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Short paper

Application of 2-Phenoxyethanol in Live Transportation of Sea Bass, *Lates calcarifer*

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There are two systems used in live transportation of fish. One is open system which utilizes fish-distribution tanks with continuous supply of oxygen. The other is a closed system which transports fish in sealed polyethylene or plastic bags containing limited amount of oxygen. In both systems, several factors must be considered to ensure good survival rate: (1) oxygen supply is adequate, (2) accumulation of toxic metabolites like ammonia (NH_3) and carbonic dioxide (CO_2) must be suppressed, (3) bacterial blooms should be prevented (Amend *et al.*, 1982). Usually, chemicals are added in tanks or bags to promote survival of fish by ways of inhibition of either metabolic rate of fish or growth of bacteria (Amend *et al.*, 1982; Teo *et al.*, 1989; Piper *et al.*, 1982). Anesthetic is one of those popular chemicals. The main purpose of adding anesthetic is to reduce metabolic rate of fish and to sedate fish to prevent injury during transportation (Piper *et al.*, 1982).

2-Phenoxyethanol (2-PE, also as ethylene glycol monophenyl ether), a common fish anesthetic, is widely applied in closed transport systems of fish (Teo *et al.*, 1989; Hseu *et al.*, 1995; Guo *et al.*, 1995a; Kaiser and Vine, 1998). Compared with quinate, MS-222, and metomidate, 2-PE is most effective in decreasing metabolic activity and mortality of transported platyfish, *Xiphophorus maculatus* (Guo *et al.*, 1995a). However, the effects of adding 2-PE in closed transport systems seemed to be influenced by a lot of factors, e.g., species, packing density, transport time, etc. What is appropriate for one species may not be suitable for the other. In previous literature on live transportation (Teo *et al.*, 1989; Guo *et al.*, 1995a; Kaiser and Vine, 1998), most of fish are small ornamental ones less than 4 g. However, for certain experimental purposes, we often need to transport bigger fish from our hatchery at Tainan to other cities. Thus, this study is designed to investigate whether 2-PE can reduce metabolic rate of sea bass (*Lates calcarifer*), which weighed about 30 g, in live transportation.

Sea bass weighing 29.17 ± 0.55 g were collected from aquacultural ponds of the Tainan Branch, Taiwan Fisheries Research Institute and were acclimated in 2-ton FRP tanks with 1,500 l of sea water for 1 week. During acclimatization, the fish were fed with commercial eel feed. Feeding was stopped three days before the experiment. Upon experiment, 8 sea bass were transferred to a 23 × 80 cm plastic bag filled with 10 l of filtrated sea water (35 ppt of salinity). In anesthetic-adding group, 2-PE was added at a concentration of 150 μl/l which was referred to prior references and experiences. Control group did not receive any anesthetic treatment. Triple bags were set for each group. Furthermore, we set up 3 bags containing only sea water as a blank group. After expulsion of air, all bags were filled with oxygen with a ratio of oxygen volume to water volume being about four. Then the bags were sealed tightly and stayed stationary. Six

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hours later, they were opened, and water in the bags was analyzed immediately. The water temperature in the bags was 28-30 ℃ during the experiment. The total ammonia-nitrogen (TAN, NH_3+NH_4^+) was determined by the phenolhypochlorite method (Solózano, 1969). The pH value and dissolved oxygen (DO) were measured with portable pH meter and dissolved oxygen meter, respectively. Initial TAN, DO, and pH of sea water were also determined right before the bags were sealed. Ammonia-N excretion rate was converted from the change of TAN concentration through 6 hours of experiment.

After 6 hours of seal treatment, the pH value and DO of the sea water in the control and 2-PE-adding groups were apparently lower than those of the initial point and blank group. The increase in acidity of water during transportation was observed by a lot of authors (Amend *et al.*, 1982; Chow *et al.*, 1994; Hseu *et al.*, 1995; Guo *et al.*, 1995a; Kaiser and Vine, 1998). This is largely due to the CO_2 excreted by the transported fish (Chow *et al.*, 1994). Although Gou et al. (1995a) reported that adding 2-PE could reduce CO_2 excretion of platyfish in a closed transport system. It did not prevent acidification of water in most closed transport systems (Teo *et al.*, 1989; Hseu *et al.*, 1995; Guo *et al.*, 1995a; Kasier and Vine, 1998; this study).

In many closed transport systems (Amend *et al.*, 1982; Teo *et al.*, 1989; Chow *et al.*, 1994), oxygen was not thought to be a critical factor. The concentration of DO in transport water usually remained high. In live transportation of clownfish, *Amphiprion ocellaris*, Chow *et al.* (1994) even found that the DO at the end of 48 hours of transportation were higher than the initial. However, in this study, the mean of total weights of the bass in the bags were 235 g, which was much higher than those in the studies of Teo *et al.* (1989) and Chow *et al.* (1994). Although the volumes of packed water and oxygen in this study were larger than those in the previous studies, we still found that the DO of water in the transport bags were quickly exhausted by the bass. After 6 hours, DO of the 2-PE-added and control groups declined to 2.33 and 2.60 mg/l, respectively. Oxygen thus could be a critical factor in determining the duration of transport in our system. 2-PE did not reduce oxygen consumption of the bass. In guppy, *Poecilia reticulata* and platyfish, 100-200 μl/l concentrations of 2-PE could effectively reduce oxygen consumption rates at 20 and 25 ℃ (Teo and Chen, 1993; Guo *et al.*, 1995b). In addition to species-specific response to 2-PE, higher temperature in our study (28-30 ℃) could be another explanation for the difference in the results between this study and the previous ones. The effects of 2-PE in depressing oxygen consumption decreased at higher temperature (Teo and Chen, 1993; Guo *et al.*, 1995b).

Table 1. pH values, dissolved oxygen (DO), and total ammonia (TAN) of sea water in sealed bags with or without adding 2-phenoxyethanol (2-PE). Values are means ± SEM (N=3)

Treatment	pH	DO (mg/l)	TAN (mg/l)
Initial sea water	8.30	6.7	ND
After 6 h transport			
Blank	8.31 ± 0.00^a	9.53 ± 0.12^a	ND
Control	7.06 ± 0.03^b	2.60 ± 0.55^b	$2.77 \pm 0.24^*$
2-PE adding	7.10 ± 0.03^b	2.33 ± 0.03^b	1.24 ± 0.08

1. ND: not detected.
2. In pH and DO, values with different subscripts in the same column are significantly different (One-way ANOVA with Duncan's multiple-range test, $p<0.05$).
3. In TAN, * means significant difference between the control group and 2-PE-adding groups (Student's *t*-test, $p<0.05$).

The ammonia-N excretion rate of the bass was suppressed by 2-PE. The mean value of ammonia-N excretion rates in the control group (12.23 $\mu g \cdot g^{-1} \cdot h^{-1}$) was significantly higher than that of 2-PE-adding group (5.15 $\mu g \cdot g^{-1} \cdot h^{-1}$) ($p < 0.05$). This phenomenon was also reported by other authors (Teo *et al.*, 1989; Hseu *et al.*, 1995; Guo *et al.*, 1995a). Teo *et al.* (1989) thought that 2-PE reduced activity of the transported fish and therefore resulted in low ammonia excretion. Kaiser and Vine (1998) reported that 2-PE did not reduce excretion of ammonia of transported goldfish, *Carassius auratus*. However, the transport bags in the study of Kaiser and Vine (1998) were kept in darkness, and this might cause similar reduction in metabolic activity as did by 2-PE.

In summary, 2-PE could not reduce oxygen consumption of the bass, nor did it prevent acidification of the transport water. However, 2-PE could effectively suppress excretion of ammonia of the bass and consequently did not cause any mortality during experiment and subsequent 3 days in our system. In addition, being cheap and easy to use (Hseu *et al.*, 1998), 2-PE is still recommended for application in live transportation of certain fishes.

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運用 2-Phenoxyethanol 於金目鱸活魚輸送之研究

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本研究之目的旨在調查，於密閉活魚輸送系統中添加 2-phenoxyethanol (2-PE) 是否可以降低金目鱸的代謝率。實驗結果顯示， 2-PE 並無法防止輸送袋中海水的酸化，也不能降低金目鱸的耗氧率，但是， 2-PE 可以有效降低鱸魚的排氨率。由於 2-PE 價格便宜，操作又容易，因此我們認爲在以活魚袋運送一些魚類時，還是可以酌量添加 2-PE ，以利輸送。

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