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Fish physiology

COMPARATIVE EFFICACY OF FOUR ANESTHETICS ON COMMON CARP *CYPRINUS CARPIO* L.

PORÓWNANIE EFEKTYWNOŚĆI CZTERECH ANESTETYKÓW ZASTOSOWANYCH U KARPIA *CYPRINUS CARPIO* L.

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Anesthetics are often used to immobilise fishes in research. Many chemicals have been tested in anesthetisation of fishes and each chemical has its own merits and demerits. In the present study, four anesthetics namely, 2-phenoxyethanol, quinaldine, MS-222, and benzocaine were selected to test the efficiency in anesthetisation of common carp *Cyprinus carpio*. Results indicated that the induction time of the fish exposed in four anesthetic solutions significantly ($P < 0.05$) decreased with increasing concentration but recovery time was independent to concentration. The effective concentration which produced anesthesia within 3 minutes and allowed recovery within 5 minutes in common carp were 600 mm^3/dm^3 (2-phenoxyethanol), 6 mg/ dm^3 (quinaldine), 50 mg/ dm^3 (MS-222), and 50 mg/ dm^3 (benzocaine). A brief comparison about the advantages and drawbacks of the four anesthetics are given in Tab. 4.

INTRODUCTION

Many chemicals are used as anesthetics in research to immobilise fishes and they are necessary to avoid stress caused by manipulation (Marking and Meyer 1985; Gilderhus and Marking 1987; Summerfelt and Smith 1990). Each anesthetic has its own merits and demerits with regard to cost, toxicity, efficacy, safety, manipulation, induction and recovery time. The aim of the present study was to determine the effective concentration of four anesthetics namely, 2-phenoxyethanol (ethylene glycol monophenyl ether), quinaldine (2-methylquinoline), MS-222 (tricainemethanesulfonate), and benzocaine (ethyl aminobenzocate) in anesthetisation of common carp *Cyprinus carpio*. These anesthetics have been demonstrated to be effective and have been widely used in many fish species (Marking and Meyer 1985; Gilderhus and Marking 1987). In this study, first effective concentration of each anesthetic was determined, then the applying cost of each anesthetic was calculated. Finally a brief comparisons of their merits and demerits were presented in a form of table.

MATERIALS AND METHODS

Good genetic strains and healthy common carp of a single egg mass were purchased from Fish Farmer's Development Authority (FFDA), Tirunelveli, South India. These fish were acclimatised to laboratory conditions in a 1000-dm^3 cement tank (525 fish) on a laboratory prepared pelleted diet containing 38% crude protein, 7.5% crude lipid, and 33% crude carbohydrate for two weeks. The uniform sized (5.15 ±0.12 g live weight) fish were randomly stocked (25 fish/concentration) into 25 dm^3 glass aquarium filled with 20 dm^3 fresh water containing different concentration of anesthetic. The physicochemical characteristics of four anesthetics and their concentrations used in this study are illustrated in Tabs. 1 and 2, respectively. Since quinaldine and benzocaine are sparingly soluble in water, they were first dissolved in 95% ethanol at 1 g/10 cm^3 and 1 g/10 cm^3, respectively. Crystalline MS-222 is readily soluble in water, so they were dissolved in water 1 g/10 cm^3 before adding to aquaria. Only 2-phenoxyethanol was added directly into the anesthetic tank. Water temperature, oxygen content, pH and salinity during the acclimatisation and the experiment were about 27 to 29°C, 6 to 7 ppm, 7.3 to 7.8 and 0.5 to 0.7 ppt, respectively.

Table 1

Physicochemical characteristics of four anesthetics used in the study (Hseu et al.1998)

Anesthetic	Physicochemical characteristics
2-phenoxyethanol	Colourless oily liquid, molecular weight 138.2; boiling point 245°C, density 1.1; faint aromatic odour; moderately soluble in water (26.7 g/dm^3) but freely soluble in ethanol
Quinaldine	Colourless oily liquid, molecular weight 143.2; boiling point 246 to 247°C, density 1.06; unpleasant odour of quinoline; practically insoluble in water but soluble in acetone and ethanol
Tricaine methanesulfonate (MS-222)	White crystalline powder, molecular weight 261.3; melting point 149 to 150°C; readily soluble in water (1250 g/dm^3)
Benzocaine	Colourless oily liquid, molecular weight 165.2; melting point 88 to 90°C; barely soluble in water (0.4 g/dm^3) but soluble in acetone and ethanol

The anesthetic induction time is the period from the time when the test fish is placed in the anesthetic tank until the time is stops swimming and its tail stops swinging (Mattson and Riple 1989). After the induction time the anesthetized fish were immediately transferred into a recovery tank. The recovery time is the period from the time when an anesthetized fish is placed in a recovery tank until it recovers from anesthetization with full equilibrium motion (Hseu et al. 1998). The effective concentration is defined as the minimum concentration required to anesthetize the fish within three minutes in the anesthetic tank and which allows recovery within five minutes in the recovery tank (Hseu et al. 1998). Data were analysed by one-way ANOVA followed by Tukey type multiple comparisons at five percent level of significant (Zar 1984).

Table 2

Induction time and recovery time of common carp exposed in different anesthetics for 10 minutes. Mean ±SD (n =10); mean in the same column having the same superscript are not significantly different (P > 0.05)

Anesthetic levels	Induction time (min)	Recovery time (min)
2-phenoxyethanol (mm^3/dm^3)		
700	0.95 ± 0.11^a	0.55 ± 0.03^a
600	2.15 ± 0.03^b	0.56 ± 0.04^a
500	3.95 ± 0.17^c	0.48 ± 0.02^a
400	9.63 ± 0.19^d	—
Quinaldine (mg/dm^3)		
10	0.62 ± 0.10^a	0.61 ± 0.02^a
8	0.92 ± 0.08^b	0.64 ± 0.02^a
6	1.92 ± 0.07^c	0.59 ± 0.01^a
4	7.81 ± 0.15^d	—
MS-222 (mg/dm^3)		
100	0.43 ± 0.12^a	0.87 ± 0.01^a
75	0.85 ± 0.06^b	0.81 ± 0.01^a
50	1.16 ± 0.04^c	1.06 ± 0.06^a
25	8.25 ± 0.15^d	—
Benzocaine (mg/dm^3)		
70	1.65 ± 0.09^a	0.88 ± 0.02^a
60	1.97 ± 0.03^b	0.74 ± 0.02^a
50	3.18 ± 0.06^c	0.80 ± 0.01^a
40	10.20 ± 0.08^d	—

RESULTS AND DISCUSSION

Generally, an ideal anesthetic should produce rapid anesthesia (within 3 or 5 minutes), a quick recovery, not be toxic to fish and users, leave low tissue residue and be inexpensive (Marking and Meyer 1985; Gilderhus and Marking 1987). Results of the present study showed that the induction time of common carp exposed to four anesthetic significantly ($P < 0.05$) decreased within increasing their concentrations (Tab. 2). The recovery time was generally within one minute and no mortality was observed during the experimental period.

Among all the four anesthetics, 2-phenoxyethanol was the most conveniently used and no preparation was necessary before treatment of fish. Guo et al. (1995) suggested that 2-phenoxyethanol was more suitable than either quinate of MS-222 to sedate non-food fishes (e.g. ornamental fishes) during live transport. A major drawback of 2-phenoxyethanol is comparatively high anesthetic doses in fishes. The effective anesthetic doses of 2-phenoxyethanol in most fishes ranged from 200 to 600 mm^3/dm^3 (Tahashima et al. 1982; Gilderhus and Marking 1987; Mattson and Riple 1989: Hseu et al. 1994, 1997, 1998; Hseu and Ting 1995; Weyl et al. 1996). In the present study, the effective concentration of 2-phenoxyethanol for common carp was 600 mm^3/dm^3.

Like MS-222, quinaldine depressed the sensory centres of the central nervous system (Locke 1969). Due to its lipid solubility, quinaldine tends to accumulate in the brain (Brandenburger Brown et al. 1972). Atlantic mackerel required 4 to 6 mg/dm^3 to completely anesthetise at 12 to 17°C (Lambert 1982). Tilapia were extremely tolerant to quinaldine and 500 mg/dm^3 was required to completely anesthetise. The effective concentration of quinaldine for goldline sea bream was 9 mm^3/dm^3 (Hseu et al. 1998). In the present study 6 mg/dm^3 quinaldine was required to anesthetise common carp and it was the lowest effective concentration among the four anesthetics.

The most widely used fish anesthetic in the USA is MS-222 (Marking and Meyer 1985). MS-222 has been used in the anesthetisation of striped mullet (Sylvester 1975), cod (Mattson and Riple 1989), halibut (Malmstrøm et al. 1993) and porgy (Oikawa et al. 1994). In the present study, the effective concentration of MS-222 for common carp was 50 mg/dm^3 and this concentration was half of the effective dosage (100 mg/dm^3) of goldline sea bream (Hseu et al. 1998). Many authors suggested that MS-222 should be neutralised with alkali, such as NaOH thus, sea water could act as a buffer to prevent excessive acidification from MS-222 (Ohr 1976; Smit and Hattingh 1979).

Benzocaine has a similar chemical structure of MS-222 but unlike MS-222, it was barely soluble in water (Summerfelt and Smith 1990). In some fish, benzocaine is more suitable than MS-222 because striped bass are very sensitive to MS-222 and benzocaine induced, anesthesia very rapidly than MS-222 in white perch. The effective concentrations of benzocaine for striped bass and goldline sea bream were 55–80 mg/dm^3 (Gilderhus et al. 1991) and 50 mg/dm^3 (Hseu et al. 1998), respectively. In the present study MS-222 also was as effective anesthetic for common carp and its effective concentration was 60 mg/dm^3.

The cost of application of each anesthetic was calculated based on the effective concentration and the price of anesthetic (Tab. 3). Among the four anesthetics, the cheapest one was quinaldine and the most expensive one was MS-222 followed by 2-phenoxyethanol. The cost of benzocaine was inexpensive. In general, the recovery time of

2-phenoxyethanol, quinaldine, MS-222, and benzocaine are not very long (Imamura-Kojima et al. 1987; Allen 1988). Benzocaine and MS-222 are comparatively safety to users (Summerfelt and Smith 1990). Quinaldine is highly irritating the mucus membrane of eyes and respiratory system (Summerfelt and Smith 1990). Morton (1990)suggested that the regular usage of 2-phenoxyethanol causes some neurophysiological syndrome to users (Tab. 4)

Table 3

Costs of four anesthetics used with common carp (US $)

Anesthetic	Price*	Content in 10 dm^3	Cost
2-Phenoxyethanol	176.00/18 dm^3	6.00 cm^3	0.59
Quinaldine	187.40/500 g	0.06 g	< 0.01
MS-222	69.80/50 g	0.50 g	0.70
Benzocaine	54.10/500 g	0.60 g	0.06

* Price of the maximum package size of each chemical in Aldrich Catalog (1998–99)

Table 4

Brief comparison of four anesthetics used with fish

Anesthetic	Induction and recovery time	Manipulation	Cost	Safety for operator
2-Phenoxyethanol	rapid	very convenient	expensive	a little dangerous
Quinaldine	rapid	convenient	cheapest	a little dangerous
MS-222	rapid	convenient	most expensive	safe
Benzocaine	rapid	convenient	cheap	safe

REFERENCES

Allen J.L., 1988: Residues of benzocaine in rainbow trout, largemouth bass, and fish meal. Progr. Fish Cult., **50**: 59–60.

Brandenburger Brown E.A., J.E. Franklin, E. Pratt, E.G. Trams, 1972: Contribution to the pharmacology of quinaldine (uptake and distribution in the shark and comparative studies). Comp. Biochem. Physiol., **42A**: 223–231.

Gilderhus P.A., C.A. Lemm, L.C. Wood, 1991: Benzocaine as an anesthetic for striped bass. Progr. Fish Cult., **53**: 105–107.

Gilderhus P.A., L.L. Marking, 1987: Comparative efficacy of 16 anesthetic chemicals on rainbow trout. N. Am. J. Fish. Manag., **7**: 288–292.

Guo F.C., L.H. Teo, T.W. Chen, 1995: Effects of anesthetics on the water parameters in a stimulated transport experiment of platyfish, *Xiphophorus maculatus* (Günther). Aqua. Res., **26**: 265–271.

Hseu J.R., Y.Y. Ting, 1995: Anesthesia of aquatic animals. Aquatic animal disease control, **2**: 4–7. (In Chinese).

Hseu J.R., S.L. Yeh, Y.T. Chu, Y.Y. Ting, 1994: The anesthetic effects of 2-phenoxyethanol in goldline sea bream (*Sparus sarba*). J. Taiwan Fish. Res., **2**, 2: 41–49.

Hseu J.R., S.L. Yeh, Y.T. Chu, Y.Y. Ting, 1995: The use of 2-phenoxyethanol as an anesthetic in the transport of black porgy *Acanthopagrus schlegeli*. J. Taiwan Fish. Res., **3**, 1: 11–18.

Hseu J.R., S.L. Yeh, Y.T. Chu, Y.Y. Ting, 1997: Different anesthetic effects of 2-phenoxymethanol on four species of teleost. J. Fish. Soc. Taiwan, **24**: 185–191.

Hseu J.R., S.L. Yeh, Y.T. Chu, Y.Y. Ting, 1998: Comparison of efficacy of five anesthetic in goldlined sea bream, *Sparus sarba*. Acta Zool. Taiwan, **9**, 1: 11–18.

Imamura-Kojima H., F. Takashima, T. Yoshida, 1987: Absorption, distribution and excretion of 2-phenoxyethanol in rainbow trout. Nippon Suisan Gakkaishi. **53**: 1339–1342.

Lambert T.C., 1982: Techniques for the capture and handling of Atlantic mackerel with special reference to use of quinaldine. Progr. Fish Cult., **44**: 145–147.

Locke D.O., 1969: Quinaldine as an anesthetic for brook trout, lake trout and Atlantic salmon. U.S. Fish. Wildl. Serv., Invest. Fish Control, 24.

Malmstrøm T., R. Salte H.M. Gjøen, A. Linseth, 1993: A practical evaluation of metomidate and MS-222 as anesthetics for Atlantic halibut (*Hippoglossus hipoglossus*). Aquaculture, **113**: 331–338.

Marking L.L., F.P. Meyer, 1985: Are better anesthetics needed in fisheries? Fisheries, **10**, 6: 2–5.

Mattson N.S., T.H. Riple, 1989: Metomidate, a better anesthetic for cod (*Gadus marhua*) in comparison with benzocaine, MS-222, chlorobutanol and phenoxyethanol. Aquaculture, **83**: 89–94.

Morton W.E., 1990: Occupational phenoxyethanol neurotoxicity. A report of three cases. J. Occup. Med., **32**: 42–45.

Ohr E.A., 1976: Tricaine methanesulfonate. I. PH and its effects on anesthetic potency. Comp. Biochem. Physiol., **54C**: 13–17.

Oikawa S., T. Takeda,, Y. Itazawa, 1994: Scale effects of MS-222 on a marine teleost, porgy *Pagrus major*. Aquaculture, 121: 369–379.

Smit G.L., J. Hattingh, 1979: Anesthetic potency of MS-222 and neutralized MS-222 as studied in three fresh water fish species. Comp. Biochem. Physiol., **62C**: 237–241.

Summerfelt R.C., L.S. Smith, 1990: Anesthesia, surgery and related techniques. In: Methods for fish biology [R.C. Shreck, P.B. Moyle (eds.)], Am. Fish. Soc., Betheseda, MD, USA: 213–272.

Sylvester J.R., 1975: Factors influencing the efficacy of MS-222 to striped mullet (*Mugil cephalus*). Aquaculture, **6**: 163–169.

Takashima F., H. Kasai, H. Asakawa, Y. Yamamoto, 1982: 2-phenoxyethanol as an anesthetic for fish. Suisanzoshoku, **30**: 48–51.

Weyl O., H. Kaiser, T. Hecht, 1996: On the efficacy and mode of action of 2-phenoxyethanol as an anesthetic for goldfish, *Carassius auratus* (L.) at different temperatures and concentrations. Aqua. Res., **27**: 757–764.

Zar J.H., 1984: Biostatistical Analysis. Prentice Hall, Inc., Engelwood Clifs, New Jersey.

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PORÓWNANIE EFEKTYWNOŚCI CZTERECH ANESTETYKÓW ZASTOSOWANYCH U KARPIA *CYPRINUS CARPIO* L.

STRESZCZENIE

Anestetyki są często stosowane do unieruchamiania ryb dla przeprowadzenia badań naukowych. Wiele związków chemicznych testowano pod kątem ich przydatności jako anestetyków. Każdy z nich ma jednak swoje strony pozytywne jak i negatywne. W niniejszych badaniach wybrano cztery anestetyki (2-fenoksyetanol, quinaldinę, MS-222 i benzokainę) w celu zbadania ich efektywności w anestezji karpia (*Cyprinus carpio*). Otrzymane wyniki wskazują, że czas indukcji u ryb poddanych działaniu roztworów anestetyków w sposób istotny ($P < 0, 05$) malał wraz ze wzrostem stężenia, zaś czas „dobudzania" był niezależny od stężenia. Skuteczne stężenia, które pozwalały osiągnąć „efekt usypiający" po 3 minutach i „dobudzenie" po 5 minutach wynosiło u karpia 600 mm^3/dm^3 w przypadku 2-fenoksyetanolu, 6 mg/dm^3 dla quinaldiny, 60 mg/dm^3 dla MS-222 oraz 50 mg/dm^3 w przypadku benzokainy. Zwięzłe porównanie zalet i wad czterech badanych anestetyków podano w tabeli 4.

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