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Responses of Red Drum *(Sciaenops ocellatus)* to Calcium and Magnesium Concentrations in Fresh and Salt Water

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ABSTRACT

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Experiments were conducted to determine some of the chemical aspects of water quality required by cultured red drum in fresh or salt water. Two studies were conducted in fresh and two in salt water differing in concentrations of calcium and magnesium. Red drum weighing 1-3 g each were stocked at 15 fish per 114-l tank. Treatments were replicated three times.

Environmental calcium significantly affected red drum survival in fresh and salt water; magnesium produced no discernible effect on performance. In saltwater [35 g/l total dissolved solids (TDS)] experiments, fish in experimental water containing less than 176 mg/l calcium exhibited 100% mortality within 96 h. Highest survivals were observed in water containing 340-465 mg/1 calcium. Red drum stocked in fresh water (0.56-1.9 g/1 TDS) with calcium concentrations 1.7 mg/1 or less performed poorly (0-33% survival after 96 h). Growth and survival were not significantly affected when calcium was between 9 and 407 mg/1. These data are consistent with the recognized physiological effects of calcium on membrane permeability and its postulated function in pore or channel mechanisms.

Generally, fish from the first fresh- and saltwater experiments had significantly better long-term (42 days) survival than those from the second set of experiments. This appeared to be related to the use of acid-washed biofiltration media (hypothetically related to the removal of essential trace components) in the second trials.

INTRODUCTION

The Texas Parks and Wildlife Department (TPWD) has been involved with the culture of red drum (*Sciaenops ocellatus*) fry for over 10 years. The technology for spawning and larval rearing is well established (Arnold et al., 1976, 1977; Colura et al., 1976; Roberts et al., 1978a,b; G. McCarty, personal communication, 1987). A number of TPWD red drum introductions have been successful in power plant cooling reservoirs, inland lakes with hard waters (high

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levels of calcium and/or magnesium) and in several low salinity impoundments of West Texas (R.L. Colura, personal communication, 1987). These introductions have demonstrated the ability of the red drum to adapt to a diverse range of environmental conditions (marine and fresh water).

Under optimal conditions, red drum have attained sizes from 0.5 to 1.4 kg in 1 year (Bearden, 1967; Luebke and Strawn, 1973; Arnold et al., 1977, R.L. Colura, personal communication, 1987). Red drum typically reach 450 g in 1 year on their natural feeding grounds (Pearson, 1929; Simmons and Breuer, 1962; Harrington et al., 1979).

Environmental calcium is required for proper development and hatching of the larvae of certain euryhaline or marine teleosts (Brown and Lynam, 1981; Lee and Hu, 1983; Lee and Krishnan, 1985). Crocker et al. (1983) observed that red drum fry transferred from salt water to fresh water exhibited a drop in blood osmolality. This decline was reduced by the addition of calcium. Miranda and Sonski (1985) indicated the importance of an optimum chloride level (above 130 mg/1) for good red drum survival in fresh water. However, they noted that some additional, unidentified ion appeared to be critical in this respect. All of those observations are consistent with the successful survival and growth of red drum in hard fresh water. Hard water can result from the presence of calcium and/or magnesium. Apparently, it is the presence of one of these ions in natural waters that improves the survival of red drum.

The purpose of this research was to evaluate the effects of environmental calcium and magnesium in fresh and salt water on the performance of red drum. The work was conducted at the Aquaculture Research Center of the Department of Wildlife and Fisheries Sciences, Texas A&M University.

MATERIALS AND METHODS

Four experiments were conducted to determine the importance and requirement of the divalent ions calcium and magnesium for juvenile red drum. Two experiments were conducted in fresh water containing 1.9 g/l or less total dissolved solids (TDS) and two in salt water (35 g/l TDS). An experimental unit was composed of a 114-l tank maintained by an undergravel airlift biofilter. Pea gravel rinsed with trace calcium water was used for biofiltration in the initial fresh- and saltwater experiments while acid-washed sand was used for the second set of experiments.

Three experimental units were assigned for each treatment in each experiment. The replicated tanks were each stocked with 15 red drum weighing from 1 to 3 g. Prior to initiation of the experiment, fish were held for 28 days in an environment similar in composition to that of the control for each trial. Fish were fed daily at the rate of 5.0-6.0% of individual tank biomass with commercial salmon feed (48% protein). Feeding rates were adjusted after biweekly weighings. Trials were continued for 56 days or until the fastest growing fish increased in weight by 500%, whichever occurred first. Food conversion efficiencies (FCE) were calculated for all experiments [(weight gained/weight of feed offered) X 100]. The studies were conducted from July 1983 to August 1985.

Water temperature and dissolved oxygen were measured in each replicate three times weekly, and total ammonia-nitrogen, total nitrite-nitrogen and pH were measured once weekly. If high levels of ammonia-nitrogen or nitrite-nitrogen were observed, all replicates were checked twice weekly for that variable. No palliative measures were taken.

Experimental water was prepared from well water containing trace quantities of calcium and magnesium (Table 1). Reagent-grade salts were used to prepare water differing in levels of calcium or magnesium. Only chloride salts were used in saltwater experiments to block the effect of other anions. The second freshwater experiment had equimolar chloride levels for all divalent ion levels to rule out any chloride effect. A representative sample was taken from each divalent ion level to check calcium and magnesium concentrations. Samples were collected at the beginning, on day 28 and at the conclusion of the experiment. Titrations were performed on samples from the initial fresh- and saltwater experiments while atomic absorption spectrophotometry was used for the second set.

Fish for freshwater trials were pre-acclimated in low-salinity water (5 g/l TDS) prepared with commercial synthetic sea salts (Table 1). The first study was to determine environmental calcium requirements, the second, magnesium. Tank water was allowed to change with respect to total dissolved solids concomitantly with increasing levels of divalent ions.

TABLE 1

Concentrations (mg/l except for pH) of major ionic constituents in well water, sea water [natural or formulated with synthetic sea salts (35 g/l TDS)] , dilute sea water (5 g/l TDS) and vertebrate extracellular fluid (ECF)

Ion	Well water	Sea water[1] (35 g/l TDS)	Dilute sea water (5 g/l TDS)	ECF[2] (9 g/l TDS)
Sodium	170	10685	1526	3265
Potassium	1	396	57	195
Calcium	Trace	410	59	100
Magnesium	Trace	1287	184	36
Chloride	62	19215	2745	3652
Bicarbonate	249	142	20	1708
Sulfate	78	2511	359	48
Nitrate	Trace	-	-	-
pH	8.4	7.8-8.4	7.8-8.4	7.4

[1]Gross (1977).

[2]Guyton (1971).

The first freshwater experiment involved exposing fish to six levels of calcium ranging from a trace to 400 mg/l. Magnesium concentrations were to be constant at 50 mg/l. Both divalent ions were added as chlorides. Fish were fed at 5.0% of biomass daily.

The second freshwater experiment had 10 levels of magnesium ranging from a trace to 240 mg/l. Calcium concentrations were formulated at 50 mg/l. Two trace calcium concentrations were included to determine if there were absolute requirements for that ion or magnesium in fresh water. Magnesium was added as sulfate while calcium was added as chloride. Fish were fed at 6.0% of biomass daily.

The control for the saltwater trials was a 35 g/l TDS formulation containing both calcium and magnesium. In each trial one group of fish was exposed to equimolar concentrations of calcium and magnesium. Any change in total dissolved solids created by varying the levels of divalent ions in the water was corrected to 35 g/l TDS by adding sodium and potassium as chloride salts in a ratio similar to that of sea water (96:4 Na:K).

The first saltwater trial was conducted to determine absolute requirements for calcium and/or magnesium with respect to presence and ratio. The control in that instance was salt prepared using commercial synthetic sea salts, offering both a natural ratio and concentration of calcium and magnesium (Table 1). Four additional ratios of calcium: magnesium were formulated. Fry were stocked at 14 per tank and fed at 5.0% of biomass daily.

The second saltwater trial consisted of nine calcium levels ranging from a trace to 400 mg/l with magnesium held constant at a calculated level of 240 mg/l. An additional trace magnesium and 400 mg/l calcium formulation was also evaluated. Fish were fed at 5.5% of biomass daily.

Final statistical analyses for all experiments were performed on percent growth and survival and involved one-way analysis of variance and Duncan's multiple range test (Ott, 1977). Survival data were transformed using the arcsine method suggested by Mostellar and Youtz (1961). In addition, one-way analysis of variance and multiple comparison tests were performed on ammonia-nitrogen and nitrite-nitrogen data from all replicates for each experiment. When data sets of equal size were compared, PROC ANOVA (Helwig and Council, 1979) and the previously mentioned multiple comparison test were performed. If data sets from replicates within an experiment were unequal, PROC GLM (Helwig and Council, 1979), and Fischer's protected least significant difference (LSD) test (Ott, 1977) were performed. Results were reported as significant with a $P \leq 0.05$ for analysis of variance and multiple comparison tests.

RESULTS

The first freshwater trial of 56 days was designed to determine the lower limit of calcium required by red drum. Calcium contamination of unknown origin affected the initial levels (designed to be trace, 25, 50, 100, 200 and 400 mg/l). Magnesium levels

TABLE 2

Mean survival, percentage weight gain, and food conversion efficiency of red drum exposed for 56 days to various initial concentrations of calcium and a calculated initial magnesium level of 50 mg/l (first freshwater experiment)

Initial calcium concentration (mg/l)	Survival (%)	Weight gain (%)	Food conversion efficiency (%)
19	60	583	83
47	67	600	83
71	53	613	91
124	69	581	83
204	67	563	91
403	78	518	83

were similar to the anticipated 50 mg/l level (ranging from 38 to 51 mg/l at the onset of the experiment). There were no significant differences among experimental groups in terms of red drum survival or growth. Mean survivals ranged from 87 to 98% at 14 days and 53 to 78% at 56 days. Fish experienced mean weight increases ranging from 518 to 613%. Mean FCE values ranged from 83 to 91% (Table 2).

Dissolved oxygen remained above 6.0 mg/l in each experimental group. Temperature ranged from 26 to 30°C with a trial mean of 27.6°C. Initial pH ranged from 8.3 to 8.4 with 56-day, log transformed means of 7.6-7.9 for all groups. Ammonia-nitrogen had a mean 56-day range of 0.05-0.2 mg/l and nitrite-nitrogen a mean range of 0.06-0.45 mg/l. No significant differences were observed among nitrogenous waste concentrations.

The second freshwater trial was a 42-day experiment in which calcium was maintained at a mean concentration of 60 mg/l in all but two experimental groups. Magnesium concentrations ranged from a trace to 268 mg/l (Table 3). In one of the other two groups, both magnesium and calcium were present at the levels in well water. In the second, the calcium level was that of well water, but magnesium was added to create a 30 mg/l level. An additional group was exposed to 5 g/l TDS formulated from synthetic sea salts (Table 3).

There were significant differences among experimental groups with respect to red drum survival and ion concentration. Survival was poor in all but the 5.0 g/l TDS low-salinity sea water. No significant differences in growth were observed. Mean weight increase among experimental groups ranged from 370 to 701%. Mean FCE values ranged from 111 to 143% (Table 3).

Mean dissolved oxygen values for the second freshwater trial ranged from 5.1 to 6.9 mg/l. Initial pH ranged from 8.4 to 8.8 with 42-day, log transformed means of 6.5-8.4 for all groups; the lowest mean (6.5) occurred in the saltwater control. All other means were at or above 7.1. Mean ammonia-nitrogen concentrations during the trial ranged

TABLE 3

Mean survival, percentage weight gain, and food conversion efficiency of red drum exposed for 42 days to various initial concentrations of magnesium and calcium (second freshwater trial)

Initial ion concentration (mg/l)		Survival (%)[1]	Weight gain (%)	Food conversion efficiency (%)
Mg	Ca			
Trace	1	2^{w}	405	125
33	1	$7^{w,x}$	370	100
1.5	60	$20^{x,y}$	470	111
8	63	$11^{w,x,y}$	476	143
15	57	$22^{x,y}$	701	125
34	63	$22^{x,y}$	548	111
70	58	$20^{x,y}$	599	143
137	58	31^{y}	551	125
268	57	36^{y}	534	125
192[2]	61	89^{z}	504	111

[1]Means within a column followed by the same superscript letters are not significantly different based on Duncan's multiple range test.
[2] Saltwater control (5 g/l total dissolved solids).

from 0.04 to 1.03 mg/l. Fischer's protected LSD test for comparing means showed that the salt water was significantly higher than all other formulations with respect to ammonia-nitrogen (1.0 as compared with ≤0.5 mg/l). Mean nitrite-nitrogen concentrations ranged from <0.01 to 0.21 mg/l during the trial. Concentrations recorded from sea water were significantly higher than those from other formulations.

In the first 42-day saltwater trial, red drum were exposed to various magnesium-to-calcium ratios (Table 4). Changes in ionic concentrations within groups over the experimental period were generally small compared with the differences between groups, with one exception. The water with initial low (trace) magnesium levels increased to 6-18 mg/l by the end of the experiment.

Survival through 30 days was significantly affected by calcium concentration (Table 4). Fish in water having calcium levels of 30 and 62 mg/l experienced 100% mortality within 6 h of exposure. Growth comparisons were difficult to interpret due to a chronic disease problem with *Amyloodinium ocellatum* (a pathogenic dinoflagellate) in the control that necessitated termination of that part of the experiment after 37 days (Table 4).

High nitrogenous waste levels (1.1 mg/l mean ammonia-nitrogen and 2.1 mg/l mean nitrite-nitrogen) occurred in water containing 425 mg/l calcium and trace magnesium (Table 4). Water with 465 mg/l calcium and 270 mg/l magnesium had a mean nitrite-nitrogen level of 0.84. Values for ammonia-nitrogen and nitrite-nitrogen from the other groups were similar to those found in the freshwater trials. No

TABLE 4

Mean survival, percentage weight gain, and food conversion efficiency of red drum exposed for 42 days to various initial concentrations of calcium and magnesium (first saltwater trial)

Initial ion concentration (mg/l)		Survival after 30 days (%)[1]	Weight gain (%)	Food conversion efficiency (%)
Ca	Mg			
30	Trace	0^x	-	-
62	257	0^x	-	-
425	Trace	37^y	248^z	83
465	270	76^z	509^y	125
445[2]	1291	73^z	364^z	100

[1]Means within a column followed by the same superscript letters are not significantly different based on Duncan's multiple range test.
[2] Weight gain percentage and food conversion efficiency values for the last (control) treatment were based upon 37 days. The treatment was discontinued early because of an epizootic.

nitrogenous waste data were collected from tanks after they had experienced total mortality.

Initial dissolved oxygen and pH levels in the first saltwater trial ranged from 4.9 to 5.4 mg/l and 8.3 to 8.6, respectively. Over the 42-day trial, temperature ranged from 24 to 31°C with a mean of 27°C. Mean dissolved oxygen was 5.4 mg/l.

The second saltwater trial was conducted for 56 days. Calcium varied from a trace to over 400 mg/l. Magnesium ranged from 260 to 306 mg/l in eight groups and occurred at trace levels in the remaining two (Table 5). There were significant differences among experimental groups with respect to fish survivals and calcium concentrations (Table 5). Fish in water containing only trace levels of calcium experienced complete mortality within 2 h of exposure, and those exposed to 55-120 mg/l calcium experienced 100% mortality within 96 h. Red drum survival improved as calcium level increased (Fig. 1). However, after 56 days, highest mean survival was only 29%. No statistically significant differences were observed in growth among experimental groups that had fish surviving at the end of the trial. Mean FCE values ranged from 34 to 71%.

As in the first saltwater trial, final survival in water with high calcium and trace magnesium appeared to be impacted by chronically high ammonia-nitrogen loads (the mean was > 2.4 mg/l). Dissolved oxygen was at or above 5.0 mg/l in all experimental groups throughout the trial. Temperatures ranged from 21 to 28ºC with a mean of 25ºC. Initial pH ranged from 8.3 to 8.5 while 56-day, log transformed means ranged from 7.9 to 8.4.

Mean ammonia-nitrogen concentrations during the first 96 h ranged from 0.07 to > 1.8 mg/l. After 56 days, mean ammonia-nitrogen in groups with surviving fish ranged from 0.3 to > 2.4 mg/l. In general, means for 96-h ammonia- nitrogen concentrations

TABLE 5

Mean survival, percentage weight gain, and food conversion efficiency of red drum exposed for 56 days to various initial concentrations of calcium and magnesium (second saltwater trial)

Initial ion concentration (mg/1)		Survival (%)[1]	Weight gain (%)	Food conversion efficiency (%)
Ca	Mg			
Trace	Trace	0^{x}	-	-
Trace	260	0^{x}	-	-
55	270	0^{x}	-	-
78	268	0^{x}	-	-
120	264	0^{x}	-	-
176	254	$5^{x,y}$	238	50
246	267	9^{y}	374	71
340	256	$16^{y,z}$	386	71
416	306	29^{z}	311	63
359	Trace	$11^{x,y}$	227	34

'Means within a column followed by the same superscript letters are not significantly different based on Duncan's multiple range test.

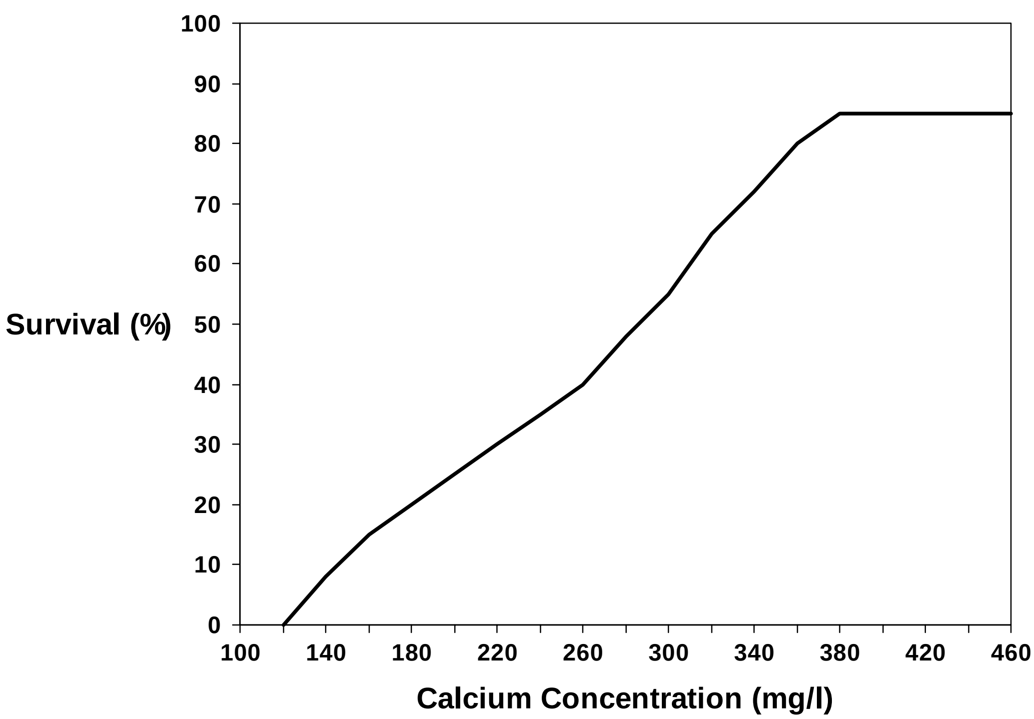

Fig. l. Survival of red drum after 96 h as a function of calcium concentration in the second seawater trial.

were greatest in those groups with high fish survival. Poor 56-day survival in the water with 359 mg/l calcium and a trace level of magnesium was coincidental with a mean ammonia-nitrogen concentration of > 2.4 mg/l. Mean nitrite-nitrogen concentrations from the first 96 h ranged from 0.13 to 0.36 mg/l. The highest values of 96-h ammonia-nitrogen and nitrite-nitrogen did not correlate with experimental groups that experienced complete mortality. No significant differences among mean nitrite-nitrogen concentrations were detected after 56 days.

Replicates in which fish were not adversely affected by divalent ion concentrations after 96 h, from all four studies, were compared for differences in survival at 42 days. Fish placed in the low-salinity (5 g/l TDS) water of the second freshwater trial had the highest mean survival (89%) which was not significantly different from the 465-mg/l calcium group in the first saltwater trial (74% mean survival) or those in the first freshwater trial that were exposed to calcium concentrations from 47 to 403 mg/l (71-82% mean survivals). Generally, mean survivals (11-47%) in groups from the second freshwater and saltwater experiments were significantly lower than those in the first trials. Mean survival in low-salinity (5 g/l TDS) water was the exception.

DISCUSSION

Calcium contamination occurred in the first saltwater and freshwater experiments. When acid-washed sand was used for biofiltration in the second set of experiments, the contamination problem was substantially reduced. It was then possible to determine absolute and basic range requirements for calcium and magnesium. The apparent increase in divalent ion concentrations over time in water initially containing trace levels may have resulted from their release by unutilized or undigested feed accumulated in biofiltration sand.

Results from the first freshwater trial indicated no significant differences in survival (Table 2) after 42 days for red drum placed in fresh water containing 19-403 mg/l calcium (53-78% survival). The second freshwater trial demonstrated that red drum performed significantly poorer (4-13% mean survival after 96 h) in fresh water containing 1.7 mg/l calcium or less. Mean survivals over 96 h at all other ion levels in the second freshwater trial ranged from 51 to 100%. Long-term survival also declined substantially in the latter study (compare Table 2 with Table 3).

Environmental magnesium offered no apparent advantage for red drum growth or survival in fresh water with concentrations between 1.5 and 268 mg/ 1 (Tables 3 and 4). In both saltwater trials, ammonia-nitrogen levels were significantly higher in water containing trace levels of magnesium than at other levels and could have affected growth and survival, particularly long-term survival. The efficiency of nitrifying bacteria appeared to be impaired in saltwater environments (35 g/l TDS) with trace concentrations of magnesium. Nitrifying bacteria oxidize ammonia through a nitrite intermediate to nitrate. Magnesium serves as a cofactor of phosphohydrolases and phosphotransferases (Lehninger, 1975). Without the ability to store or release energy in phosphate bonds, it would not be possible for nitrifying bacteria to carry out the most basic metabolic functions. Sufficient magnesium may have accumulated (feed and feces) in biofiltration sand by the end of both experiments for partial nitrification to occur, converting high ammonia-nitrogen to high nitrite-nitrogen concentrations.

Except for water with trace magnesium levels, in saltwater trials, water quality was poorest in experimental groups that demonstrated highest survivals, presumably due to greater fish biomass and corresponding larger feed and waste loads. In both saltwater trace magnesium groups, ammonia-nitrogen concentrations remained elevated until the final 7-14 days of the experiment when nitrite-nitrogen levels peaked. Trial means for ammonia-nitrogen and nitrite-nitrogen concentrations in all experiments were, in most instances, well below values reported to be deleterious by Holt and Arnold (1983).

There is considerable evidence to indicate that the ionic composition of a teleost's environment directly affects either gill ionic exchange mechanisms and/or their permeability (passive) to certain ions and water (Pickford et al., 1966; Potts and Fleming, 1970, 1971; Bornancin et al., 1972; Fleming et al., 1974; Eddy, 1975; Ogawa, 1975; Carrier and Evans, 1976; Isaia and Masoni, 1976; McWilliams and Potts, 1978; Gallis et al., 1979; Pic and Maetz, 1981). The results of our experiments appear to substantiate that hypothesis. The concentration of environmental calcium appears to directly affect survival of red drum in both salt and fresh water.

Cell membranes, vital to the maintenance of intra- and extracellular ion concentrations, appear to be important ion permeability barriers in fish. The presence of specific cellular pores in or channels from excitable tissues has been studied by Matsuda and Noma (1984) and Moczydlowski et al. (1985). It is postulated that the membrane pore is lined or gated with positively charged prosthetic groups. Further, it is theorized that the prosthetic groups are divalent cations; calcium, in particular. These ions are attached - presumably to binding sites - along the surface of the pore (Frankenhaeuser and Hodgkin, 1957; Solomon, 1960; Guyton, 1971; Moczydlowski et al., 1985). It is believed the charged fields of divalent calcium ions allow only certain molecules to pass through the membrane pore. Positively charged calcium ions lining the pore restrict the passage of other positively charged ions on the basis of the magnitude of their charged sphere. Ostensibly, the pore (passive mechanism) coupled with energy-dependent ion pumps (active mechanism) sustain ionic gradients. It has been hypothesized (Frankenhaeuser and Hodgkin, 1957; Guyton, 1971) that a sudden yet temporary removal of the charged calcium that lines the membrane pore allows sodium to more readily penetrate or move through the membrane.

The results of the ion experiments presented above can be related to the ion pore theory. In low-calcium aquatic environments, the ion pores of the surface epithelia would be submaximally saturated with calcium. This would lower the kinetic energy necessary to strip calcium from the pore. If environmental calcium concentrations were sufficiently low, a rapid and spontaneous flux of sodium (possibly potassium as well) could occur. Diffusion would be rapid enough that active (energy dependent) uptake or elimination of ions could not compensate. Death would occur as a result of altered circulatory volume and/or disrupted ion metabolism. This is consistent with the results observed in the present study.

It has been recognized that the intensity of stimulus necessary to initiate sodium influx in nerve cells can be reduced by lowering the concentration of calcium in the extracellular fluid (Frankenhaeuser and Hodgkin, 1957; Guyton, 1971). Presumably, this occurs in response to an altered binding affinity for calcium in the membrane pores. If calcium concentrations are sufficiently low in the extracellular fluid, spontaneous sodium influx will occur (Frankenhaeuser and Hodgkin, 1957; Guyton, 1971).

The diffusional gradients for sodium and calcium across the surface epithelia of fish placed in low-calcium (less than 100 mg/l) sea water (35 g/l TDS) are in opposite directions. Sodium ions diffuse from sea water into the extracellular fluid while calcium would be driven towards sea water. The sharp sodium gradient causes sodium to influx with high energy. Low calcium concentrations in the environment would tend to dislodge calcium from its ion pore binding sites. As calcium begins to flux, the influx of sodium would become rapid, pushing free calcium ions toward the extracellular fluid. Apparently, the force of influxing sodium is so great that a minimum concentration of 176 mg/l calcium (lowest calcium level that supported red drum survival) in sea water is necessary to begin saturating ion pore binding sites, thus retarding the influx of sodium and perhaps, that of potassium. Sodium influx and the concomitant efflux of water are too great in saltwater environments containing 120 mg/l calcium or less (groups with 100% mortality). The resultant loss of fluid volume and the increased sodium (and potassium) content of the extracellular fluid may cause circulatory shock and cardiac failure (Guyton, 1971), resulting in death.

In fresh water, the diffusional gradients for sodium, potassium and calcium are in the same direction across the surface epithelia. This unidirectional ion flow, through the ion pores, is the most reasonable explanation that the euryhaline red drum can tolerate much lower calcium concentrations in fresh water than in salt water, 19 mg/l as opposed to 176 mg/l, respectively. Since calcium diffuses in the same direction as sodium and potassium and at a relatively constant concentration, it would keep ion pore binding sites in a state of comparative saturation, thus retarding ion effluxes. However, when the environmental concentration falls below a minimum level (1.7 mg/l in this study), the kinetic energy driving calcium and monovalent ions through the pore would tend to continuously desaturate calcium binding sites allowing sodium and potassium (to a lesser extent, calcium) to diffuse into the environment at a rate greater than active uptake mechanisms could replace them. There would be a simultaneous water influx. The net effect would be the reduction in concentration of these ions in the extracellular fluid. When ionic concentrations reach a critical low level, cardiac spasms (low ionic tetany) result (Guyton, 1971), causing death.

Fish in the low-salinity (5 g/l TDS) control from the second freshwater trial had the highest overall survival after 42 days. That was in water formulated with synthetic sea salts. One contention might be that performance was best in the control because fish had been adapted to that salinity prior to the experiment. However, there is an equally compelling physiological argument.

A physiological saline solution formulated with sodium chloride is approximately isotonic with vertebrate extracellular fluid and has a salinity of approximately 9.0 g/l TDS. If one makes a salt solution (using sodium chloride) equimolar with respect to sodium, as found in vertebrate extracellular fluids (Guyton, 1971), the resultant salinity would be 8.3 g/l TDS. Sea water diluted to 5 g/l TDS has concentrations of sodium, potassium and calcium of 1526, 57 and 59 mg/l, respectively. Vertebrate extracellular fluid contains sodium, potassium and calcium at 3265, 195 and 100 mg/l (Table 1). While extracellular fluid is higher in these ionic components, the 5 g/l TDS synthetic seawater formulation provided fish with an ample environmental reservoir of these ions for active uptake and/or exchange. The presence of these environmental ions in comparatively high concentrations reduced the diffusional gradient, relative to sea water or fresh water, driving their efflux from fish extracellular fluid and provided the postulated ion pore (permeability barrier) with an external supply of calcium. Since the ionic composition of the environment was lower than fish extracellular fluid and was separated from it by a semi-permeable membrane or pores, water was drawn into the extracellular fluid by osmosis. This would create a condition of fluid loading which could be beneficial in preventing reduction of circulatory volume and cardiovascular failure as a result of shock (Guyton, 1971) which might be induced by handling stress. Perhaps what is most beneficial is the calcium: sodium ratio, 410:10685 (sea water 35 g/l TDS), 59:1526 (dilute sea water, 5 g/l TDS) or 0.038:1. This may be the optimal ratio for maintaining the integrity of ion pores.

Another notable disparity from the overall comparison of red drum survivals is that survival at 42 days was significantly lower for fish in the second set of saltwater and freshwater experiments except in the low-salinity (5 g/l TDS) control. The most obvious difference between the first and second set of ion studies was the use of acid-washed sand for biofiltration media in the second series of trials.

Several researchers (Pang et al., 1980; Payan et al., 1981; Mayer-Gostan et al., 1983) have noted that fish can absorb calcium directly from the environment. Fish may absorb trace elements not supplied by their diet directly from the environment. Studies by Phillips et al. (1959, 1960, 1961), and Podoliak and McCormick (1967) have shown that trout can absorb calcium, strontium, chloride, phosphorus and sulfate from their environment. Berman (1969) observed that manganese and zinc are readily absorbed from the water by *Abramis brama* and *Esox lucius*. It is possible that acid washing removed some vital component, not supplied by the diet, that was supplied to the water by biofiltration gravel or sand much as calcium was in the first studies. Trace elements are routinely added to commercial synthetic sea salts of the type used to formulate the low-salinity control which displayed good survivals.

It is evident that environmental calcium concentration can profoundly affect red drum survival. This effect appears to be related to a calcium mediated, passive permeability barrier to monovalent ions (i.e., sodium and potassium) at gill and body surfaces. High levels of calcium decrease passive permeability.

In fresh water, the minimum calcium concentration for red drum culture should be no less than 25 mg/l; levels from 50 to 100 mg/l appear to be more desirable. Saline waters with 35 g/l TDS should have calcium concentrations no less than 340 mg/l, with a preferred concentration of 400 mg/l. It is possible that long-term acclimation (28 days) might lower these requirements. However, the need for rapid adaptation and stocking in aquaculture may render long-term tempering impractical.

When saline waters containing 9-35 g/l TDS are being considered for red drum culture, it is advisable to seek a calcium: sodium ratio approximating 0.038:1. In waters with levels of TDS below 9 g/l, the calcium concentration recommended for fresh water should be adequate. It is important to test for the presence of other ions as well, particularly those of physiological importance (e.g., sodium, potassium, bicarbonate and chloride). Some minimum trace element profile may also be required. Further research in both of these areas is needed.

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