese et al. 1986). Variation in shell morphology has been used to monitor variation in water chemistry and water turbulence (McCuaig and Green 1983; Mitchell and Collins 1984; Mitchell 1984; Hinch et al. 1986; Hinch and Bailey 1988).

To assess the relative contribution of environment and genotype to bivalve response (e.g., physiology, contaminant uptake, growth, survival), researchers have transplanted bivalves from one habitat to another. Results from transplant studies are often more convincing than *in situ* studies because they are the product of an experimental rather than an observational approach. Studies using reciprocally transplanted *Mytilus edulis* have shown that certain physiological characters (i.e., respiration and excretion rates, absorption efficiency) are primarily determined by environmental factors (Widdows et al. 1984), although environment and genotype contribute equally to changes in biomass (Dickie et al. 1984). A similar experiment with freshwater clams (*Lampsilis radiata*) revealed that shell growth was related to the original source, while shell shape was a function of environment (Hinch et al. 1986). However, the actual contribution of genotype to clam growth has not been examined in such transplant experiments.

The most common reason to transplant bivalves is to assess the distribution and quantity of biologically available contaminants among sites (Adams et al. 1981; Roesijadi et al. 1984; Cain and Luoma 1985; Johnson and Lack 1985; Kauss and Hamdy 1985; Creese et al. 1986). These studies usually entail taking clams from a relatively nonpolluted source and transplanting them to destinations where contaminant levels

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are to be determined. It is assumed that contaminant levels in the transplants will, after a certain amount of time, reflect levels of biologically available contaminants at the destination site. However, in addition to the environmental contaminant levels, there are several physiological factors that can influence contaminant uptake in bivalves, for example metabolic changes associated with sexual maturity (Cossa et al. 1980), reproductive state, and seasonal growth rate patterns (Strong and Luoma 1981; Popham and D'Auria 1983; Cain and Luoma 1986) and size and age variation (Hinch and Stephenson 1987, and references within). Even after controlling for these factors, levels of certain contaminants in transplanted bivalves are often more related to the source environment than to the destination environment. Cain and Luoma (1985) suggest that history of contaminant exposure may modify the ability of transplanted clams to accumulate or lose contaminants. That is, the transplants may require more time to acclimatize than is provided in many studies. Alternatively, contaminant uptake may be related to clam genotype. The latter hypothesis has never been investigated in freshwater bivalves.

Our purpose was to assess the relative contributions of genotype and environment to growth and metal uptake in freshwater clams. We reciprocally transplanted *Elliptio complanata* among three south central Ontario lakes differing in alkalinity. *Elliptio complanata* is a unionid bivalve that is widely distributed across northeastern North America (Clark 1973) and can be very abundant in low alkalinity lakes (Mackie and Flippance 1983a). Clams were transplanted across an alkalinity gradient because both shell morphology (Green 1972, 1980; Singer 1981; Mackie and Flippance 1983b; Hinch et al. 1989) and concentrations of metals in clam tissue (Forester 1980; Hinch and Stephenson 1987) are known to vary with differences in lake alkalinity.

### Methods

Thirty lakes that were intermediate in size, relative to other lakes in the area, were chosen from topographic maps of the Muskoka-Haliburton region of south central Ontario. Morphometric similarity of the lakes was assessed using an average sum-of-squares cluster analysis of three variables: surface area, maximum fetch, and perimeter length (Hinch 1987). After preliminary sampling of most of these lakes, Beech Lake (45°05'N, 78°42'W), Bark Lake (44°56'N, 78°28'W), and Tock Lake (45°16'N, 78°53'W) were selected as sites for the transplant experiment because they were similar in shape and size, and because they represented high, medium, and low positions on an alkalinity gradient (see Table 1).

In August 1985, a low exposure (low water turbulence) site was selected on the southwest shore in each lake, away from inflows and outflows (Hinch and Bailey 1988). Each site was similar in substrate composition based on the percentage of a sediment sample that passed through a 125- $\mu$ m sieve (Beech Lake 25.3%, Bark Lake 26.9%, Tock Lake 22.7%). See Allison and Harvey (1981) for a description of the sediment coring devices. Therefore, the effects of water turbulence at the three sites are approximately the same.

All clams that were visible were collected from each site by hand using SCUBA. Two hundred clams were randomly selected from the samples. Shell length, height, and width (as defined in Hinch et al. 1986) were measured to the nearest 0.01 mm for each clam using digital calipers. Clams ranged in length from 36.1 to 75.4 mm, 49.9 to 71.6 mm, and 48.5 to 84.9 mm for Beech, Bark, and Tock lakes, respectively. An identifying code was scribed onto each shell. Fifty randomly selected clams were transplanted to each of the other two sites, while 50 were left at each source site as controls. Clams were placed by hand in their normal living position around underwater

TABLE 1. Selected physical and chemical characteristics of Tock, Bark, and Beech lakes

	Tock Lake	Bark Lake	Beech Lake
pH	6.0	6.3	7.2
Alkalinity, $\mu$ equiv. $\cdot$ L <sup>-1</sup>	22	153	238
Conductivity, $\mu$ mho $\cdot$ cm <sup>-1</sup>	26	22	58
Area, km <sup>2</sup>	1.2	1.7	1.4
Perimeter, km	7.2	9.0	6.6
Volume, km <sup>3</sup> ( $\times 10^{-3}$ )	6.9	7.7	14.1
Mean depth, m	5.9	4.6	10.4
Maximum fetch, km	2.5	2.0	2.2
Approx. max epilimnetic volume, km <sup>3</sup> ( $\times 10^{-3}$ )	5.3	6.9	6.6

NOTE: Lake morphometry data were provided by the Ontario Ministry of Natural Resources. Water chemistry values are averages of Ontario Ministry of the Environment data collected between 1978 and 1983 (Ontario Ministry of the Environment 1983).

buoys. Water depth at all sites was approximately 1.5 m. The remaining 50 clams were brought back to the laboratory, and the shells were aged using the thin section method (Hinch and Stephenson 1987).

In August 1986, 1 year later, marked clams were recovered from the transplant sites. Length, height, and width of each clam were redetermined. Clams were brought back to the laboratory and kept alive for 1 week so that they could purge their digestive systems. The foot tissue from each clam was removed and stored frozen (-40°C) until it was used in electrophoretic analyses. Remaining clam tissue was frozen alive in the shells and kept frozen (-20°C) until it was used in trace metal analyses.

Levels of Cu, Cd, Zn, and Mn in the gills and remaining visceral mass (body) were determined following the methods in Hinch and Stephenson (1987). The gills were analysed separately because they often contain large concentrations of metals (Hemefraad et al. 1986) and because metals can diffuse out of gill tissue at a faster rate than from most other organs (Smith et al. 1975).

Electrophoretic analyses were carried out on the supernatant of macerated and centrifuged foot tissue of each clam using cellulose acetate gels. We followed the methodology of Hebert and Payne (1985). Thirteen loci, coding for the following enzymes, known to be polymorphic in aquatic invertebrates were investigated: aldehyde dehydrogenase (AO), fumarate hydratase (FUM), glucose-6-phosphate dehydrogenase (G-6-PDH), isocitrate dehydrogenase (IDH), hexokinase (HEX), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucosmutase (PGM), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucoseisomerase (PGI), malate dehydrogenase-NADP (ME), esterase (EST), and leucineaminopeptidase (LAP-2). Loci coding for LDH and 6-PGDH were not present. Loci coding for MDH, PGI, ME, FUM, and HEX were monomorphic. Loci coding for G-6-PDH, AO, EST, and IDH may have been polymorphic, but they produced weak, fuzzy bands that we were unable to read. The remaining 2 loci were polymorphic (*Pgm*, *Lap-2*) and their bands were clear.

To compare the pretransplant, log-transformed shell morphology measurements among the three populations, a multivariate analysis of variance (MANOVA) was performed. This was followed by a canonical variates analysis that described the differences with respect to shell dimensions.

To determine the statistical significance and the nature of the differences in growth related to the transplant source and destination of the clams, a factorial multivariate analysis of covariance (MANCOVA) was carried out. This analysis compares growth differences after correcting for among-population differences in initial shell morphology. Log-transformed final length, height, and width of the clams were used as dependent variables and log-transformed initial length, height, and width were the covariates. This is a multivariate factorial analogue of the Walford Plot ANCOVA (McCuaig and Green 1983;

TABLE 2. Standardized and structure coefficients for the first canonical axis from the canonical variates analysis of pretransplant length, height, and width (the lake means along the canonical axis are provided)

	Standardized	Structure	Lake mean
Length	-0.430	0.347	1.051 (Tock)
Height	2.674	0.574	0.539 (Beech)
Width	-1.822	-0.111	-1.808 (Bark)

Green 1987) using log-transformed data. It has been used on clam transplant data of a similar nature (Hinch et al. 1986).

We wished to assess the effects of transplant source and destination on final metal concentrations in clam tissues. Of course we had no measure of initial metal concentrations, which would have required killing the clams. We do have final concentrations for control clams. Initial metal concentrations were, however, determined on representative individuals from Beech and Tock lakes that were not used in the experiment. These results are presented in Hinch and Stephenson (1987).

Tissue metal concentrations are correlated with clam size, in a metal- and tissue-specific manner (Hinch and Stephenson 1987). In our statistical analysis we wished to remove any effects of variation in both initial clam size and growth rate during that year. To eliminate dependence on initial size, log-transformed final metal concentration was regressed on initial clam size (length) and the *y*-residuals were calculated. Then, to eliminate dependence on growth rate, these *y*-residuals were regressed on a measure of relative growth rate (derived from a Walford Plot), and the *y*-residuals from that regression were used as the dependent variable in a source-by-destination factorial ANOVA. This analysis was done for each unique combination of metal (Cu, Cd, Zn, and Mn) and tissue type (gill and body tissues).

## Results

### Pretransplant morphology and age

Clams from the three sites differed in morphology (based on Wilks criterion, MANOVA  $F(6,782) = 119.15, P < 0.0001$ ). The first canonical axis accounted for 77% of the shell morphology variation and described a gradient of increasing shell height relative to shell width (Table 2). Clams from Tock Lake, the most acidic lake, were proportionately tall and thin. Clams from Bark Lake, intermediate in acidity, were proportionately wide and short. Clams from Beech Lake, which is circumneutral, were intermediate in morphology.

The age structure of the three populations is illustrated in Fig. 1. Tock Lake had a relatively old age distribution and the greatest mean age ( $P < 0.05$ ). Beech Lake had slightly more individuals in younger age classes than the other sites. Young individuals (less than 2 years old) were absent from the samples.

### Post-transplant shell morphology

Eighty-eight percent of all marked clams was recovered. However, identification and (or) remeasurement of some individuals were hampered by shell etching that had occurred over the year. Shell etching is the loss of periostracum and underlying shell layers (Hinch and Green 1988). Therefore, we could only use 73% of the clams for the statistical analyses. The number of individuals used in the analyses and the average amount of shell growth in each dimension is listed by source and destination in Table 3.

The factorial MANCOVA on the transplant growth data revealed that both clam source and destination had significant effects ( $P < 0.0001$ ) on shell growth (Table 4). A significant

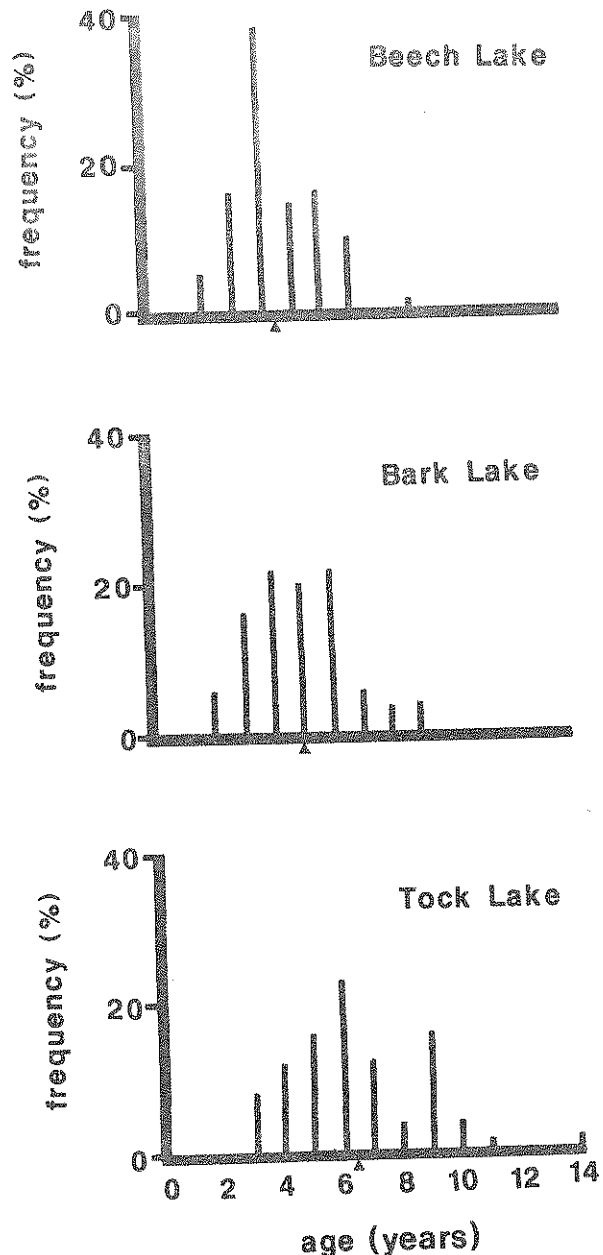


FIG. 1. Population age structure in Beech, Bark, and Tock lake clams. At each site  $n = 50$ . Arrows indicate the mean age of the population.

interaction ( $P < 0.0003$ ) also existed between these two factors. The source effect is very strong and quite clear. Regardless of transplant destination, clams from Bark Lake had more growth in width relative to growth in length than did clams from Beech or Tock lakes (Fig. 2A). The destination effect is much more difficult to interpret (Fig. 2B). Effects of destination are more evident, however, in the interaction between the source and destination main effects (Fig. 2C). Clams that were placed into Bark Lake and came from Bark and Tock lakes grew larger in all dimensions and grew more streamlined (length increasing relative to height, and width increasing relative to height) relative to clams from Bark and Tock lakes that were placed into Beech and Tock lakes. Clams from Beech Lake did not show this pattern (a differing response when the destination was Bark Lake).

TABLE 3. Mean (SD) shell growth (cm) in each dimension based on clams recovered from the reciprocal transplant experiment

Source	Destination	Length	Height	Width	n
Beech	Beech	0.76 (0.42)	1.21 (0.42)	0.43 (0.25)	36
	Bark	1.85 (1.41)	1.80 (3.12)	0.84 (0.39)	39
	Tock	1.42 (0.88)	1.28 (0.53)	0.57 (0.29)	41
Bark	Beech	0.58 (0.31)	1.35 (0.53)	0.47 (0.26)	39
	Bark	0.77 (0.33)	0.96 (0.61)	0.67 (0.41)	36
	Tock	0.64 (0.28)	1.23 (0.52)	0.36 (0.24)	39
Tock	Beech	0.53 (0.26)	1.13 (0.43)	0.28 (0.22)	32
	Bark	0.79 (0.45)	1.03 (0.56)	0.37 (0.25)	36
	Tock	0.79 (0.28)	1.50 (0.55)	0.33 (0.22)	32

#### Post-transplant metal levels

Clam source accounted for a great deal of the variation in gill metal concentrations (Table 5). Clams from Tock Lake tended to have higher Cu concentrations than Bark and Beech lake clams (Fig. 3A). Clams from Tock and Bark lakes had higher Cd concentrations than Beech Lake clams (Fig. 3B). Zinc and Mn concentrations tended to be highest in clams from Tock Lake (Fig. 3C and 3D).

Clam destination accounted for some of the variation in gill metal concentrations. Clams placed into Tock Lake tended to have greater Cu and Mn concentrations than clams placed into the other lakes (Fig. 3A and 3D).

Clam source and destination were both important in explaining variability in body metal concentrations. Clams from Tock Lake had greater Cu concentrations than clams from the other two lakes (Fig. 4A). Clams placed into Tock Lake had greater Cu concentrations than clams placed into the other two lakes. Zinc concentrations were greatest in clams that were placed into Beech Lake (Fig. 4C).

Body Cd and body Mn concentrations were influenced by an interaction between clam source and destination. Clams from Beech Lake that were placed into Bark Lake had the lowest Cd concentration (Fig. 4B). Clams from Tock Lake that were placed into Tock and Bark lakes had the lowest Mn concentration (Fig. 4D).

#### Allozyme variation

Allelic frequencies at the *pgm* locus, which is a four-allele monomer, significantly differed among Beech, Bark, and Tock lake clams ( $\chi^2_{(6df)} = 154.82, P < 0.001$ ). Allelic frequencies at the *Lap-2* locus, a three allele monomer, marginally differed among the three sites ( $\chi^2_{(4df)} = 8.32, P = 0.08$ ). Pairwise comparisons of allelic frequencies (Table 6) revealed that clams from Beech and Tock lakes were similar in allelic frequencies at the *Lap-2* locus. A paper containing a more extensive examination of within and among population genotypic differences among Beech, Bark, and Tock lake clams is in preparation.

#### Discussion

##### Shell morphology

The source populations differ in shell morphology. Shell growth patterns during the transplant experiment were also strongly influenced by population source. If proximate water chemistry influences clam growth, one would expect that clam growth would be ordered along the destination axis according to water chemistry. Clams that were placed in the highest alkalinity lake (Beech Lake) should have exhibited the most

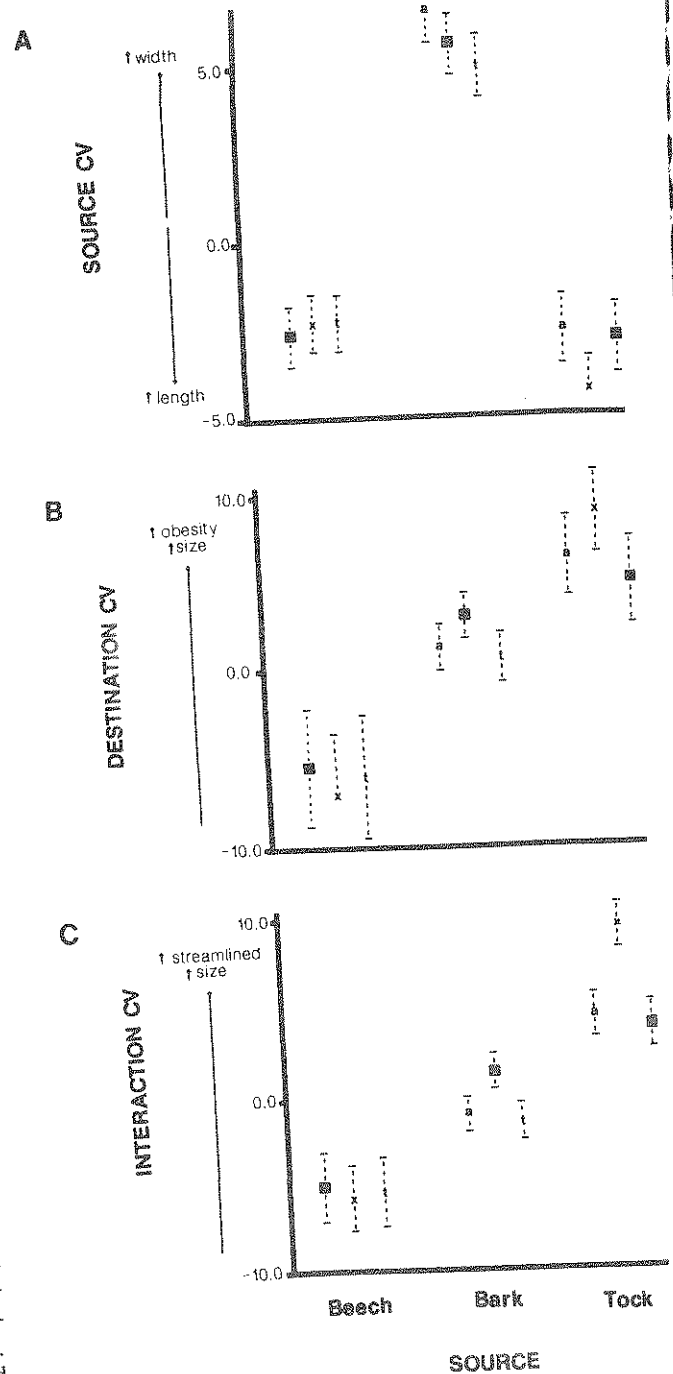


FIG. 2. First canonical score means (SE) of the source (A), destination (B), and interaction (C) effects. Means are labelled by clam destination. a, Beech; x, Bark; t, Tock;  $\blacksquare$ , control clams at each site. Obesity represents growth in width relative to growth in length. Streamlined represents growth in length relative to growth in height. CV, canonical variate.

growth, in length, height and width, while clams placed into the lowest alkalinity lake (Tock Lake), should have exhibited the least. This pattern could also have manifested itself in the interaction axis. For instance, within one or two of the population sources, clam growth would be ordered according to lake destination water chemistry. None of these scenarios occurred. Clams that were placed into Beech and Tock lakes

TABLE 4. Standardized and structure coefficients and probability levels for factorial MANCOVA of length, height, and width growth of the transplanted clams

	Length		Height		Width		P
	Standardized	Structure	Standardized	Structure	Standardized	Structure	
Source	-8.99	-0.49	-0.72	-0.51	8.50	0.15	<0.0001
Destination	5.32	0.93	-0.70	0.82	6.09	0.96	<0.0001
Source × destination	5.26	0.97	1.70	0.90	4.40	0.92	<0.0003

TABLE 5. Factorial ANOVA results on clam tissue metal concentrations

Metal	Source of variation	Gill tissue			Body tissue		
		Sum of squares	F	P	Sum of squares	F	P
Cd	Source	3.59	12.52	<0.01	2.44	7.12	<0.01
	Destination	0.46	1.61	0.20	9.26	27.00	<0.01
	Source × destination	0.44	0.76	0.55	2.47	3.60	<0.01
Cu	Source	3.57	5.83	<0.01	3.74	9.13	<0.01
	Destination	6.08	9.93	<0.01	6.17	15.06	<0.01
	Source × destination	1.17	0.95	0.43	0.87	1.05	0.38
Zn	Source	2.68	7.97	<0.01	<0.01	<0.61	0.99
	Destination	0.25	0.74	0.48	4.14	22.38	<0.01
	Source × destination	0.55	0.81	0.52	0.59	1.60	0.17
Mn	Source	1.43	5.94	<0.01	8.70	17.87	<0.01
	Destination	0.84	3.46	0.03	1.35	2.77	0.06
	Source × destination	0.41	0.85	0.50	2.26	2.32	0.06

grew about the same amount in overall size, while clams placed into Bark Lake grew the most in overall size. Since Bark Lake is intermediate in alkalinity between the other two lakes, there is no relationship between clam growth patterns and lake alkalinity.

Relationships between *E. complanata* shell morphology and alkalinity have been observed in large scale observational studies where differences in lake alkalinity were at least 10 times greater than those in the present study. Mackie and Flippance (1983b) noted that shorter, heavier shells were associated with decreasing calcium content of the water in relation to alkalinity and pH in streams and lakes in south central Ontario. Hinch et al. (1989) sampled 40 lakes in central Ontario and were able to relate larger and thicker shelled clams to higher alkalinity environments.

Differences in population age structure were probably not responsible for growth differences in the experiment. Clams from Beech Lake, the youngest population, grew the least in overall size, while clams from Tock Lake, the oldest population, grew the most. If age structure were important, one would expect more growth in the younger clams. This same rationale would probably apply to the initial differences in clam size. Beech Lake clams were the smallest, and they probably had the greatest potential for growth. In the experiment they grew the least. In any event, our analysis should have corrected for differences among populations in initial clam size.

#### Trace metals

Both clam source and destination were important predictors of tissue metal concentrations. In nearly every instance (the exceptions being body Mn and Zn), clams from or placed into Tock Lake had the highest tissue metal concentrations. Tock Lake with its low pH is presumably the most contaminated by

aqueous trace metals. This presumption is based on known relationships between acidity and trace metal concentrations in the water of south central Ontario lakes (Zimmerman et al. 1983; Stokes et al. 1985). Therefore, clams placed into Tock may be reflecting environmental levels of biologically available trace metals. Forester (1980) speculated that relatively high clam tissue metal concentrations in freshwater clams from south central Ontario may be attributable to the region's susceptibility to acid precipitation and the concurrent effects of metal mobilization in the lakes. On the other hand, Servos et al. (1987) reported that *E. complanata* transplanted among south central Ontario streams of differing pH and metal concentration did not differentially accumulate trace metals. Low water temperatures and short-term exposure (about 2 months) may have contributed to their failure to detect differences.

Water metal levels were determined once in the summer for Beech and Tock lakes, the highest and lowest in pH, respectively. At that time metal levels were either below detectable limits or did not differ between lakes (Hinch and Stephenson 1987). Differences in water metal levels between lakes are usually greatest during spring snowmelt (Haines 1981), and data are not available to examine seasonal differences in the metal concentrations in these lakes.

In marine clams, the history of metal exposure can determine body burdens of metals in transplants and may influence physiological or behavioural response to metals (Ritz et al. 1982; Worrall and Widdows 1983; Widdows et al. 1984; Cain and Luoma 1985). Cain and Luoma (1985) estimated that it would take approximately the entire life of their transplanted *Macoma balthica* (transplanted to a metal contaminated site in San Francisco Bay) for their body burdens to equal those of the resident clams. They suggest that in some contaminated environments, transplanted clams may never reach body bur-

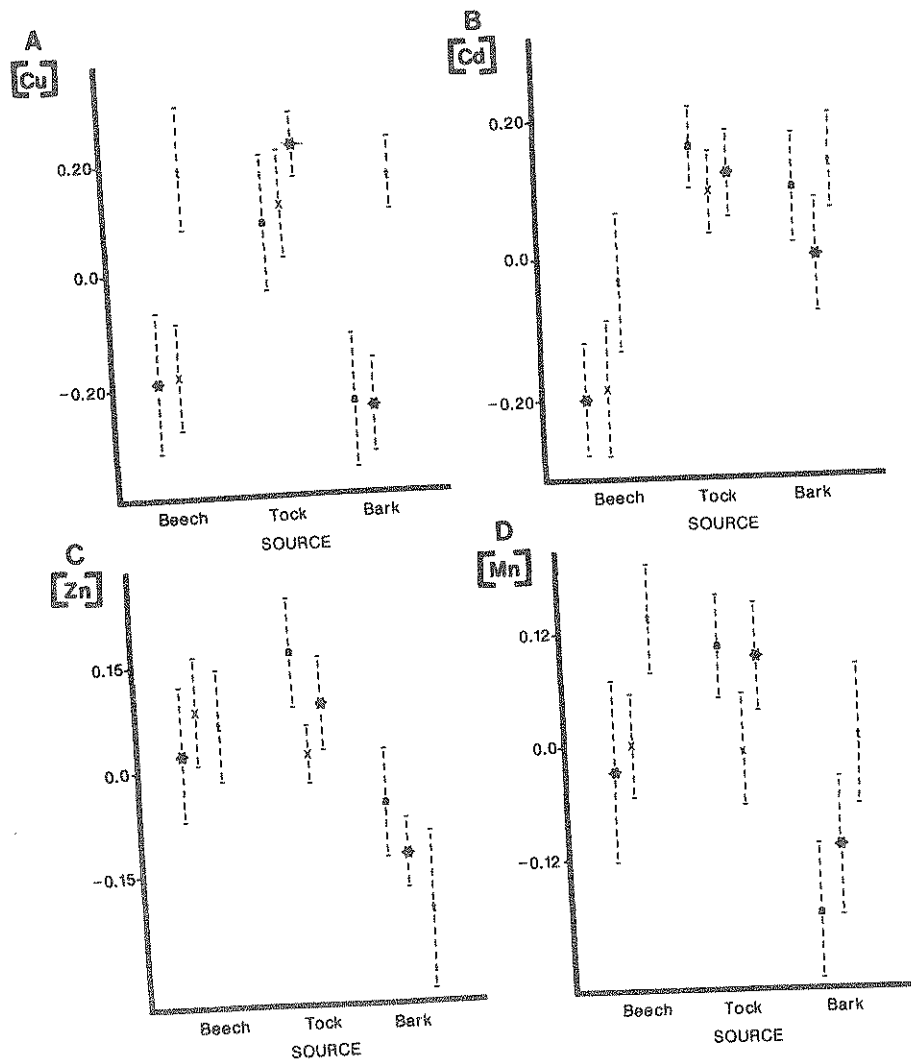


FIG. 3. Post-transplant mean (SE) log-transformed metal concentrations ( $\mu\text{g} \cdot \text{g}^{-1}$ ) in gill tissues. Means are labelled by clam destination. a, Beech; x, Bark; t, Tock. Asterisks represent control clams at each site.

dens of the resident clams. Assuming that Tock Lake is the most contaminated of the three lakes, clams whose original source was Tock Lake may be reflecting Tock Lake metal levels regardless of transplant destination. One year may be insufficient time for body burdens to equilibrate with the new proximate environment.

#### Allozyme variation

The source effect that is evident in the shell morphology and metal uptake data may represent genetic differences in growth patterns and in metal uptake patterns among the populations. The allelic frequency data suggest that these populations are genetically different. They are probably under different selection pressures because of differences in lake water chemistry. It is interesting to note that a balanced polymorphism is maintained in the Beech Lake population for the *Pgm* and *Lap-2* loci, while the populations from the two more acidic lakes do not conform to Hardy-Weinberg equilibrium because of an excess in homozygosity (manuscript in preparation). This suggests that selection may be occurring in Bark and Tock lake populations. Koehn (1978) has shown that the activity of LAP-1 in marine mussels is correlated with environmental salinity, and it is possible that a similar relationship between

TABLE 6. Probability levels from pairwise comparisons of Beech, Bark, and Tock lake allelic frequencies at the *Pgm* and *Lap-2* loci

	Bark	Tock
Beech	$P < 0.001^*$	$P < 0.001^*$
	$P = 0.050^\dagger$	$P = 0.860^\dagger$
Bark		$P < 0.001^*$
		$P = 0.038^\dagger$

\**Pgm* locus.

†*Lap-2* locus.

alkalinity (pH) and allozyme activity may exist in freshwater clams.

#### Applications to biomonitoring

We detected a strong source component to growth and metal uptake. The source effect could be a result of incomplete acclimatization of the transplant clams to their new environment. Allowing the experiment to run over several years (as opposed to 1 year) may have reduced or eliminated this problem. Most researchers who transplant freshwater clams

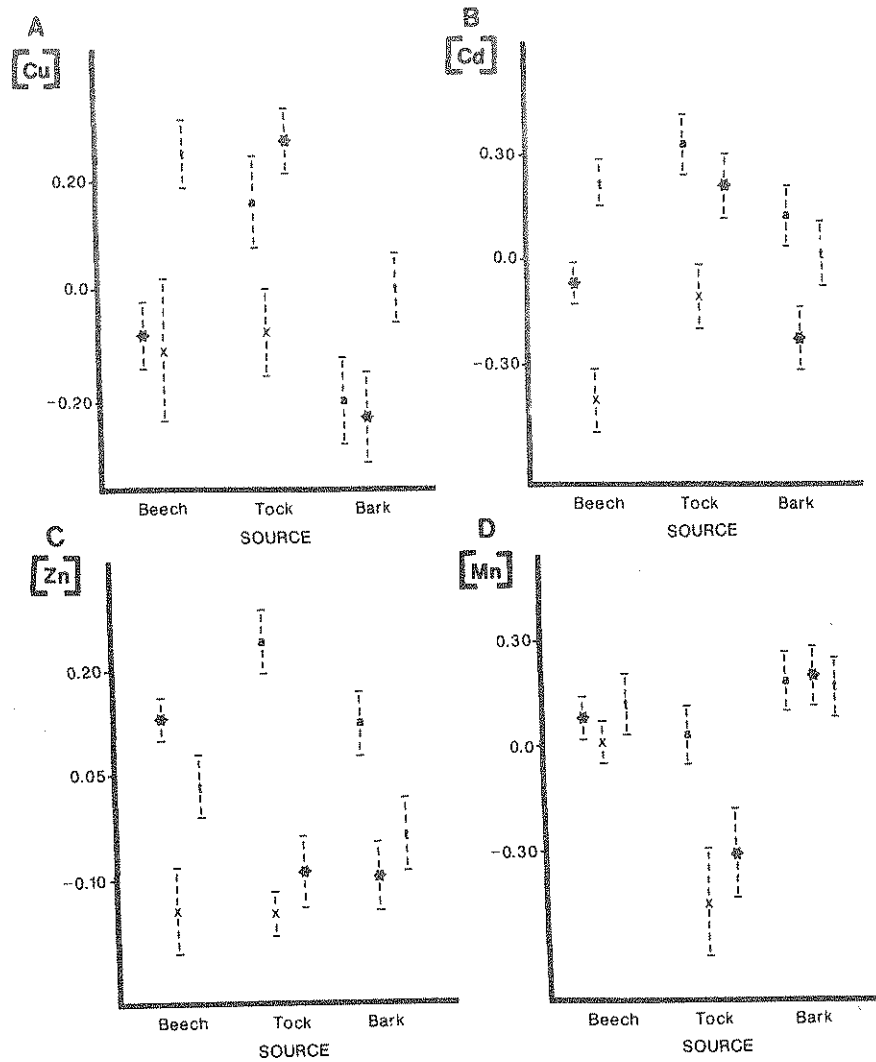


FIG. 4. Post-transplant mean (SE) log-transformed metal concentrations ( $\mu\text{g} \cdot \text{g}^{-1}$ ) in body tissues. Means are labelled by clam destination. a, Beech; x, Bark; t, Tock. Asterisks represent control clams at each site.

for contaminant monitoring conclude their experiments after only 1–12 weeks (see Adams et al. 1981; Kauss et al. 1981; Kauss and Hamdy 1985). Their inability to detect differences in tissue metals (i.e., Kauss et al. 1981) between transplant sites may be a result of the relatively short post-transplant exposure time.

Underlying genetic differences among the source populations could also be responsible for the source effect. It is probably impractical to standardize transplanted clams by genotype, but care should be taken to collect all clams from the same site in the same lake and to randomly assign clams to transplant destinations. Thus, genetic variation will not be confounded with destination effects, though it may inflate the error and make statistical tests more conservative.

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