In other work, repetitive pH depressions (i.e. pH 4.0–4.5) were not toxic to brook and rainbow trout (*Salmo gairdneri*) at durations shorter than 24 h until months of cyclic exposure (Curtis et al., unpubl. data). Considering the magnitude of the environmental acidification problem and the complexities of pH–Al synergism, further investigation of these time–concentration relationships appears to be warranted.

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References

Baker, J. P., and C. L. Schofield. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 18: 289–309.

Brown, D. J. A. 1983. Effects of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Environ. Contam. Toxicol. 30: 582–587.

Brown, V. M., D. H. M. Jordan, and B. A. Tiller. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol, and zinc. J. Fish Biol. 1: 1–9.

Chapman, G. A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc. 107(6): 841–847.

Coffin, D. L., D. E. Gardner, G. I. Sidorenko, and M. A. Pinigin. 1977. Role of time as a factor in the toxicity of chemical compound in intermittent and continuous exposures. Part II. Effects of intermittent exposure. J. Toxicol. Environ. Health 3: 821–828.

Curtis, L. R., W. K. Seim, and G. A. Chapman. 1985. Toxicity of fenvalerate to developing steelhead trout following continuous or intermittent exposure. J. Toxicol. Environ. Health 15: 445–457.

Driscoll, C. T. Jr., J. P. Baker, J. J. Bisogni, and C. L. Schofield. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

Gee, A. S., and K. R. Wade. 1984. The effects of acidification on the ecology of streams in the upper Tywi catchment in west Wales. Environ. Pollut. 35: 135–157.

Gingerich, W. H., W. K. Seim, and R. D. Schonbrod. 1979. An apparatus for the continuous generation of hydrophobic chemicals. Bull. Environ. Contam. Toxicol. 23: 685–689.

Groves, A. B., and A. J. Novotny. 1965. A thermal-marking technique for juvenile salmonids. Trans. Am. Fish. Soc. 94: 386–389.

Gunn, J. M., and W. Keller. 1984. Spawning site water chemistry and lake trout (*Salvelinus namaycush*) sac fry survival during spring snowmelt. Can. J. Fish. Aquat. Sci. 41: 319–329.

Haines, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110: 669–707.

Harriman, R., and B. R. S. Morrison. 1982. Ecology of streams draining forested and non-forested catchments in an area of central Scotland subject to acid precipitation. Hydrobiologia 88: 251–263.

Ingersoll, C. G., and B. W. Winner. 1982. Effect on *Daphnia pulex* (De Greer) of daily pulse exposures to copper or cadmium. Environ. Toxicol. Chem. 1: 321–327.

Lacroix, G. L. 1985. Survival of eggs and alevins of Atlantic salmon (*Salmo salar*) in relation to the chemistry of interstitial water in redds in some acidic streams of Atlantic Canada. Can. J. Fish. Aquat. Sci. 42: 292–299.

Muniz, I. P., and H. Leivestad. 1980. Toxic effects of aluminum on the brown trout (*Salmo trutta*) L., p. 320–321. *In* D. Drablos and A. Tollan [ed.] Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. SNSF Project, Oslo, Norway.

Robertson, C. E., and J. D. Hem. 1969. Solubility of aluminum in the presence of hydroxide, fluoride, and sulfate. U.S. Geological Survey Water-Supply Paper 1827-C, U.S.G.S.

Rogers, D. W. 1984. Ambient pH and calcium concentration as modifiers of growth and calcium dynamics of brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–366. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY.

Schofield, C. L., and J. R. Trojnar. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–3562. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY. 500 p.

Seim, W. K., L. R. Curtis, S. W. Glenn, and G. A. Chapman. 1984. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously or intermittently exposed to copper. Can. J. Fish. Aquat. Sci. 41: 433–438.

Sharpe, W. E., D. R. DeWalle, R. T. Leibfried, R. S. Dinicola, W. G. Kimmel, and L. S. Sherwin. 1984. Causes of acidification of four streams on Laurel Hill in southwestern Pennsylvania. J. Environ. Qual. 13(4): 619–631.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

Staurnes, M., T. Sigholt, and O. B. Reite. 1984. Reduced carbonic anhydrase and Na-K-ATPase activity in gills of salmonids exposed to aluminum-containing acid water. Experentia 40: 226–227.

Warren, C. E. 1971. Biology and water pollution control. W. B. Saunders Co., Philadelphia, PA. 434 p.

Variation in Major Ion Concentration of *Cambarus robustus* and *Orconectes rusticus* Following Exposure to Low pH

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Hollett, L., M. Berrill, and L. Rowe. 1986.Variation in major ion concentration of *Cambarus robustus* and *Orconectes rusticus* following exposure to low pH. Can. J. Fish. Aquat. Sci. 43: 2040–2044.

Cambarus robustus is more tolerant of low environmental pH than *Orconectes rusticus* and this tolerance reflects a difference in ion regulation physiology. Chronic exposure (96 h) of the acid-tolerant *C. robustus* to pH 3.8 soft water did not significantly change haemolymph $[Na^+]$ or $[Ca^{2+}]$ of the adults or total body $[Na^+]$ of the juveniles relative to the control (pH 6.5). In contrast, the intolerant *O. rusticus* showed a significant decrease in $[Na^+]$ and increase in $[Ca^{2+}]$ in adult haemolymph (Wood and Rogano. 1986. Can. J. Fish. Aquat. Sci. 43: 1017–1026) and an increase in total body [Na^+] of stage III juveniles following acute exposure to pH 3.8 compared with the pH 6.5 control.

Cambarus robustus est une espèce plus tolérante d'un faible pH environnemental que *Orconectes rusticus*, et cette tolérance reflète une différence dans la physiologie de la régulation des ions. Une exposition chronique (96 h) de *C. robustus*, qui tolère un milieu acide, à une eau douce présentant un pH de 3,8 n'a pas modifié de façon notable l'ion [Na^+] ni le [Ca^{+2}] dans l'hémolymphe des adultes ni le [Na^+] de l'ensemble du corps des juvéniles par rapport au groupe témoin (pH 6,5). Par opposition, l'espèce peu tolérante *O. rusticus* a montré une nette baisse de [Na^+] et une augmentation de [Ca^{+2}] dans l'hémolymphe des adultes (Wood et Rogano. 1986. Can. J. Fish. Aquat. Sci. 43 : 1017–1026) et une augmentation de [Na^+] de l'ensemble du corps chez les juvéniles de stade III à la suite d'une exposition aiguë à un pH de 3,8 par opposition au groupe témoin soumis à un pH de 6,5.

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Crayfish vary in their tolerance of pH stress with respect to species and life history stage. Adult and stage III juvenile *Cambarus robustus* are more tolerant of laboratory and in situ low pH than those of *Oronectes* species, and this variation in tolerance is reflected in species distribution with respect to pH (Berrill et al. 1985). Gradual acidification of Experimental Lakes Area Lake 223 to pH 5.1 resulted in a progressive decrease in population numbers of *Orconectes virilis* due to failed recruitment and possibly the disruption of ion regulation (Schindler et al. 1985). Further experiments indicate that juvenile *O. virilis* (France 1984), *O. rusticus*, and *O. propinquus* (Berrill et al. 1985) are more sensitive to low pH than adults. While the major effect of environmental acidity is severe haemolymph acidosis, an accompanying disruption of ion regulation in adults of several intolerant crayfish species, including the intolerant *O. rusticus*, has been reported (Appelberg 1985; Morgan and McMahon 1982; Wood and Rogano 1986). Typically, [Na^+] and [Cl^-] decreases while [Ca^{2+}] increases in the haemolymph. These results appear to be independent of water hardness in adult crayfish (Morgan and McMahon 1982; Wood and Rogano 1986). Similar physiological studies with tolerant species are required to establish that interspecific differences in pH tolerance observed in the field are correlated with differences in physiology. To date, no physiological studies have been done on the adults or juveniles of acid-tolerant crayfish.

In this study we examine the effect of acid exposure on haemolymph [Na^+] and [Ca^{2+}] in the relatively acid-tolerant *C. robustus* adult and on the total body [Na^+] of stage III juveniles of *O. rusticus* and *C. robustus*. Stage III juveniles were tested in varying [Ca^{2+}] in order to determine the effect of ambient [Ca^{2+}] on exposure to low pH. Results of Wood and Rogano (1986) for adult *O. rusticus* are used for comparative purposes with adult *C. robustus*.

Given the small body size of juvenile crayfish, whole body analysis is an attractive method for measuring ion concentration. Individual variation in carapace and claw development and the localization of Ca^{2+} in these structures meant that changes in [Ca^{2+}] could not be accurately measured in juveniles.

Methods

Experimental animals and water — In our laboratory experiments we used sexually mature, intermoult adults and stage III juveniles. Early stage III juveniles are particularly attractive for laboratory experiments, for they are capable of independent existence prior to their dispersal, they carry yolk reserves for the first 1–2 wk of that stage and therefore do not need feeding, and they can be collected in relatively large numbers from a single female. All adult crayfish were collected approximately 24 h prior to experimentation. *Cambarus robustus* were collected from Gull River (Victoria County) and *O. rusticus* was collected from Thompson's Creek (Peterborough County), both hardwater streams in southern Ontario with similar water chemistry to that of the Otonabee River (Table 1).

Water used for the exposure of adult *C. robustus* was laboratory-constituted soft water made by adding a specified quantity of each element (usually in a compound form) to deionized water (Table 1). In order to establish a possible [Ca^{2+}] mitigating effect, three natural water sources were selected for exposure of stage III juveniles: the hardwater Otonabee River, the relatively soft Kosh Lake water, and the very soft Plastic Lake water (Table 1). Water pH was established and maintained within 0.2 pH unit using 1 N H_2SO_4 and 1 M KOH. pH 3.8 was selected as the test pH with an intermediate pH of 5.0 for juvenile exposure. Although pH 3.8 is lower than levels found in acid waters, this level allows comparison with other studies (i.e. Wood and Rogano 1986: pH 4.0; Morgan and McMahon 1982: pH 3.8). All water was decarbonated, aerated continuously, and maintained at 15°C.

Adult exposure — Twenty *C. robustus* adults (male and females) were collected in late May and were placed in the test water at a pH of 6.5 for a 7-d acclimation period. Water was renewed on the fourth day. Test chambers were static 10-L aquaria, each holding 10 crayfish. Following acclimation, 10 individuals were exposed to pH 3.8 for 4 d. The remaining 10 crayfish were left at pH 6.5 for the same 4-d period to act as a control group. Chambers were checked twice daily for pH fluctuations or mortality. After the exposure period, a 0.1-mL haemolymph sample was drawn from near the basipodite of a walking leg of each crayfish using a 1-mL syringe. Haemolymph samples were transferred to 10 mL of deionized water and diluted to a detectable level (0.006–0.026 μmol/mL for Na^+ and 0.025–0.100 μmol/mL for Ca^{2+}). In order to suppress sodium and calcium ionization, potassium (0.05 mmol/mL) was added to each sample, and chemical interference in the determination of [Ca^{2+}] was prevented by the addition of strontium chloride (0.06 mmol/mL). Samples were then analysed for [Na^+] and [Ca^{2+}] by atomic emmission and atomic absorption, respectively, on a Varian 375 atomic

TABLE 1. Major ion concentration of the four experimental water sources in mmol/L (mg/L in parentheses).

Water source	Ca^{2+}	Na^+	Cl^-	K^+	Mg^{2+}	SO_4^-	Al ($\times 10^{-3}$)
Artificial soft	0.05 (2.35)	0.04 (0.92)	0.18 (6.38)	0.01 (0.39)	0.01 (0.24)	0.04–0.09 (3.83–8.62)	—
Plastic Lake	0.05 (2.35)	0.02 (0.46)	0.01 (0.35)	0.01 (0.39)	0.02 (0.49)	0.07 (6.70)	0.37–3.70 (10–100)
Kosh Lake	0.18 (7.21)	0.10 (2.30)	0.06 (2.13)	0.03 (1.17)	0.04 (0.97)	0.07 (6.70)	—
Otonabee River	0.95 (38.01)	0.13 (2.99)	0.10 (3.55)	0.03 (1.17)	0.03 (0.73)	0.10 (9.60)	—

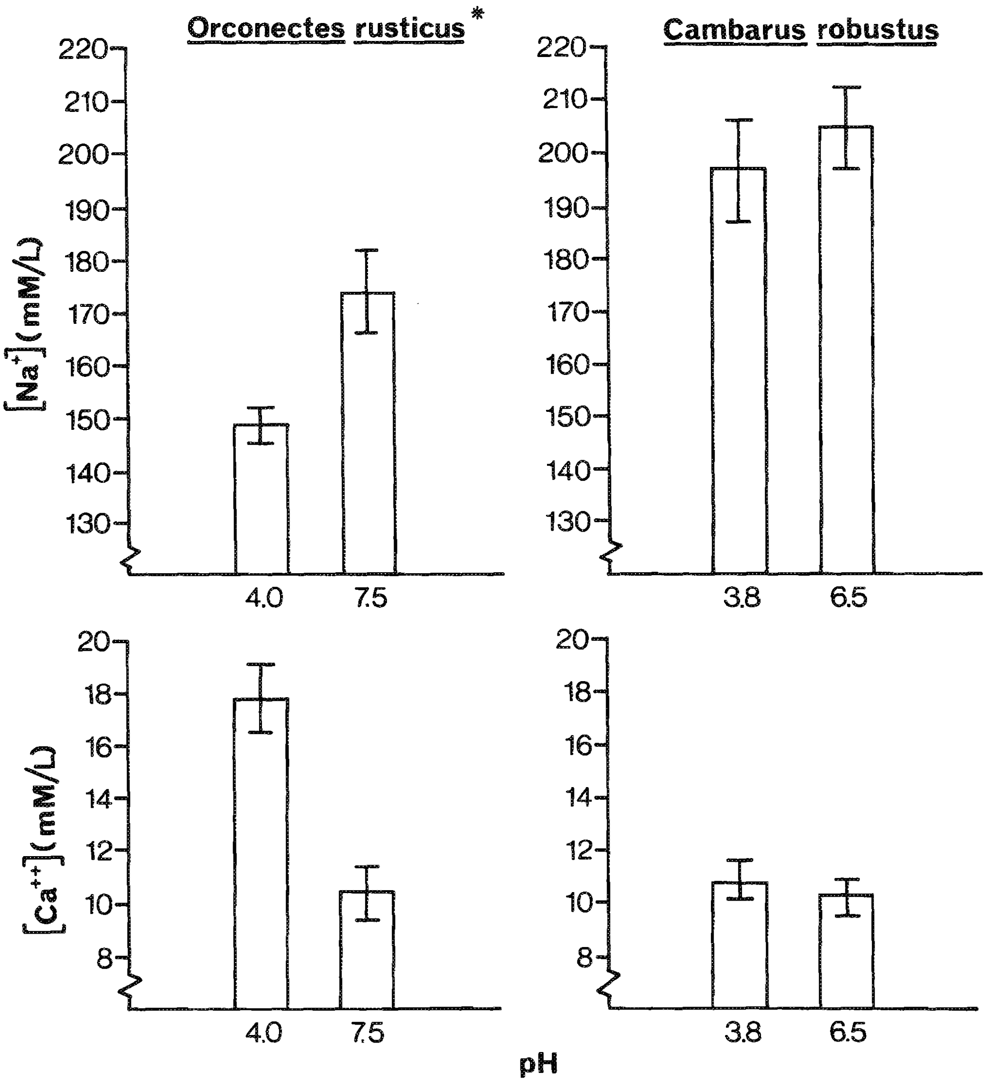

FIG. 1. Haemolymph [Na^+] and [Ca^{2+}] in *O. rusticus* and *C. robustus* following 96-h exposure to three pH levels. Bars represent means ± 95% confidence intervals. (*Results for *O. rusticus* are taken with permission from Wood and Rogano 1986)

absorption spectrophotometer. Actual concentrations were determined comparing sample results to a similar range of known N.B.S. standards.

Juvenile exposure — Female *O. rusticus* with eggs were collected in May 1984 and kept in static 10-L aquaria in each of the three water types at pH 6.5 until the young crayfish had moulted to stage III (approximately 2 wk). Water was renewed approximately every 5 d. Female *C. robustus* with stage II young were collected in mid-August 1984 and similarly kept until the juveniles moulted to stage III (approximately 4 d). Stage III juveniles of each species were exposed to three pH

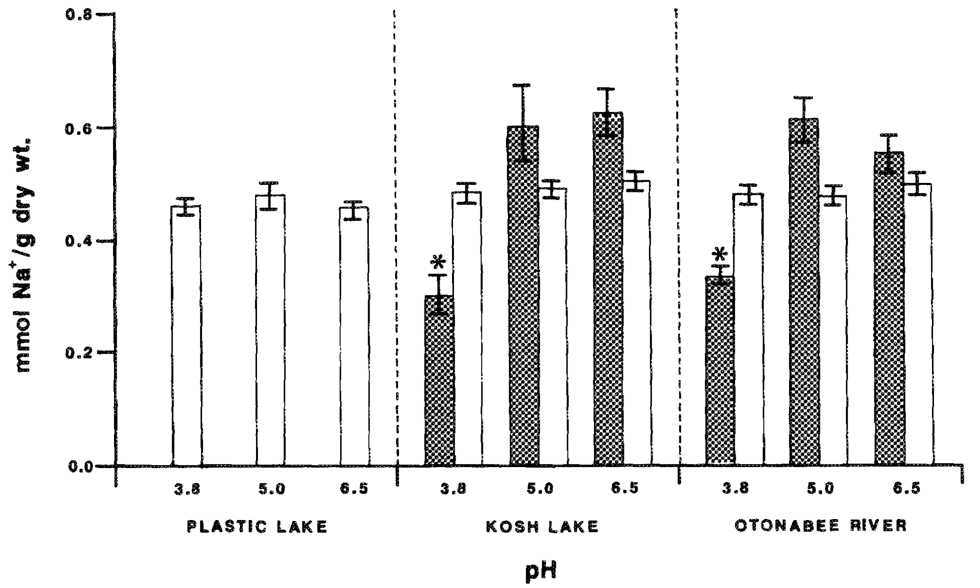

FIG. 2. Concentration of total body $[Na^+]$ in stage III juvenile *O. rusticus* (shaded bars) and *C. robustus* (open bars) following 96-h exposure to three pH levels. Bars represent means ± 95% confidence intervals. *Result for 48 h.

levels (3.8, 5.0, and 6.5) in each water type for 4 d. Enough *O. rusticus* juveniles were obtained to allow us to expose a total of 450 individuals in replicates of 25 per 2-L container of water, at each pH in each water type. The limited number of stage III *C. robustus* which we obtained allowed us to test only 25 in each treatment. pH was checked and maintained daily, and any dead juveniles were removed. After 4 d, surviving juveniles were dried to a constant weight at 60°C. However, in Plastic Lake water, *O. rusticus* mortality was too great to allow the exposure to last for more than 24 h at all pH levels or for more than 48 h in the other two water types at pH 3.8. *Orconectes rusticus* juveniles were digested in groups of three by refluxing with 1 mL of concentrated HNO_3 over a hot plate for 0.5 h (one blank was run with each set of digestions). Each solution was then diluted with 0.1 *N* HCl such that final solutions were within the detectable range (0.006−0.026 μmol/mL). Potassium (0.05 mmol/mL) was again added to each sample, and the emission of each solution was measured for $[Na^+]$ on a Varian 375 atomic absorption spectrophotometer. *Cambarus robustus* were treated in a similar manner except they were digested in pairs because of their larger size.

Statistical analysis — For both experiments, sample means were compared using Student's t-Test. Differences were considered significant at the 95 percent confidence level.

Results and Discussion

Adult *C. robustus* appeared unaffected by exposure to low pH. Four-day exposure of adult *C. robustus* to pH 3.8 soft water $[Ca^{2+}] = 0.05$ mmol/L) had no significant effect on haemolymph $[Na^+]$ and $[Ca^{2+}]$ relative to control animals (pH 6.5; Fig. 1). In comparison, 4-d exposure of adult *O. rusticus* to pH 4.0 soft water ($[Ca^{2+}] = 0.1$ mmol/L) resulted in significant loss in haemolymph $[Na^+]$ and significant gain in haemolymph $[Ca^{2+}]$ (Wood and Rogano 1986).

Stage III juvenile *C. robustus* total body $[Na^+]$ remained remarkably consistant in all treatments and no mortality occurred, while juvenile *O. rusticus* suffered significant losses of Na^+ and increased mortality in treatments of low pH and low $[Ca^{2+}]$, independent of low pH (Fig. 2). Juvenile *O. rusticus* did not survive the (96-h) exposure to pH 3.8 in either the hard water of Otonabee River (10% mortality) or the relatively soft water of Kosh Lake (15% mortality). Those exposed to pH 3.8 in both hard and soft water lost approximately 50% of their total body $[Na^+]$ after 48 h compared with the controls (pH 6.5) after 96 h, a highly significant difference ($p < 0.01$, Otonabee water; $p < 0.01$, Kosh Lake water). $[Na^+]$ did not change in juveniles exposed to pH 5.0 relative to those exposed to pH 6.5 in these two water types. There was no significant difference between results for Otonabee River and Kosh Lake waters. Only 5 of the 150 stage III *O. rusticus* exposed to the three pH levels in the very soft Plastic Lake water survived for 24 h (1 at pH 3.8, 3 at pH 5.0, and 1 at pH 6.5). Although too few to consider statistically, all five had lost at least 70% of their total body $[Na^+]$. Although aluminum concentrations are higher in Plastic Lake, increased aluminum concentration at pH 4.5−5.0 has been shown to have no affect on mortality of *O. rusticus* (Berrill et al. 1985). It appears that low ambient $[Ca^{2+}]$, independent of pH level, is lethal to *O. rusticus*, since mortality was equally high at all pH levels in Plastic Lake.

The results reported here clearly illustrate that interspecific differences in low pH tolerance by crayfish reflect differences in their ion regulation physiology. Net losses of Na^+ are due mainly to an inhibition of Na^+ uptake while efflux rates remain largely unchanged (Shaw 1960; Wood and Rogano 1986). Since *C. robustus* showed no change in blood Na^+ concentration, it may be able to maintain Na^+ influx in low pH water. Elevated haemolymph $[Ca^{2+}]$ following low pH exposure has been reported for the intolerant *O. rusticus*, *O. propinquus* (Wood and Rogano 1986), and *Procambarus clarkii* (Morgan and McMahan 1982) and appears to result from dissolution of $CaCO_3$ from the carapace to act as a buffer against blood acidosis which occurs in acid-stressed crayfish (Wood and Rogano 1986; Morgan and McMahon 1982). Since haemolymph $[Ca^{2+}]$ did not increase in *C. robustus*, this may indicate that this species is also resistant to blood acidosis.

There is growing evidence indicating that *Cambarus* as a genus is tolerant to low pH, while *Orconectes* is not (Berrill et al. 1985), and the data reported here suggest that the difference is one of ion physiology. Evolutionary events may account for these physiological differences between genera. The genera *Cambarus* and *Orconectes* both belong to the relatively recently evolved Cambaridae family of crayfish, but whereas *Orconectes* apparently originated in the central basin of the three great rivers of central North America (the Mississippi, Ohio, and Missouri), *Cambarus* appears to have originated in the mountainous regions of the Southern Appalachians and Ozarks (Hobbs 1942, 1974). The two genera have therefore evolved under quite different conditions, including those of water chemistry. It is possible that *Cambarus*, evolving under the softer water conditions characteristic of mountain streams, evolved ionoregulation mechanisms that preadapted it to withstanding low pH stress more successfully than *Orconectes*.

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References

APPELBERG, M. 1985. Changes in haemolymph ion concentrations of *Astacus astacus* L. and *Pacifastacus leniusculus* (Dana) after exposure to low pH and aluminum. Hydrobiologia 121: 19–25.

BERRILL, M., L. HOLLETT, A. MARGOSIAN, AND J. HUDSON. 1985. Variation in tolerance to low environmental pH by the crayfish *Orconectes rusticus*, *O. propinquus*, and *Cambarus robustus*. Can. J. Zool. 63: 2586–2589.

FRANCE, R. L. 1984. Comparative tolerance to low pH of three life stages of the crayfish *Orconectes virilis*. Can. J. Zool. 62: 2360–2363.

HOBBS, H. H. JR. 1942. A generic revision of the crayfishes of the subfamily Cambarinae (Decapoda, Astacidae) with the description of a new genus and species. Am. Midl. Nat. 28(2): 334–357.

1974. Synopsis of the families and genera of crayfishes. Smithson. Contrib. Zool. 164: 1–32.

MORGAN, D. O., AND B. R. MCMAHON. 1982. Acid tolerance and effects of sublethal acid exposure on ionoregulation and acid–base status in two crayfish *Procambarus clarki* and *Oronectes rusticus*. J. Exp. Biol. 97: 241–252.

SCHINDLER, D. W., K. H. MILLS, D. F. MALLEY, D. L. FINDLEY, J. A. SHEARER, I. J. DAVIES, M. A. TURNER, G. A. LINSEY, AND D. R. CRUIKSHANK. 1985. Long-term exosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

SHAW, J. 1980. The absorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. II. The effect of the external anion. J. Exp. Biol. 37: 534–547.

WOOD, C. M., AND M. S. ROGANO. 1986. Physiological responses to acid stress in crayfish (*Orconectes*): haemolymph ions, acid–base status, and exchanges with the environment. Can. J. Fish. Aquat. Sci. 43: 1017–1026.

High Precision Microcomputer Based Measuring System for Ecological Research

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Roff, J. C., and R. R. Hopcroft. 1986. High precision microcomputer based measuring system for ecological research. Can. J. Fish. Aquat. Sci. 43: 2044–2048.

A semiautomated measuring system is described which allows precise multiple measurements on organisms of biological interest. It consists of a microscope with drawing tube positioned next to a digitizing tablet which is interfaced to a personal computer; a TV camera and monitor are optional additions. Light from an LED fitted cursor on the digitizing pad is focused through the drawing tube and combined with the microscope image. Measurement signals are sent to the computer when the cursor button is depressed. Data storage, calculation, and display can be performed on-line as data are entered. Maximum precision of repeated measurements is ±0.04%; in routine use an accuracy of <±0.25% is achieved. An example of its precision compared with a conventional eyepiece micrometer is given. The system has been used for measurement of a diversity of aquatic particles including phytoplankton, zooplankton, fecal pellets, and stream benthic invertebrates at magnifications from 10 to 1250 power.

Nous décrivons un système de mesure semi-automatique qui permet d'effectuer des mesures multiples et précises sur des organismes intéressant le biologiste. Il se compose d'un microscope avec tube de tracé localisé près d'un numériseur qui possède une interface avec un ordinateur personnel; une caméra de télévision et un écran peuvent y être ajoutés. La lumière provenant d'un curseur à DEL sur le numériseur est focalisée par le tube de traçé et se combine à l'image du microscope. Les signaux de mesure sont envoyés à l'ordinateur lorsque le bouton du curseur est enfoncé. Il est possible d'effectuer l'archivage des données, le calcul et l'affichage en direct à mesure que les données sont entrées. La précision maximum de mesures répétées est de ±0,04 %; dans l'emploi ordinaire, on obtient une précision de <±0,25 %. Nous donnons un exemple de ce degré de précision comparé