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Acute toxicity of Calcium chloride on different stages (Egg, Spawn, Fry and Fingerling) of rohu (*Labeo rohita*, Hamilton)

Mallick A.[1,2*], Mohapatra B.C.[2] and Sarangi N.[2]
[1]Department of Environmental Science, University of Kalyani, Kalyani, West Bengal -741235, INDIA
[2]Central Institute of Freshwater Aquaculture, (Indian Council of Agricultural Research), Bhubaneswar- 751 002, Odisha, INDIA

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Abstract

The acute toxicity of calcium chloride ($CaCl_2$) was studied on the early stages of Indian major carp rohu, Labeo rohita (egg, spawn, fry and fingerling) through bioassay tests. Percentage of dead or damaged egg, spawn, fry and fingerling at 6, 12, 18, 24, 36, 48, 60, 72 and 96 hours were recorded for the calculation of LC_{50}. The increase in $CaCl_2$ concentration in water increased the toxicity and reduced the duration to damage 50% of the eggs, spawn, fry and fingerling. Low hatchability, delayed hatching, poor survival observed in the test solutions >1000ppm concentration. The deformed and dead larvae were observed through microscopic examinations. The behavioural studies such as swimming, surfacing, activeness and survival were also observed in spawn, fry and fingerling. The LC_{50} values of $CaCl_2$ for egg, spawn, fry and fingerling were 3743.79-28.93ppm, 7249.78-1260.73ppm, 9872.44-6384.12ppm and 12276.52-9987.67ppm in 6 - 96 hours respectively.

Keywords: Acute toxicity, calcium chloride, Rohu, Bioassay, LC_{50}

Introduction

Aquatic organisms differ from terrestrial organisms in their metabolic requirements for calcium that typically can be met by absorbing this mineral from the water in which they live. Both shrimp and fish can absorb some minerals from the water via drinking (primarily marine organisms), and by direct absorption via gills, fins and skin. The gills are the most important sites of calcium regulation in freshwater and marine fish. Freshwater of moderate hardness (50 mg/l as $CaCO_3$) as well as brackish water and sea water, contain much higher levels of dissolved calcium; therefore, dietary supplementation of calcium for various fish and crustacean species is generally not necessary. The calcium helps to protect the freshwater fish against environmental toxicants, osmotic and ionic losses. The excessive levels of dietary calcium should be avoided because it can negatively affect the utilization of other minerals. The uptake of ions from the water may be driven by the perivitelline potential, ion exchange, transporting enzyme or by any combination of these[1-3]. Calcium has a marked effect on sodium fluxes in both marine and freshwater organisms[4-6]. In few fish species, which have so far been examined, the effects of external calcium appear to be primarily on sodium efflux, which is reduced in the presence of calcium[4,6]. Fish embryos maintain the constancy in the ion concentrations and osmolality through their body fluids and the skin chloride is responsible for the active transport of ions[7-9]. The physicochemical parameters of the water body affects the fish production and diversity[10,11]. The acute toxicity of mercury to *Clarias gariepinus* and tannery chromium to *Labeo rohita* were studied by Guedenon et al and Praveena et al respectively[12,13]. Eknath[14] studied the toxicity of detergents on *Mystus montanus* fish and the behavioural changes. In the present investigation studied the toxicity of calcium chloride concentrations on different stages of rohu and the LC_{50} dose over a period of 6-96 hours.

Material and Methods

Test organism: The rohu (*Labeo rohita*) was selected as test organism. The egg, spawn, fry and fingerling of appropriate quality of rohu were selected for the experiment. Spawn, fry and fingerlings were collected from the hatchery of Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar. The organisms were brought to the laboratory for in vitro study. The spawn were acclimatized in the glass aquaria for one day, but in case of fry and fingerlings they were acclimatized in plastic pool for a period of two days before bioassay.

Test container: Glass jar tanks of 20 liters capacity were used as test containers. Before using these were cleaned with laboratory detergents, then with 100% acetone and tap water. After each test, the containers were washed appropriately with acid to remove metals, bases and organic compounds. Each of the test containers were provided with facilities of continuous aeration and covered with velon screen netting to prevent the test organisms from jumping out.

Test concentration: Selections of test concentrations were made following the APHA method[15]. At least five different concentrations were taken in each experiment. While designing, minimum of five exposure (treatment) concentrations of a test substance and one control was taken for bioassay test. In the present study the test organisms were exposed to a wide range of

calcium concentrations in different stages of rohu. The concentrations were as far as possible selected in logarithmic or geometric scale. For egg, spawn, fry and fingerling 0.5- 10000, 20-20000, 0.75-11250, and 200-11250ppm of $CaCl_2$ were selected respectively.

Bioassay: The bioassay was conducted for different stages of rohu (egg, spawn, fry and fingerling). Four experiments were conducted for bioassay test. These experiments were egg bioassay (experiment-1), spawn bioassay (experiment-2), Fry bioassay (experiment-3) and Fingerling bioassay (experiment-4). Different concentrations of $CaCl_2$ were used as the test solutions.

Data analysis: The data obtained from the experiments were processed by Probit analysis for determination of LC_{50} values using SPSS statistical software[16-18]. The lethal concentrations were plotted against time in hours to get "Toxicity curve"[19].

Results and Discussion

The embryonic development and LC_{50} values were determined by applying $CaCl_2$ as a toxic agent to the developmental stages of rohu (figure 1-4). The toxic effect of $CaCl_2$ was observed in different concentrations and exposure time. The percentages of damaged or dead eggs were varied in different concentrations. In higher concentration (>1000 ppm) the percentage of damaged eggs were increased, eggs became smaller, whitish just before death and coelomic content turned opaque or white. In the hatchling stage the tail bended towards the back (figure 5a-d). Early life stages of the teleosts are known to be very sensitive stages. The sensitivity depends on the developmental stages and the time of exposure, physico-chemical characteristic of water, toxicant concentration and fish species[20-22]. In higher salt concentrations, high percentage of embryos exhibited deformation in the spinal cord and larval tail bended towards backside. The present findings corroborated with the observation of Hodson et al[23], in rainbow trout exposed to lead (Pb) at different pH. The exchange of cations like Ca^{2+} and Mg^{2+} is crucial for normal embryonic development[24]. A decrease in heart rate and pigmentation has also been reported for early life stages of freshwater rainbow trout exposed to a low pH or to a low ambient calcium concentration[25].

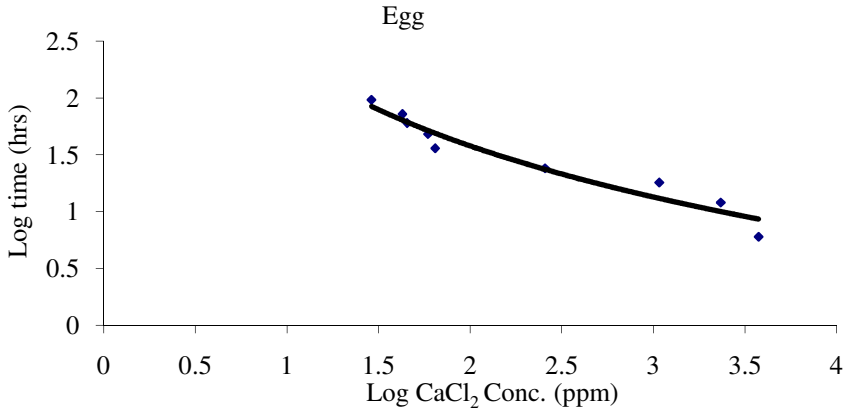

Figure-1
Toxicity curve of rohu eggs exposed to different lethal concentration of $CaCl_2$ solution

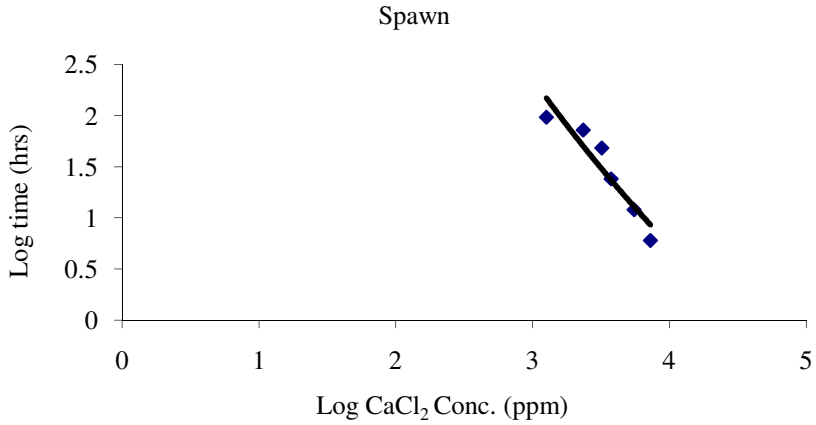

Figure-2
Toxicity curve of rohu spawn exposed to different lethal concentrations of $CaCl_2$ solution

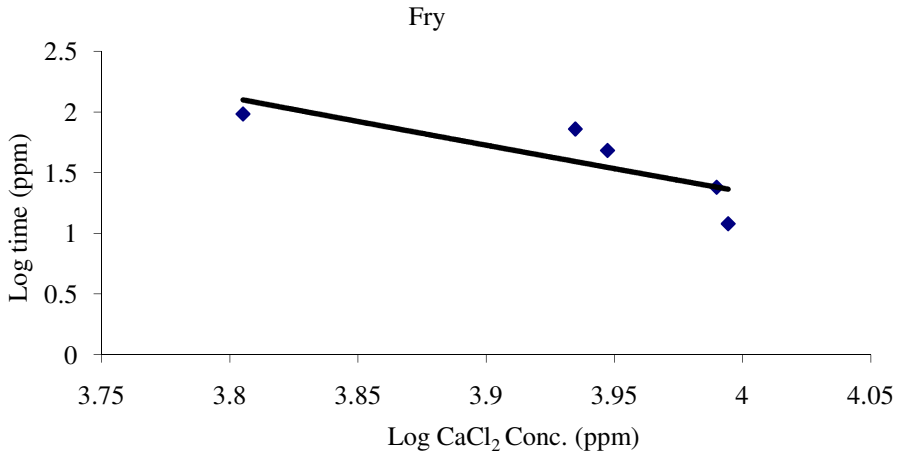

Figure-3
Toxicity curve of rohu fry exposed to different lethal concentrations of $CaCl_2$ solution

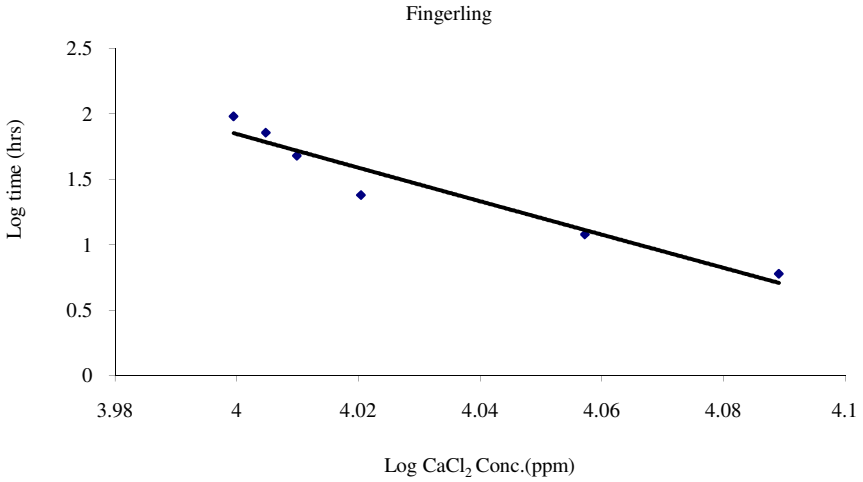

Figure-4
Toxicity curve of rohu fingerlings exposed to different lethal concentrations of $CaCl_2$ solution

The results of toxicity studies of $CaCl_2$ expressed in terms of LC_{50} values obtained from probit analysis shown in figure 1-4. The lethal concentration (LC_{50}) of $CaCl_2$ decreased gradually with the increase in exposure time from 6 to 96 hours (table 1). The LC_{50} values of $CaCl_2$ in case of egg were 3743.79, 2326.36, 1075.07, 255.95, 64.47, 58.89, 45.34, 42.72 and 28.93ppm after 6, 12, 18, 24, 36, 48, 60, 72 and 96hrs respectively. In case of spawn the LC_{50} values of $CaCl_2$ were 7249.78, 5507.77, 3738.12, 3192.77, 2343.94 and 1260.73ppm after 6, 12, 24, 48, 72 and 96 hrs respectively. The LC_{50} values of $CaCl_2$ in case of fry were 9872.44, 9770.46, 8858.51, 8605.69 and 6384.12ppm after 12, 24, 48, 72 and 96hrs respectively and in case of fingerling were 12276.52, 11407.75, 10481.15, 10229.91, 10110.34 and 9987.67ppm after 6, 12, 24, 48, 72 and 96hrs respectively. Metelev et al[25] observed that10000ppm $CaCl_2$ is toxic for white salmon, carp and perch after 16 to 29 hrs. Symptoms of poisoning in carp begin to appear at a concentration of 7000ppm $CaCl_2$. 13900ppm $CaCl_2$ is toxic after 10 days for juvenile brown trout. At 15000ppm $CaCl_2$ proved toxic for all fish in period ranging from one hour to several days[26]. According to Mohapatra[27] $CaCl_2$ is toxic to *Catla catla* at 7500, 6300, 4950, 4100 and 3950ppm after 12, 24, 48, 72 and 96hrs respectively and 24400, 23000, 21800, 21700 and 21400ppm after 12, 24, 48, 72 and 96hrs respectively in case of *S. mossambicus*. Eel can tolerate 27000ppm $CaCl_2$ $2H_2O$[25.] There are close inverse relationships between calcium concentration of water and chloride cell density in teleosts[28].

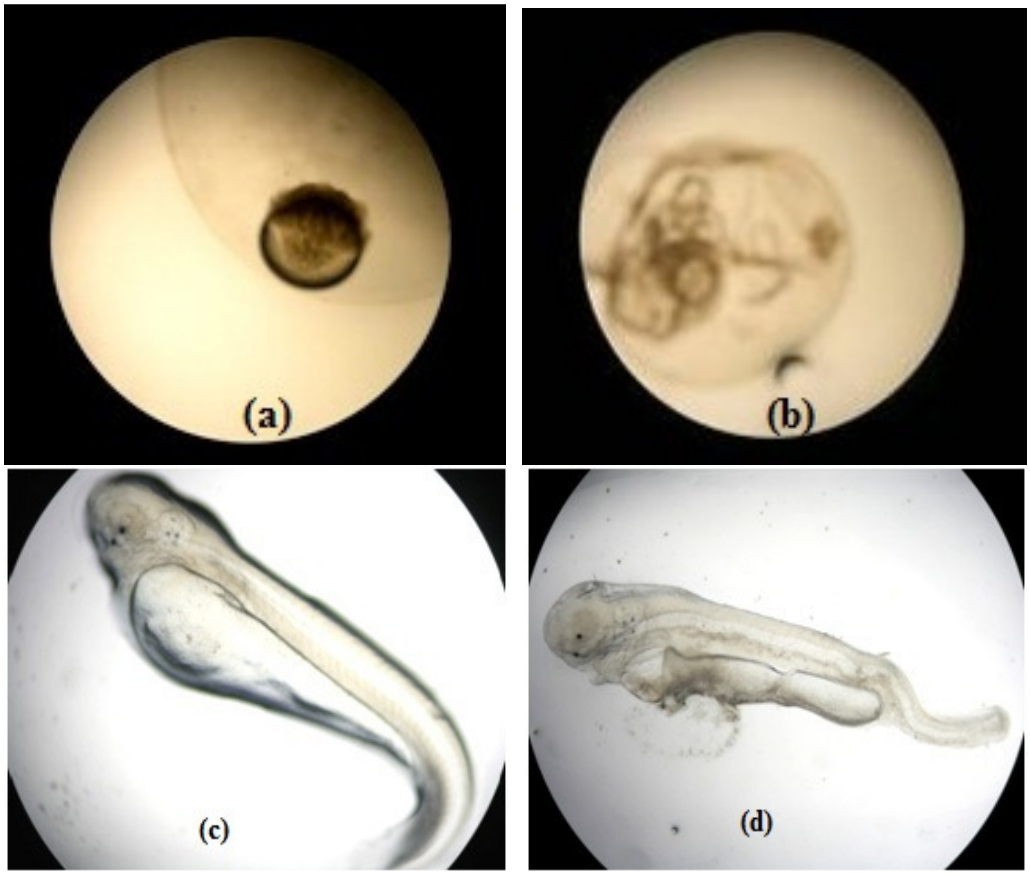

Figure-5
(a) Normal egg; (b) Deformed egg; (c) Normal spawn with fully developed yolk sac and other internal organs; (d) Deformed spawn with irregular yolk sac and bended tail

Table-1
LC_{50} values of calcium chloride ($CaCl_2$) for rohu egg, spawn, fry and fingerling

Exposure periods (hr)	LC_{50} values of $CaCl_2$ (ppm)			
	Egg	Spawn	Fry	Fingerling
6	3743.79	7249.78	-	12276.52
12	2326.36	5507.77	9872.44	11407.75
18	1075.07	-	-	-
24	255.95	3738.12	9770.46	10481.15
36	64.47	-	-	-
48	58.89	3192.77	8858.51	10229.91
60	45.34	-	-	-
72	42.72	2343.94	8605.69	10110.34
96	28.93	1260.73	6384.12	9987.67

Conclusion

Early larval stages are the most crucial and vulnerable one in the life cycle of fish. Hence, the rate of survival in these stages depends on the maintenance of water quality parameters such as alkalinity, pH and hardness in the hatchery system. Hardness (calcium and magnesium) is very important in hatchery system. The optimum water quality should be maintained by the farmer before going to operate a hatchery system. It is essential to know the calcium content in pond water as it is one of the important water quality parameter for fish culture. From the findings of this study it was concluded that > 1000 ppm of calcium in the aquatic environment can affect the larval stage (hatchling stage), but the LC_{50} values are varied in different stages.

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References

1. Peterson R.H. and Martin-Robichaud D.J., Perivitelline and vitelline potentials in teleost eggs as influenced by ambient ionic strength, natal salinity and electrode electrolyte; and the influence of these potentials on cadmium dynamics within the egg, *Can. J. Fish. Aquat. Sci,* **43,** 1445-1450 **(1986)**

2. Eddy F.B., Ward M.R., Talbot C. and Primmett D., Ionic movements across the chorion in newly shed salmon eggs *(Salmo salar L.). J. Comp. Physiol*, **159(B),** 771-776 **(1990)**

3. Shephard K.L., Ion-exchange phenomena regulate the environment of embryos in the eggs of freshwater fish, *Comp. Biochem. Physiol*, **88(A),** 659-662 **(1987)**

4. Fleming W.R., Nichlols J. and Potts W.T.W., The effect of low-calcium seawater and actinomycin-D on the sodium metabolism of *Fundulus kansae, J. exp. Biol,* **60,** 257-273 **(1974)**

5. Carrier J.C. and Evans D.H., The role of environmental calcium in freshwater survival of the marine teleost *Lagodon rhomboids, J. Exp. Biol,* **65**, 520-538 **(1976)**

6. Isaia J. and Masoni A., The effects of calcium and magnesium on water and ionic permeabilities in the seawater adapted eel, *Auguitla anguilla, L. J. comp. Physiol ,***109**, 221-233 **(1976)**

7. Hwang P.P. and Hirano R., Effects of environmental salinity on intercellular organization and functional structure of chloride cells in early stages of teleost development, *J. Exp. Zool*., **236**, 115–126 **(1985)**

8. Hwang P.P., Salinity effects on development of chloride cells in the larvae of ayu (*Plecoglossus altivelis*), *Mar Biol*, **107**, 1-7 **(1990)**

9. Ayson F.G., Kaneko T., Hasegawa S. and Hirano T., Differential expression of two prolactin and growth hormone genes during early development of tilapia (*Oreochromis mossambicus*) in fresh water and seawater: implications for possible involvement in osmoregulation during early life stages, *Gen. Comp. Endocrinol,* **95,** 143-152 **(1994)**

10. Deepak S. and Singh N.U., The Relationship between Physico-chemical Characteristics and Fish Production of Mod sagar Reservoir of Jhabua District, MP, India, *Research Journal of Recent Sciences*, **3(ISC-2013)**, 82-86 **(2014)**

11. Eknath C.N., The seasonal fluctation of physico-chemical parameter of Mula-mutha at Puna, India and their impact on fish biodiversity, *Res. J. Animal,Veterinary and Fishery Sci,* **1(1)**, 11-16 **(2013)**

12. Guedenon P., Edorh A.P., Hounkpatin A.S.Y., Alimba C.G., Ogunkanmi A., Nwokejiegbe E.G. and Boko M., Acute toxicity of mercury ($HgCl_2$) to African catfish, Clarias gariepinus, *Research Journal of Chemical Sciences*, **2(3)**, 41-45 **(2012)**

13. Praveena M., Sandeep V., Kavitha N. and Jayantha and Rao K., Impact of Tannery Effluent, Chromium on Hematological Parameters in a Fresh Water Fish, Labeo Rohita (Hamilton), *Research Journal of Animal, Veterinary and Fishery Sciences Veterinary and Fishery Sciences,* 1**(6)**, 1-5 **(2013**

14. Eknath C.N., Studies on Toxicity of Detergents to Mystusmontanus and Change in behaviour of Fish, *Research Journal of Animal, Veterinary and Fishery Sciences*, **1(9),** 14-19 **(2013)**

15. APHA, Standard methods for the examination of water and wastewater, 16th ed., American Public Health Association Water Pollution Central Federation and American Water Works Association: New York, USA **(1998)**

16. Finney D.J., A statistical treatment of the sigmoid response curve, *Probit analysis, 3rd edn. Cambridge University Press, London*, *333*, **(1971)**

17. Reish D.L. and Oshida P.S., Manual of Methods in Aquatic Environment Research, Part10, Short-term Static Bioassay, *FAO Fish. Technical paper,* **247,** 1-62 **(1987)**

18. Mohapatra B.C. and Rengarajan K., A Manual of Bioassays in the Laboratory and their Techniques, *CMFRI Spec. Pub*. **64**, CMFRI, Cochin, India, 75 **(1995)**

19. Seegert G.L., Brooks A.S., Castle I.R.V. and Gradall K., The effects of monochloramine on selected riverine fishes, *Transactions of the American Fisheries Society,* **108**, 88-96 **(1979)**

20. McKim J.M., Eaton J.G. and Holcombe G.W., Metal toxicity of embryos and larvae-early juveniles of eight species of freshwater fish II: copper, *Bull Environ Contam Toxicol,* **19**, 987–993 **(1978)**

21. Hutchinson M.J., Murr D., Krishnaraj S., Senaratna T. and Saxena P.K., Does ethylene play a role in thidiazuron-regulated somatic embryogenesis of geranium (Pelargonium x hortorum Bailey) hypocotyl cultures? In Vitro Cellular and Developmental Biology – Plant, **33**, 2, 136-141**(1997)**

22. Alabaster J.S. and Lioyd D.R., Water quality criteria for freshwater fish, *Eifac report* (FAO), 2nd edition, London, Butter Worth scientific, 315-332 **(1980)**

23. Hodson P.V., Borgmann U. and Shear H., Toxicity of copper to aquatic biota. Pages 307-372 in J. O. Nriagu, editor. *Copper in the environment,* Part 2: Health effects, John Wiley, New York **(1979)**

24. Vander Velden J.A., Kolar Z.I. and Flik G., Intake of magnesium from the water by freshwater tilapia fed on a

low Mg-diet. *Comp. Biochem. Physiol*, **99(1-2),** 103-105 **(1991)**

25. Nelson J.A., Physiological observations on developing rainbow trout, *Salmo gairdneri* (Richardson), exposed to low pH and varied calcium ion concentrations, *J. Fish Biol*, **201**, 359-372 **(1982)**

26. Metelev V.V., Kanaev A.I. and Dzasokhova N.G., Water Toxicology, Amerind Publishing Co. pvt. Ltd., New Delhi, 216 **(1983)**

27. Mohapatra B.C., Purna Saline Tract, Maharastra state: Assessment of ground water quality through fish bioassays. D.Sc. Thesis, Berhampur Univ., Berhampur, 101 **(1999)**

28. Perry S.F., Goss G.G. and Fenwick J. C., Interrelationships between gill chloride cell morphology and calcium uptake in freshwater teleosts, *Fish Physiol. Biochem,* **10**, 327-337 **(1992)**