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Influence of the Anesthetic, 2-Phenoxyethanol, on Hematological Parameters of Black Porgy *Acanthopagrus schlegeli*

Abstract

In this study, we examined changes of hematological parameters in black porgy *Acanthopagrus schlegeli* after anesthetization in 2-phenoxyethanol (2-PE) of various concentrations. In 400 and 600 ppm 2-PE solutions, black porgies were anesthetized to total loss of equilibrium within 3 min. The all values of hematological parameters of the anesthetized fishes were not significantly different between the anesthesia and control groups ($p > 0.05$). In 200 ppm 2-PE solution, since the fish could not be anesthetized to total loss of equilibrium within 30 min, we tested the black porgies which have been anesthetized for 15 min in this solution and found that the fish showed significantly decrease in hematocrit and increase in plasma glucose. It is, therefore, concluded that higher dosages of 2-PE will take shorter time to induce less stress effect on the anesthetized fish.

Key words: Anesthetic, 2-phenoxyethanol, *Acanthopagrus schlegeli*

Anesthetics are often used to immobilizing fish in a lot of manipulations (e.g., artificial insemination, surgery, and biopsy). In fish, many studies have demonstrated that anesthetization cause some alterations in a number of hematological parameters such as hematocrit, hemoglobin, red blood cell count, glucose, ion concentration, osmolarity, etc.[1-11]. The mostly used anesthetics were MS-222 (Tricaine methanesulfonate) as well as benzocaine (Ethyl aminobenzoate), and the experimental species were nearly all freshwater fishes such as salmon, trout, and carp. Studies concerning the effects of other anesthetics on the hematological changes in marine fish were rarely reported.

2-Phenoxyethanol (2-PE, also as ethylene glycol monophenyl ether; molecular weight 138.16) is a very commonly used anesthetic for fish. It is a colorless and oily liquid with a faintly aromatic odor[12]. The anesthesia effect of 2-PE on fish was incidentally discovered by Idler et al.[13]. Because of its effectiveness, safety, and low price in fish anesthetization[14,15], 2-PE has been widely applied to life transport[16-18] and general anesthesia[14-15,19-21]. In addition, it is also used for treatment of some fish diseases[22]. In this study, we used different concentrations of 2-PE to anesthetize black porgy *Acanthopagrus schlegeli* a marine fish and examined the changes of hematological parameters in the anesthetized specimens.

Materials and methods

The black porgies which weighted 70-90 g were collected from aquacultural ponds of the Tainan Branch, Taiwan Fisheries Research Institute (TFRI) and

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were acclimated in 2-ton FRP tanks with 1,500 *l* of sea water for 1 week before the experiment began. During acclimatization, fish were fed with commercial eel feed (Prince Feed Co., Tainan, Taiwan). Feeding was stopped two days before experiment commenced. Every fish of the anesthesia groups was transferred with a net from the acclimation tanks to the anesthetic tanks which were 90-*l* plastic tanks filled with 60 *l* sea water containing 3 different concentrations of 2-PE. In 400 and 600 ppm 2-PE solutions, we sampled fish in the anesthetic tanks while they reached stage 3B of anesthesia (i.e., total loss of equilibrium) defined by Mattson and Riple[23]. At this stage, fish stops swimming but its caudal peduncle still responds to the mechanical stimulus by syringe needle. The black porgy exposed to 200 ppm 2-PE solution would not reach stage 3B of anesthesia within 30 min according to our previous experience. Thus, we sampled the fish at 15 min after anesthetization. The fish of control group were sampled directly from the acclimation tanks. Seven fishes were sampled for each group. The salinity and water temperature of the water in the acclimation tank were 36 ppt and 30 °C.

Whole blood was sampled from caudal vein with 1 m*l* heparinized plastic syringes (Teruma Co., Japan), and the values of hematocrit and hemoglobin were immediately determined. Plasma for measurements of glucose, protein, chloride ion, and osmolarity was obtained after centrifugation of the blood. Hematocrit was determined by centrifuging microhematocrit capillary tubes at 12,000 × g for 5 min. Hemoglobin, glucose, chloride ion, and protein were determined by commercial biochemical kits (Sigma Company, USA). The analysis methods were described in our previous study[24]. Osmolarity was measured by Osmometer (Advanced Instrument Inc., Model 3D II).

All the hematological values were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's multiple-range test. The level of statistical significance was set at $p < 0.05$[25].

Results and Discussion

The times needed for inducing the black porgies into stage 3B of anesthesia respectively were 2.46 ± 0.14 and 0.92 ± 0.05 min in 400 and 600 ppm concentrations of 2-PE. In our study, all the anesthetized black porgies had lower means of hematocrit and hemoglobin than the control group (Fig. 1A-B). But only the hematocrit of 200 ppm anesthesia group was significantly different from control group. Houston et al.[1] reported that MS-222 could increase hematocrit and hemoglobin of brook trout *Salvelinus fontinalis* after anesthetization because of rapid hemoconcentration. This phenomenon of increasing hemoglobin and hematocrit also was observed by many other authors[3,5-6,9,11]. Nevertheless, a lot of factors such as concentration of anesthetic, experimental method, and species of the tested specimens, would produce variable results. Values of the both parameters were not always increased, and sometimes those could be lower in anesthetized fish than in the control[4,7-8,10]. In this study, hemoconcentration did not seem to happen.

Hyperglycemia is an index which indicated fish is in stress[26]. Anesthetic usually was considered a kind of chemical stressor for fish[27]. However, reviewing prior literature[1-8,10-11], we find that anesthetization may or may not raise glucose concentration in plasma. In this study, only the anesthesia group in 200 ppm 2-PE had significantly higher serum glucose level than the control (Fig. IC). The result apparently indicated that higher dosages of 2-PE took shorter induction time to induce less stress effect on the anesthetized porgies. Smit et al.[4] and Ferreira et al.[5] also found the similar phenomenon with MS-222 and benzocaine hydrochloride on freshwater fishes.

Some anesthetics, such as MS-222 and benzocaine, are well known that can cause changes of osmolarity and ion concentrations in plasma[1-5,7,9]. Our previous study indicated that in four species of marine fish, i.e., grey mullet, milk fish, red snapper and black porgy, when anesthetized with 400 ppm 2-PE solution, the black

porgy was the only one showed significant difference in osmolarity between the anesthesia and the control groups[11]. Nevertheless, in the present study, the osmolarity, chloride ion, and protein did not differ significantly between the anesthesia and control groups (Fig. 1D-F).

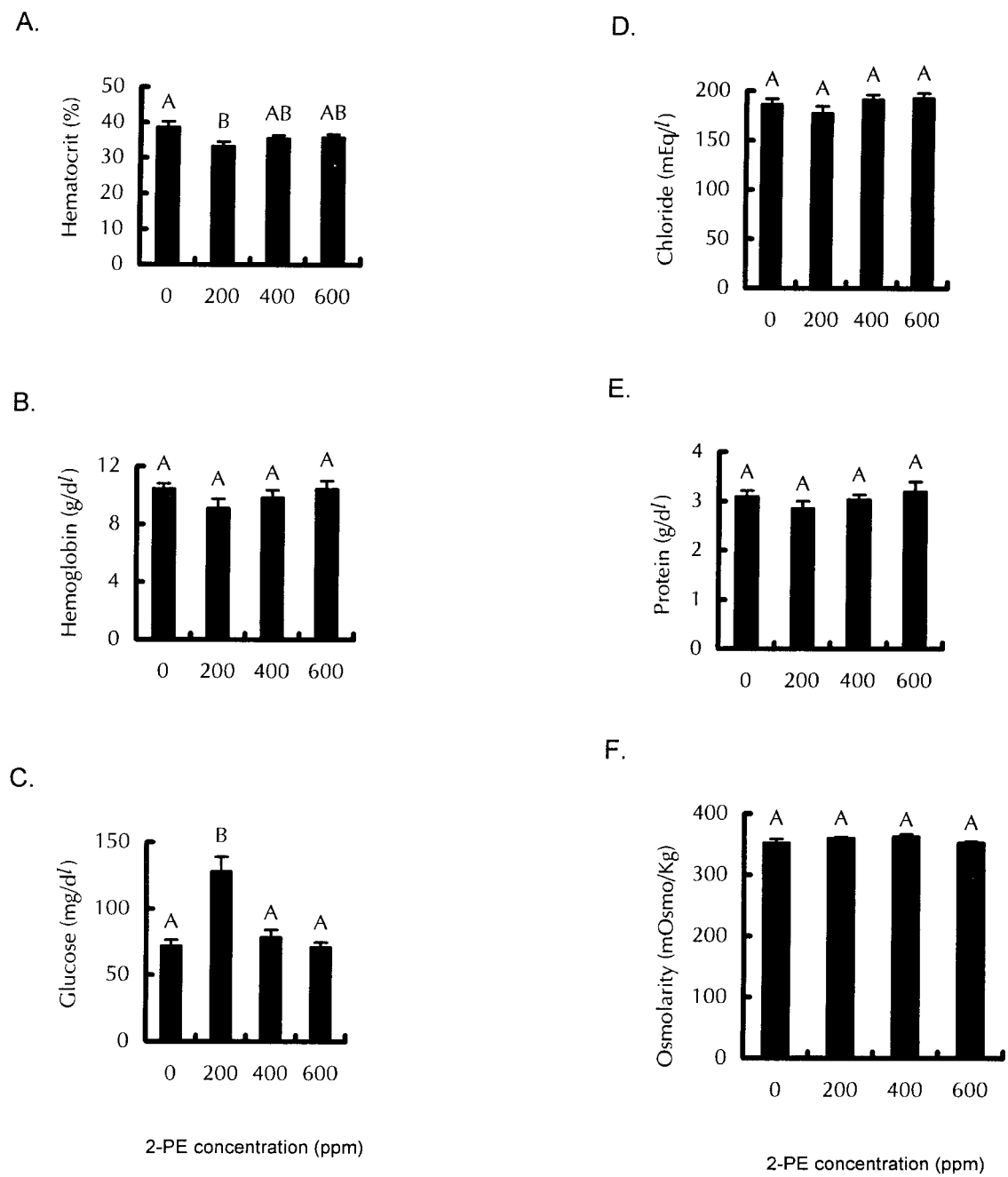

Fig. 1. The effects of different concentrations of 2-phenoxyethanol (2-PE) on the hematological parameters of black porgies. Values with same letters are not statistically different ($p > 0.05$, n=7).

In summary, the results showed that 2-PE, like other anesthetics, causes some changes of certain hematological parameters in fishes. However, the hematological responses to anesthetization need time to develop[5]. That is, higher dosages of 2-PE which take shorter time will induce less stress effect on the anesthetized fishes. We therefore recommend the use of higher dosages of 2-PE (e.g. 400 and 600 ppm) for anesthetization in fishes.

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References

1. Houston, A. H., J. A. Madden, R. J. Woods and H. M. Miles (1971) Some physiological effects of handling and tricaine methanesulphonate anesthetization upon the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Can., **28**: 625-633.
2. Houston, A. H., J. A. Madden, R. J. Woods and H. M. Miles (1971) Variations in the blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia, and surgery. J. Fish. Res. Bd. Can., **28**: 635-642.
3. Soivio, A., A. Nyholm and M. Huhti (1977) Effects of anesthesia with MS-222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. J. Fish Biol., **10**: 91-101.
4. Smit, G. L., J. Hattingh and A. P. Burger (1979) Haematological assessment of the effects of the anesthetic MS-222 in natural and neutralized form in three freshwater fish species: interspecies difference. J. Fish Biol., **15**: 633-643.
5. Ferreira, J. T. G. L. Smit and H. J. Schoonbee (1981) Haematological evaluation of the anesthetic benzocaine hydrochloride in the freshwater fish *Cyprius carpio* L. J. Fish Biol., **18**: 291-297.
6. Nieminen, M., M. Laitinen and P. Pasanen (1982) Effects of anesthesia with tricaine (MS 222) on the blood composition of the splake (*Salvelinus fontinalis* × *Salvelinus namaycush*). Comp. Biochem. Physiol., **73C**: 271-276.
7. Bourne, P. K. (1984) The use of MS-222 (Tricaine methanesulfonate) as an anesthetic for routine blood sampling in three species of marine teleosts. Aquaculture, **36**: 313-321.
8. Iwama, G. K., J. C. Mcgreer and M. P. Pawluk (1989) The effects of five anesthetics on acid-base balance, hematocrit, blood gases, cortisol and adrenaline in rainbow trout. Can. J. Zool., **67**: 2065-2073.
9. Hunn, J. R. and I. E. Greer (1991) Influence of sampling on the blood chemistry of Atlantic salmon. Prog. Fish Cult., **53**: 184-187.
10. Malmstrsymbøl, T., R. Salte, H. M. Gjøen and A. Linseth (1993) A practical evaluation of metomidate and MS-222 as anesthetics for Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture, **113**: 331-338.
11. Hseu, J. R., S. L. Yeh., Y. T. Chu and Y. Y. Ting (1996) Effects of anesthesia with 2-phenoxyethanol on the hematological parameters of four species of marine teleosts. J. Fish. Soc. Taiwan., **23**: 43-48.
12. Merck & Company (1989) 2-phenoxyethanol in The Merck index, 11th ed., Rahway, New Jersey, USA., 1153.
13. Idler, D. R., J. R. McBride, R. E. E. Jonas and N. Tomlinson (1961) Olfactory perception in migrating salmon. Can. J. Biochem. Physiol., **20**: 95-100.
14. Takashima, N. H., Kasai, H. Asakawa and Y. Yamada (1982) 2-phenxyethanol as an anesthetic for fish. Suisanzoshoku, **30**: 48-51. (in Japanese)
15. Guo, F. C., L. H. Teo and T. W. Chen (1994) Comparison of the effects of four anesthetics on platy *Xiphophorus maculatus*. *In* The third Asian fisheries forum, (L. M. Chou, A. D. Munro, T. J. Lam, T. W. Chen, L. K. K. Cheong, J. K. Ding, K. K. Hooi, H. W. Khoo, V. P. E. Phang, K. F. Shim and C. H. Tan eds.). Asian Fish. Soc., Manila, Philippines, 900-903.
16. Gilderhus, P. A. and L. L. Marking (1987) Comparative efficacy of 16 anesthetic chemicals on rainbow trout. N. Am. J. Fish. Manage., **7**: 288-292.

17. Teo, L. H., T. W. Chen and B. H. Lee (1989) Packaging of the guppy, *Poecilia recticulata*, for air transport in a closed system. Aquaculture, **78**: 321-332.
18. Guo, F. C., L. H. Teo and T. W. Chen (1995) Effects of anesthetics on the water parameters in a simulated transport experiment of platyfish *Xiphophorus maculatus* (Gunther). Aqua. Res., **26**: 265-271.
19. Hseu, J. R., S. L. Yeh, Y. T. Chu and 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. (in Chinese with English abstract)
20. Hseu, J. R., S. L. Yeh, Y. T. Chu and Y. Y. Ting (1994) The anesthetic effect of 2-phenoxyethanol in goldlined sea bream (*Sparus sarba*). J. Taiwan Fish. Res., **2(2)**: 41-49. (In Chinese with English abstract)
21. Josa, A., E. Espinosa, J. I. Cruz, L. Gill, M. V. Falceto and R. Lozano (1992) Use of 2-phenoxyethanol as an anesthetic agent in goldfish (*Cyprinus carpio*). Vet. Rec., **131**: 468.
22. Herwig, N. (1979) Handbook of drugs and chemicals used in the treatment of fish diseases: A manual of fish pharmacology and materia medica. Thomas Press,Springfield, Illinois, U.S.A., 272 pp.
23. Mattson, N. S. and T. H. Riple (1989) Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and 2-phenoxyethanol. Aquaculture, **83**: 89-94.
24. Hseu, J. R., S. L. Yeh, Y. T. Chu and Y. Y. Ting (1995) Application of sodium bicarbonate and sulfuric acid for anesthetization of black porgy (*Acanthopagrus schlegeli*). J. Taiwan Fish. Res., **3**(2): 151-159. (in Chinese with English abstract)
25. Kirk, R. E. (1982) Experimental design: Procedures for the behavioral sciences, 2nd ed. Books/Cole Publishing Company, Pacific groove, California, U.S.A., 911pp.
26. Wedemeyer, G. A. and D. J. McLeay (1981) Methods for determining the tolerance of fishes to environmental stressors. *In* Stress and Fish, (A. D. Pickering ed.). Academic Press., New York, U.S.A., 247-268.
27. Summerfelt, R. C. and L. S. Smith (1990) Anesthesia, surgery, and related techniques. *In* Methods for Fish Biology, (R. C. Schreck and P. B. Moyle eds.). Am. Fish. Soc., Bethesda, Maryland, U.S.A., 213-272.

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麻醉劑 2-phenoxyethanol 對黑鯛血液學指數的影響

摘 要

本實驗中，利用不同濃度的麻醉劑 2-phenoxyethanol (2-PE) 對黑鯛進行麻醉後，檢測其血液學指數的變化。實驗結果顯示，在 400 及 600 ppm 的 2-PE 溶液中，黑鯛在 3 分鐘內即進入完全失去平衡後期；牠們的各項血液學指數與對照組之間並無顯著差異 ($p > 0.05$)。在濃度爲 200 ppm 中，由於黑鯛無法在 30 分鐘內被麻醉至完全失去平衡後期，因此，在麻醉進行 15 分鐘後即進行抽血採樣。結果發現，本麻醉組魚的血容積比明顯低於對照組，而血糖值則顯著高於對照組。綜合上述結果，我們認爲以較高濃度麻醉劑快速麻醉魚類時，引起其所產生之壓迫反應較小。

關鍵詞：麻醉劑，2-phenoxyethanol，黑鯛

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