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SENSITIVITY OF JUVENILE FRESHWATER MUSSELS TO HYPOXIC, THERMAL AND ACID STRESS

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Abstract: Tissue culture techniques were employed to rear juvenile mussels from glochidia larvae of the freshwater unionid mussels Utterbackia imbecillis and Pyganodon cataracta. The tolerance of one-week old juveniles (96-hour exposure) to hypoxia, thermal stress and acidic conditions then was assessed. Juveniles of both species succumbed to anoxia (<0.1 ppm O_2) within 24 hours. The 96-hour LT_{50} for U. imbecillis acclimated to 20°C was approximately 31.5°C; and for P. cataracta it was 33°C. Exposure to pH 4.0 resulted in greater than 50% mortality for both species within 72 hours. The 96-hour LC_{50} for exposure to acidic conditions was estimated to be pH 4.5 for both species of mussels. These data constitute the first of their kind for juvenile unionid mussels, and suggest that juveniles may be far more vulnerable to particular environmental stresses than are adults.

Key Words: Unionidae, Utterbackia, Pyganodon, tolerance, juveniles, stress, LT_{50}

INTRODUCTION

The freshwater mussel fauna (Mollusca: Bivalvia) consists primarily of species in the unionacean family Unionidae. In contrast to marine and some other freshwater bivalves, the ecology of unionid mussels is complicated by the inclusion in their life history of a parasitic larval stage, the glochidium. In most species this larva is an obligate parasite of fish, subsequent to its release from the parent mussel within the gills of which early development occurs (Kat, 1984; Tankersley and Dimock, 1992,1993a). Following the few days to several weeks parasitic association with a fish, metamorphosed juvenile mussels detach from the host and join the benthic fauna.

Although aspects of the behavior and physiology of unionid glochidia have been studied (e.g., Wood, 1974; Silverman et al., 1987; Hoggarth and Gaunt, 1988), young post-glochidial mussels (i.e., juveniles from a few hundred μm to a few mm in length) are rarely observed in nature (Neves and Widlak, 1987). As a result of the low probability of recovering juvenile mussels from field collections (Young and Williams, 1984), and the difficulty of rearing juveniles from fish that have been infected with glochidia in the laboratory (Howard, 1922; Trdan and Hoeh, 1986), ~~the observation by~~ Russell-Hunter (1964) ~~that essentially~~ little is known ~~about~~ the biology of juvenile unionid mussels ~~remains true 30 years later.~~

In contrast to the lack of data for juvenile unionid mussels, the physiological ecology of adult freshwater bivalves, and of larvae and adults of numerous marine species, has been studied extensively. Some adult freshwater bivalves can withstand extreme environmental conditions. For example, Anodonta cygnea can withstand immersion at 13°C in anoxic water under paraffin oil for at least 6 days (Holwerda and Veenhof, 1984), while some pisidiid clams can tolerate complete anoxia for 5-10 days at 20°C and increase their resistance to more than 200 days at 0°C (Holopainen, 1987). The unionid Ligumia subrostrata can survive at least 5 days out of water under an atmosphere of pure nitrogen (Dietz, 1974).

Studies of thermal tolerance of adult unionid mussels have focused primarily on mechanisms of thermal resistance by isolated tissues, typically, ciliated structures such as gill

filaments (Precht et al., 1973; Lagerspretz, 1985). The small freshwater Asiatic clam, Corbicula fluminea, has an upper lethal limit between 24 and 34°C, depending upon prior acclimation conditions (Mattice and Dye, 1976). The zebra mussel, Dreissena polymorpha, has an upper lethal limit near 30°C (Iwanyzki and McCauley, 1993). Several freshwater mussels also tolerate severe acid stress, including Anodonta cygnea which can withstand pH 3 for at least 12 days (Machado et al., 1988). Three additional species can tolerate pH 4.0 (Pynnonen, 1990), while several pisidiids can withstand exposure to pH 2.3 for at least 96 hours (Mackie, 1989).

The development of techniques for the in vitro transformation of glochidia larvae to juvenile mussels (Hudson and Isom, 1982; Keller and Zam, 1990) has made it possible to obtain juveniles in sufficient numbers for laboratory experimentation. The present study has addressed the tolerance of juveniles of two species of unionid mussels to extreme hypoxia, thermal stress and to acidic conditions. The results are the first data of their kind on the physiological ecology of this important stage in the life histories of unionid bivalves.

METHODS AND MATERIALS

Juvenile Mussels

All experiments were conducted with juvenile mussels (Utterbackia imbecillis from Shallowford Lake, Winston-Salem, Forsyth County, NC, and Pyganodon cataracta from Speas' Pond, Booneville, Yadkin County, NC) that had been transformed in vitro using tissue culture techniques modified from the procedures of Isom and Hudson (1982) and Hudson and Shelbourne (1990). Commercial Eagles Minimal Essential Medium (MEM) (Sigma Chemical Co.) was substituted for the combination of essential and non-essential amino acids, vitamins, glucose and unionid mussel Ringers solution of Hudson and Shelbourne (1990). Rabbit serum (Sigma Chemical Co.) was added as the protein source, and the mixture was enriched with the serum supplements TCH and TCM (Celox Corporation). The medium included the antibiotics carbenicillin, gentamicin, rifampicin and amphotericin B. The final culture recipe was Eagles MEM, rabbit serum, antibiotic mix, TCH and TCM at a volumetric ratio of approximately 27:8:6:1:1, respectively.

Juvenile mussels were cultured from mature glochidia excised from the marsupial demibranchs of gravid parental mussels. Glochidia were washed in sterile pond water and transferred to 60 mm polystyrene dishes in 3 ml of the culture medium. Approximately 300-400 larvae were incubated in each culture dish in a 5% CO_2 atmosphere at 23°C for 7 days. The medium was diluted to 50% on day 8 with 3 ml of artificial pond water (APW: 0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCL, 0.25 mM $CaCO_3$; pH 7.8; total Ca = 25 mg/l).

Newly metamorphosed juveniles were transferred into 100% APW on day 9 and were held at 20°C. The juvenile mussels were fed daily a concentrated algal suspension, and were provided with a layer of fine (<60 μm) pond silt (Isom and Hudson, 1982). All experiments were conducted on juveniles (~300-350 μm in length) that were 7-10 days post-metamorphosis at the beginning on each experiment.

Bioassays

For each assay, groups of 10 juveniles were placed in 12 mm x 50 mm open-ended glass tubes fitted with 200 μm Nitex mesh as a floor (Fig. 1). Sets of 12 tubes were established in each of two replicate experimental or control chambers as described below. Thus, at the beginning of each experiment 240 juveniles of each species were subjected to each treatment condition. The juveniles were transferred directly from the rearing conditions (e.g., 20°C) into the specific treatments and were maintained therein, unfed, for up to 96 hours.

Fig. 1 near here

At 24 hour intervals, three tubes of juveniles were removed from each of two experimental and two control chambers, and the animals (N=60 for treatment or control) were assessed for mortality. Each set of 10 mussels was examined with a dissecting microscope (Nikon SMZ-2T), and living vs dead was determined by reference to foot extension, valve adduction, heart beat, rotation of protostyle in the stomach, or metachronal activity of cilia on the gills. The relatively translucent shells of young juveniles facilitated visual assessment of viability. However, any individuals that could not with confidence be appraised with the dissecting microscope were examined with an American Optical Co. inverted compound microscope at 40 or 100X. The data

are presented as percent survival for N=60 (two replicates of 30 mussels) assayed at each observation interval. Where appropriate, the condition resulting in 50% mortality at 96-hours exposure (96-h LT_{50} or LC_{50}) has been determined graphically by probit analysis (Sokal and Rohlf, 1969).

Experimental Treatments

Hypoxia.--Juveniles were held at 20°C in 8 l of APW in glass chambers (25 cm i.d. x 20 cm deep) fitted with vented lucite lids. Control chambers were aerated and maintained at full saturation (P_{O_2} > 8.6 ppm). Experimental chambers were de-oxygenated (P_{O_2} < 0.1 ppm) by continuous stripping with nitrogen gas. The P_{O_2} of each chamber was monitored with an oxygen sensor (YSI Model 58) that was calibrated and checked against a zero-oxygen solution (4% $NaSO_3$).

Thermal Stress.--Sets of mussels were held in 4 l of APW in water-jacketed lucite chambers (23 cm L x 15 cm W x 16 cm H) similar to the illustration in Figure 1. Each chamber was continuously aerated. Temperatures were held at 20, 28, 30, 32 or 34 ± 0.2°C by refrigerated, circulating water baths (Fisher Model 90).

Acidity.--Mussels were held in 8 l of continuously aerated APW in glass chambers at 20°C, as described above under Hypoxia. Three sets of chambers were established: pH 7.8, 5.0 and 4.0, ± 0.05 pH unit.. APW was acidified with H_2SO_4. The pH was monitored at 12 hour intervals (Corning Model 230 pH meter) and adjusted with H_2SO_4 or NaOH, as necessary.

RESULTS

Neither Utterbackia imbecillis nor Pyganodon cataracta tolerated exposure to the nearly anoxic conditions. Mortality was 100% for both species within 24 hours; whereas, more than 88% of control mussels survived.

Thermal tolerance followed classic patterns, with diminished survival accompanying acute exposure to increased temperature (Figs. 2 and 3). For U. imbecillis, exposure to 34°C resulted in >50% mortality within 48 hours, and more than 50% of these mussels also succumbed at 32°C

by 96 hours (Fig.2). Mortality did not exceed 25% at 30°C or less. The 96-hour 50% tolerance limit (LT_{50}) for one-week old juvenile U. imbecillis was estimated to be 31.5°C.

~~The resistance~~ of P. cataracta to thermal stress ~~is depicted in Figure 3.~~ Exposure ~~to~~ 34°C was lethal to 46% of the mussels within 24 hours, and mortality reached 100% by 72 hours (Fig. 3). No other temperature induced >50% mortality. The 96-hour LT_{50} for this species was approximately 33°C.

Figs. 4+5 NEAR here

Juvenile mussels tolerated pH 5.0 or greater for the entire exposure period (>70% survival at 96 hours; Figs. 4 and 5). However, both species succumbed to pH 4.0, with >50% mortality occurring by 72 hours. The 96-hour LC_{50} was approximately pH 4.5 for both species.

DISCUSSION

The husbandry of freshwater mussels using in vitro culturing techniques now makes it feasible to use juvenile unionids in basic and applied molluscan research. Juvenile Utterbackia imbecillis [=Anodonta imbecillis, (Hoeh, 1990)] have been used successfully in toxicological studies, and have been found to be as sensitive or more so than traditional bioassay species (Keller and Zam, 1991). Comparable studies with other laboratory reared species of unionids are becoming increasingly common (Jacobson et al., 1993).

The data from the present study represent the first quantitative expression of the physiological ecology of very young unionid mussels, as related to hypoxia, thermal stress and exposure to acidic conditions. In contrast to the often noteworthy tolerance of adult mussels to environmental stresses, one-week old juvenile U. imbecillis and P. cataracta are ~~shown to be~~ very sensitive to hypoxia and to moderately low pH. Although nothing is known about the respiratory physiology of juvenile freshwater mussels, it is clear that one-week old individuals of these two species do not have the anaerobic capacity that typifies some adult unionids (Holwerda and Veenhof, 1984); rather, they appear to be completely intolerant of anoxia. The only published report on the respiratory physiology of Utterbackia imbecillis suggests that adults can maintain a constant rate of oxygen consumption in declining oxygen tensions (i.e., are oxyregulators), but apparently cease aerobic metabolism at a P_{O_2} approximating 10% of air

saturation (Hiestand, 1938). Adult Pyganodon cataracta have recently been shown to be oxyconformers whose rate of aerobic metabolism varies directly with ambient P_{O_2} (Tankersley and Dimock, 1993b).

There are no comparative data available for the effects of exposure to low pH on juvenile bivalve molluscs. Embryonic development of the freshwater gastropod Amnicola limosa fails at pH 5.0 or lower, and hatching success is impaired even at pH 5.5 (Servos, Rooke and Mackie, 1985). The juvenile unionids of the present study failed to survive exposure to pH 4.0.

In contrast to their acute sensitivity to hypoxia and pH <5.0, juvenile U. imbecillis and P. cataracta were comparatively more resistant to elevated temperatures (Figs. 2 & 3). Both species tolerated 32°C for at least 72 hours, even though they were not fed in the interim. Juvenile P. cataracta withstood exposure to 32°C for 96 hours (Fig. 3), and were relatively more resistant than U. imbecillis (LT_{50} = 33°C for P. cataracta vs 31.5°C for U. imbecillis). The LT_{50} for each of these species equaled or exceeded that of adult Corbicula fluminea (LT_{50} ~32°C, Mattice and Dye, 1975) acclimated to 20°C.

The environmental conditions of the microhabitat of recently metamorphosed juvenile mussels are unknown. The chemistry of interstitial water of sediments inhabited by adult mussels can experience moderately low pH and oxygen tensions of 5-10% of air saturation, or less (Buddensiek et al., 1990). Obviously the thermal conditions of the benthic sediments will vary with geography, season, water depth and depth within the soil profile. Since nothing is known about the behavior of juvenile mussels in the field, including an understanding of how deeply individuals may burrow, comments about the in situ conditions experienced by juvenile mussels must remain strictly speculative. However, the apparent rarity of young unionids in the field (Russell-Hunter 1964; Negus 1966; Neves and Widlak, 1987), with perhaps fewer than 0.0000001% of glochidia surviving even to their first year of life (Young and Williams, 1984), may in part reflect narrow physiological tolerance limits for young individuals of many species of mussels. In addition to examining other species, an obvious direction for future research is to follow the ontogeny of increased physiological tolerance as juvenile mussels mature. Limited

comparable studies of larval and juvenile marine bivalves clearly illustrate the development of physiological competence with age in some species (Widdows et al., 1989; Wang and Widdows, 1991; Baker and Mann, 1992).

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FIGURE LEGENDS

Figure 1. Schematic drawing of the system for exposing juvenile mussels to various experimental conditions. Removable glass tubes holding mussels are positioned in a rack and are immersed in chambers appropriate to the specific experiment.

Figure 2. Histograms depicting the survivorship of juvenile Utterbackia imbecillis at four experimental and one control temperature. Percentage based on N=60.

Figure 3. Histograms depicting the survivorship of juvenile Pyganodon cataracta at four experimental and one control temperature. Percentage based on N=60.

Figure 4. Histograms depicting the survivorship of juvenile Utterbackia imbecillis at three values of pH. Percentage based on N=60.

Figure 5. Histograms depicting the survivorship of juvenile Pyganodon cataracta at three values of pH. Percentage based on N=60.

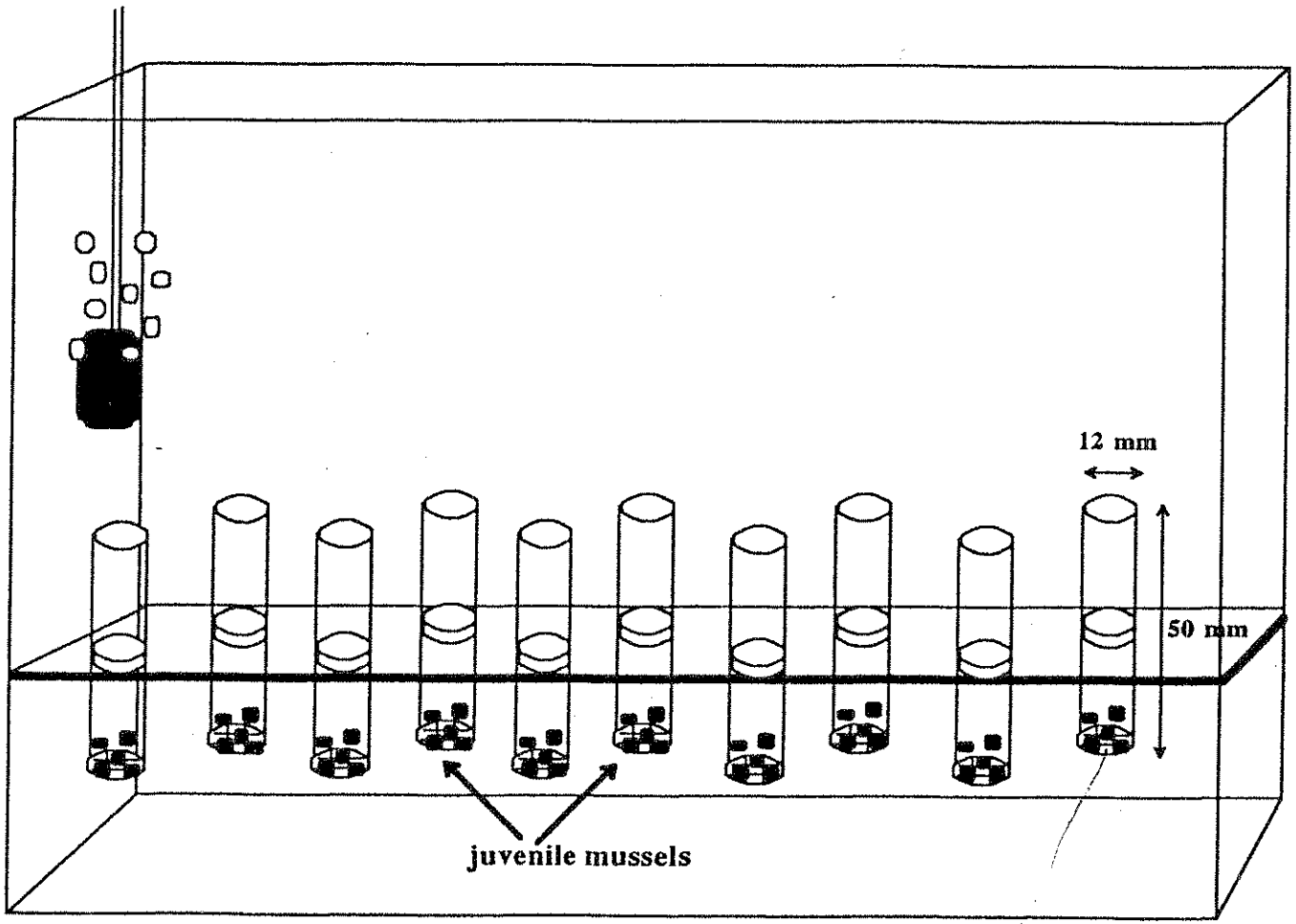

Fig. 1

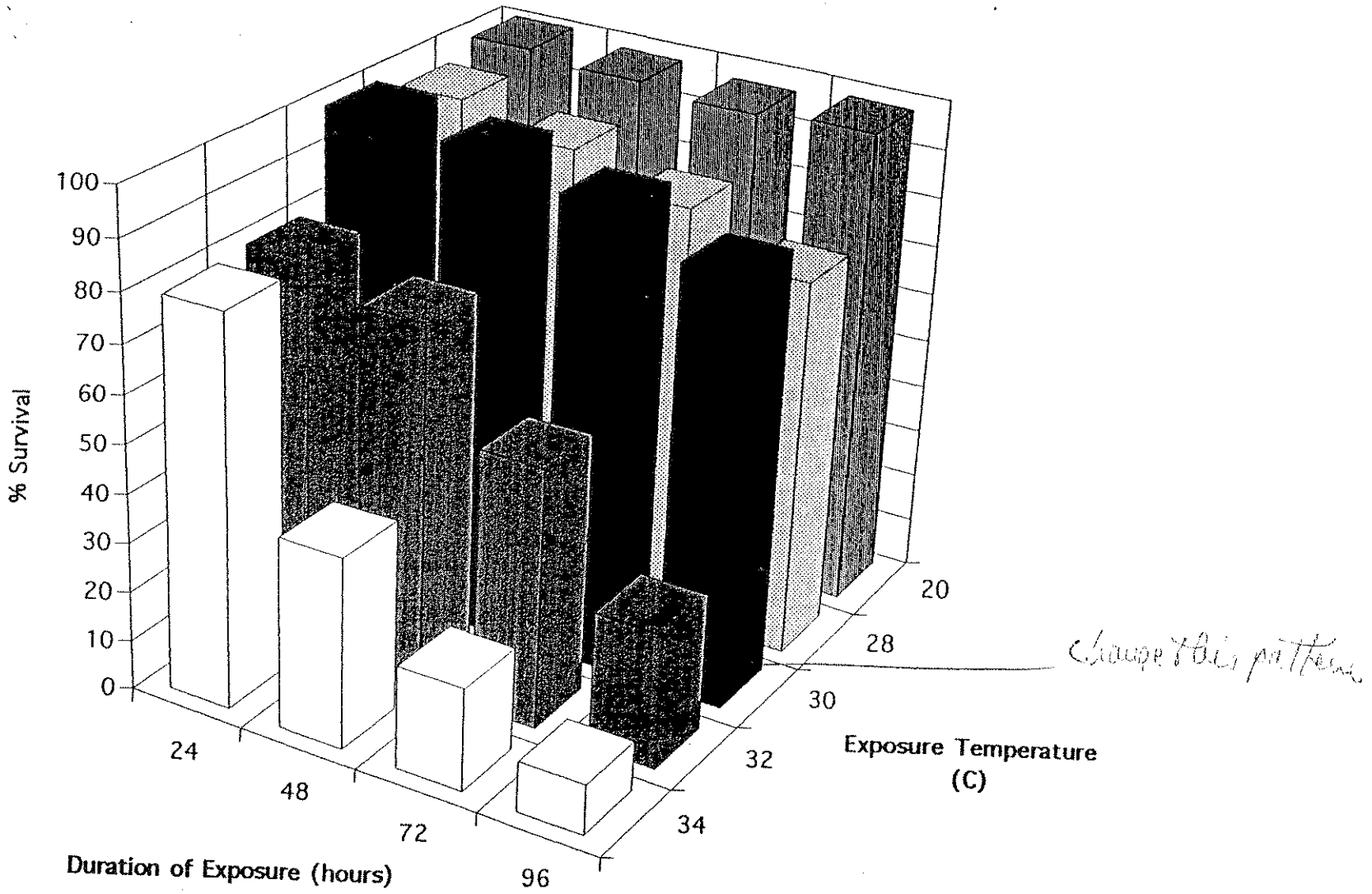
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Fig. 2

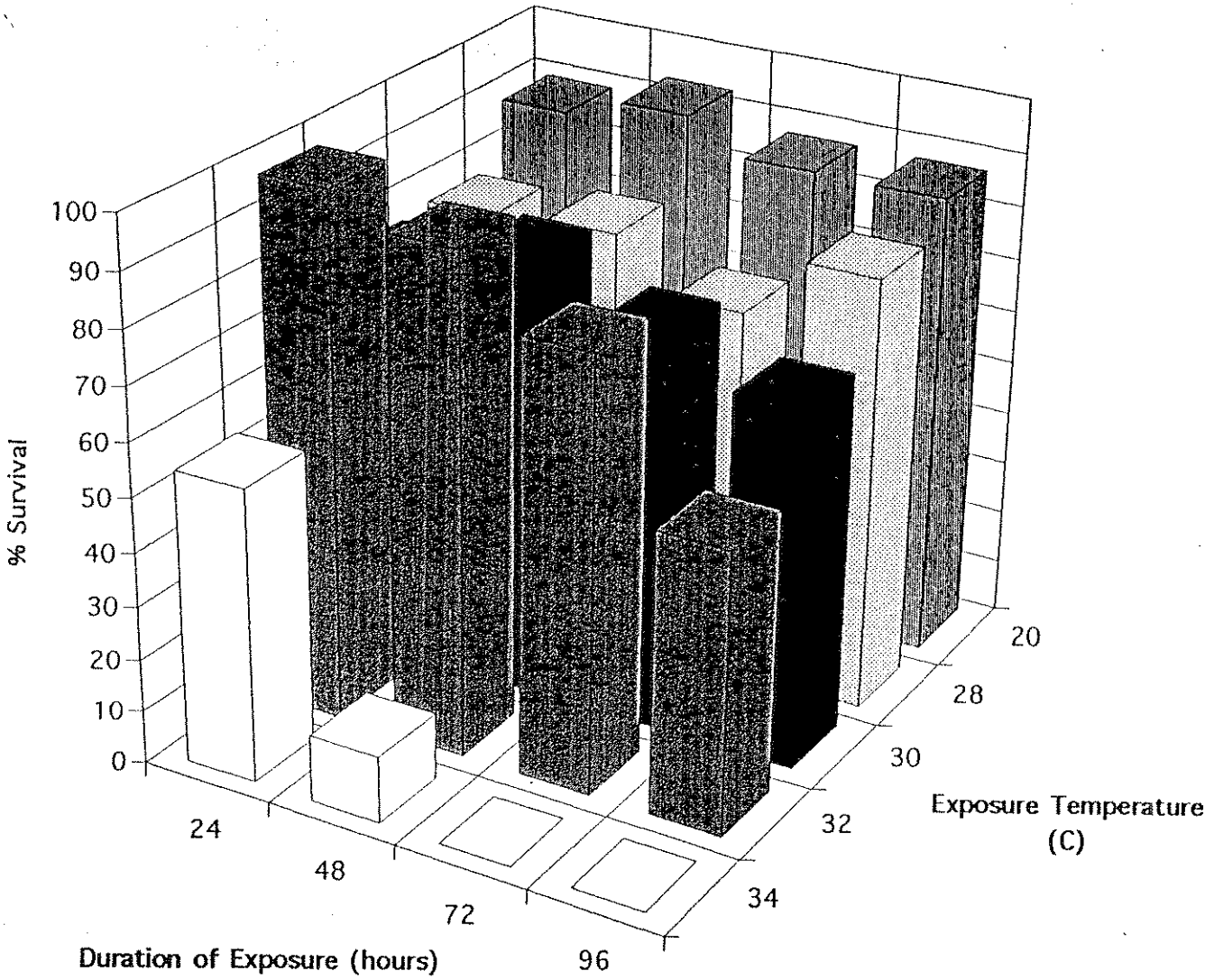
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Fig. 3

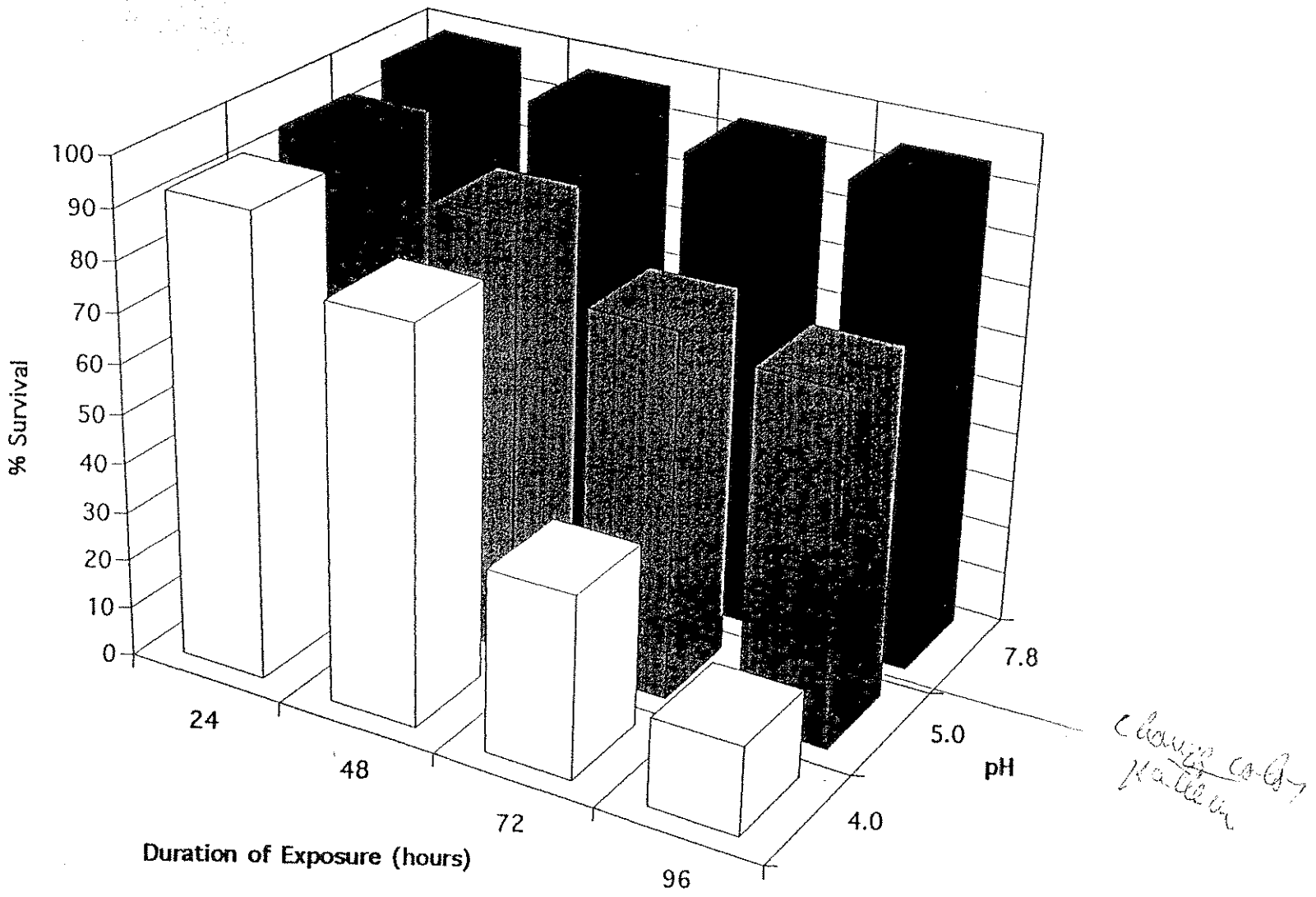
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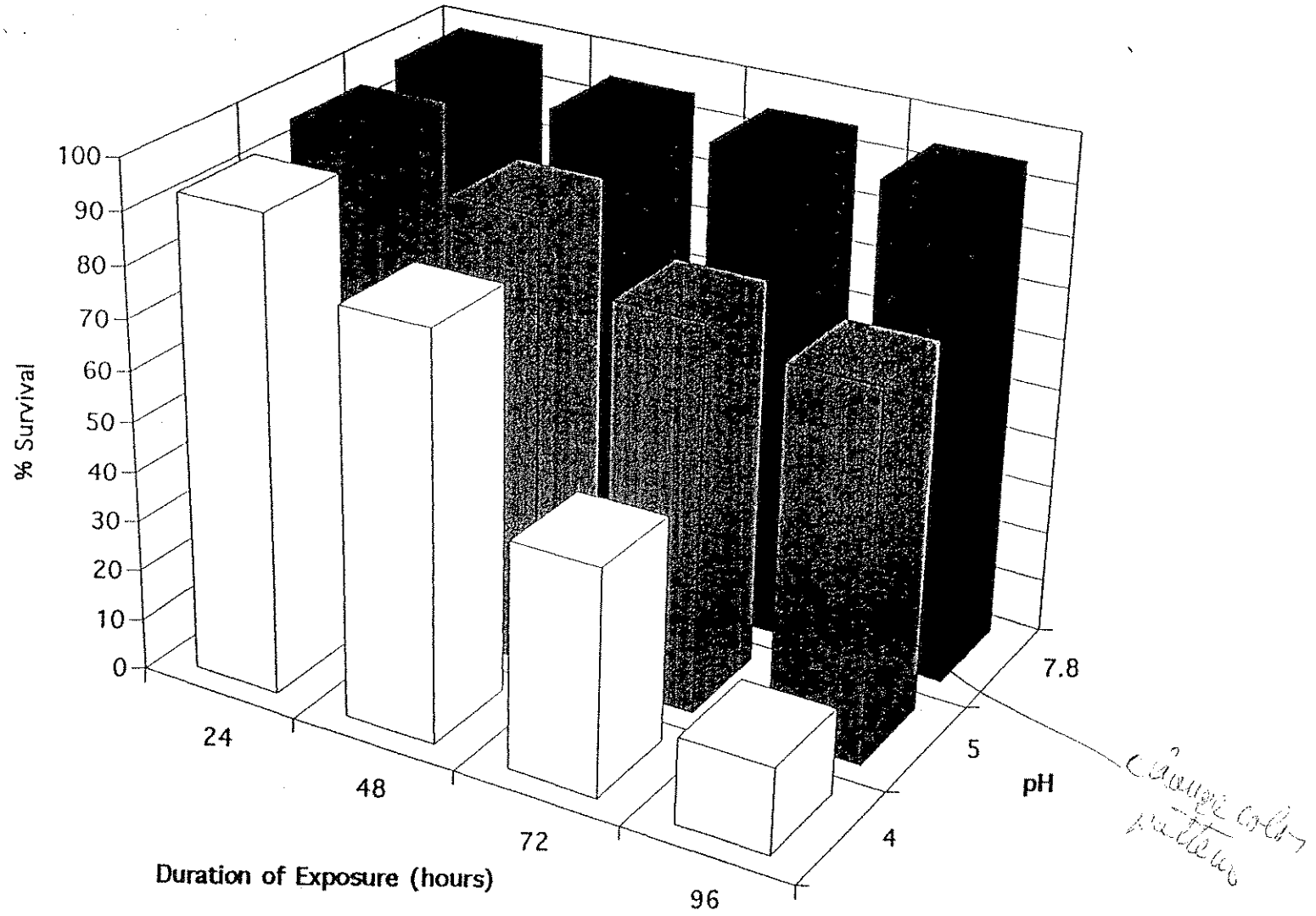
Fig. 4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7.8
pH

Fig. 5

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24 September 1993

Dr. Richard Neves
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You may feel free to correct, write on, and make suggestions on this copy of the manuscript. Please however use a cover sheet to make your recommendations and other comments about the paper. We are particularly interested in your opinion concerning whether we should publish the paper as is or after some revisions or not publish it at all.

I will be pleased to receive your review by mid-October or sooner. Thank you again.

Sincerely,

Robert R. Bryden

Robert R. Bryden

Sensitivity of Juvenile Freshwater Mussels to Hypoxic, Thermal and Acid Stress

Dimock and Wright

This manuscript is a welcomed addition to the scarce body of literature on young freshwater mussels. The paper is well written and concise, providing new data on environmental tolerances of juvenile mussels. In addition to marginalia included on the manuscript, I provide the following comments and questions:

1. Is the medium used more similar to Keller and Zarn (1990) than to Hudson and Shelbourne (1990)? Because Hudson's paper is an unpublished report, reference to the published literature would be more beneficial to interested readers.

2. Were the juveniles examined within the tubes or removed; i.e., were conditions maintained throughout the 96 hr period?

3. I wish the experiments had run for 7 days to provide better information on tolerance to stressed conditions.

4. On Figures 2-5, is there a better color pattern than solid black in the histograms? It may be just the photocopy, but the black and near black bars detract from the visual clarity of expressed results.

This paper is an important contribution to science and definitely worthy of publication in JEMSS.