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RESPONSE TO 2-PHENOXYETHANOL IN JUVENILE *VIMBA VIMBA* (L.)

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ABSTRACT. The anaesthetic effect of 2-phenoxyethanol on juvenile *Vimba vimba* (L.) aged 38-179 days post-hatch (26-56 mm TL) was studied at 25°C. The concentration which anaesthetized 100% of the fish within 10 min without causing mortality after 15 min of exposure ranged from 0.35 g dm⁻³ to 0.48 g dm⁻³ in 38 day-old vimba and from 0.33 g dm⁻³ to 0.43 g dm⁻³ in older fish. The induction and recovery times were shorter in the initial phase of vimba juvenile life than in older fish. In fish of the same age, induction time or recovery time did not depend on their size or condition (Fulton's coefficient). At 25°C, 2-phenoxyethanol at 0.40 g dm⁻³ may be used to efficiently and safely anaesthetize vimba juveniles.

Key words: *VIMBA VIMBA*, JUVENILES, 2-PHENOXYETHANOL, ANAESTHESIA

INTRODUCTION

Anaesthetics are widely used in aquaculture. They alleviate stress in fish during harvest, grading, transport or artificial breeding, and even allow complex surgery to be performed (Gomułka and Antychowicz 1999). Among the anaesthetics used on fish, 2-phenoxyethanol is one of the most popular. Initially used for treatment of certain fish diseases (van Duijn 1956, Wolf and Dunbar 1959), 2-phenoxyethanol came to be used as an anti-stress agent (Sehdev et al. 1963) after its anaesthetic properties had been discovered (Idler et al. 1961).

All anaesthetics used on fish must perform satisfactorily and be safe and economical to use. According to Guilderhus and Marking (1987), an efficient anaesthetic must meet the following requirements: induction time of general anaesthesia cannot exceed 3 min; recovery of normal swimming after 15 min of exposure cannot last more than 10 min; 15 min of exposure cannot induce mortality.

The time of inducing general anaesthesia depends on various factors, e.g. fish age and size, water temperature, and the concentration of the anaesthetic solution. The reaction of fish to 2-phenoxyethanol is species-specific (Kamiński et al. 2000); this means that the conditions of anaesthetic use for each species must be established experimentally before practical application. Until now, no such data have been avail-

able for *Vimba vimba* (L.), a highly endangered species in Poland (Witkowski et al. 1999). Meanwhile, vimba stocking material production methods are being developed in our country which necessitates finding an efficient and safe anaesthetic.

The aim of the present study was to establish the concentration range of 2-phenoxyethanol that provides efficient and safe anaesthesia in juvenile vimba, and to evaluate induction and recovery times. The relationship between the sensitivity to anaesthetic and fish age was also studied.

MATERIAL AND METHODS

FISH

Vimba larvae were obtained from the artificial spawning of one female and three males in a commercial hatchery. The fertilized eggs were incubated at 14°C. About 500 larvae were reared in a flow-through aquarium ($V = 40 \text{ dm}^3$) at 25°C. Beginning from the fifth day post-hatch, the larvae were fed newly hatched brine shrimp *Artemia* sp. nauplii, and from the thirtieth day with the commercial carp feed Carp Starter made by Aller Aqua. During the preliminary period, both the experiment water quality and fish health were acceptable.

The study was done on juvenile vimba aged 38, 63, 115, and 179 days post-hatch. The characteristics of all the groups are shown in Table 1.

TABLE 1
Age, average total length (TL), body weight (BW), and Fulton's coefficient (K) of tested juvenile vimba

Age (days)	TL ± SD (mm)	BW ± SD (mg)	K* ± SD
38	25.7 ± 1.2	122.7 ± 20.5	0.72 ± 0.05
63	34.8 ± 2.2	325.3 ± 64.5	0.77 ± 0.06
115	42.7 ± 2.5	503.5 ± 85.8	0.64 ± 0.03
179	56.0 ± 5.0	1221.4 ± 366.0	0.68 ± 0.03

* $K=100 \text{ BW TL}^{-3}$ where BW in mg, TL in mm

2-PHENOXYETHANOL

Under normal conditions, 2-phenoxyethanol is a colorless oily liquid with a distinctive rose scent and a density of 1.11 kg dm^{-3} . Its water solubility at 25°C is 2.67%. In the present study 2-phenoxyethanol with a purity of over 99% (MERCK-Schuchardt) was used.

TEST PROCEDURES

In the experiment it was assumed that:

- the fish reached general anaesthesia at the moment of immobilization, with the exception of respiratory movements;
- the fish recovered when they regained a stable horizontal position with their backs upward;
- at an effective concentration all the fish were fully anaesthetized within 10 min and remained so for 15 min of exposure;
- safe concentration does not kill the fish during 15 min of exposure.

The fish were subjected to two types of tests. In the first, the safety and efficiency of 2-phenoxyethanol was evaluated, and in the second anaesthesia induction time and recovery time were measured.

The necessary amount of anaesthetic was weighed on an analytical scale to the nearest 0.0001 g. The solution was prepared 30 min before the test, and strongly aerated to provide dissolved oxygen saturation of about 100%. The fish were transferred from the rearing tank to the test tank without taking them out of the water. All the tests were carried out at 25°C. After the tests, the fish were measured and weighed separately. Each fish was tested only once.

LOWEST EFFECTIVE CONCENTRATION AND HIGHEST SAFE CONCENTRATION

These tests were carried out in 2.5 dm³ aquaria. Thirty fish were tested in two replicates of 15 fish each. The fish were exposed to a series of 2-phenoxyethanol solutions at 0.01 g dm⁻³ intervals to determine the lowest effective concentration (LEC) and the highest safe concentration (HSC).

INDUCTION AND RECOVERY TIMES

The fish were anaesthetized and recovered in transparent plastic tanks with a volume of 0.25 dm³. The concentration of 2-phenoxyethanol was 0.40 g dm⁻³. Thirty vimba individuals were tested separately. The times of inducing general anaesthesia and recovery after 15 min of exposure were measured to the nearest 0.01 min.

Statistical significance of the differences among the average values of induction and recovery times for various vimba age groups were evaluated with Duncan's test at $P \leq 0.05$. The relationship between the induction or recovery

time and fish size (total length, body weight) or their condition (Fulton's coefficient) was also evaluated assuming a level of 0.05. The correlation between induction and recovery time was also calculated.

RESULTS

EFFECTIVE AND SAFE CONCENTRATIONS

For the youngest vimba, the lowest effective concentration (LEC) and the highest safe concentration (HSC) were 0.35 and 0.48 g dm⁻³, respectively. For all remaining groups, the LEC was 0.33 g dm⁻³ and the HSC was 0.43 g dm⁻³.

ANAESTHESIA INDUCTION AND RECOVERY TIMES

Up to the age of 63 days, vimba showed no differences in average general anaesthesia induction time or average recovery time (Table 2). Older fish reached general anaesthesia and recovered later. The longest individual induction time was 6.22 min and the maximum recovery time was 3.00 min.

TABLE 2

General anaesthesia induction time (IT) and recovery time (RT) in juvenile vimba exposed to 2-phenoxyethanol at 0.40 g dm⁻³ (temperature 25°C, exposure time 15 min)

Age (days)	IT average ± SD (min)	RT average ± SD (min)
38	2.53 ± 0.90 ^a	1.21 ± 0.33 ^a
63	2.80 ± 0.65 ^a	1.37 ± 0.26 ^a
115	4.34 ± 1.01 ^c	1.70 ± 0.35 ^b
179	3.67 ± 1.00 ^b	2.08 ± 0.46 ^c

Data with the same superscripts are not significantly different at $P = 0.05$, (Duncan's multiple range test, $N = 30$)

No relationship was found between the induction or recovery time and fish size or condition (Fulton's coefficient) for any age group. Only the youngest fish showed a negative correlation between induction time and recovery time (Fig. 1).

DISCUSSION

According to Trevor and Miller (1987), the effect of anaesthetic on fish depends on its concentration in the central nervous system tissues. Bonath (1977) stated that the differences in sensitivity of various fish species to anaesthetic are related to the differences in

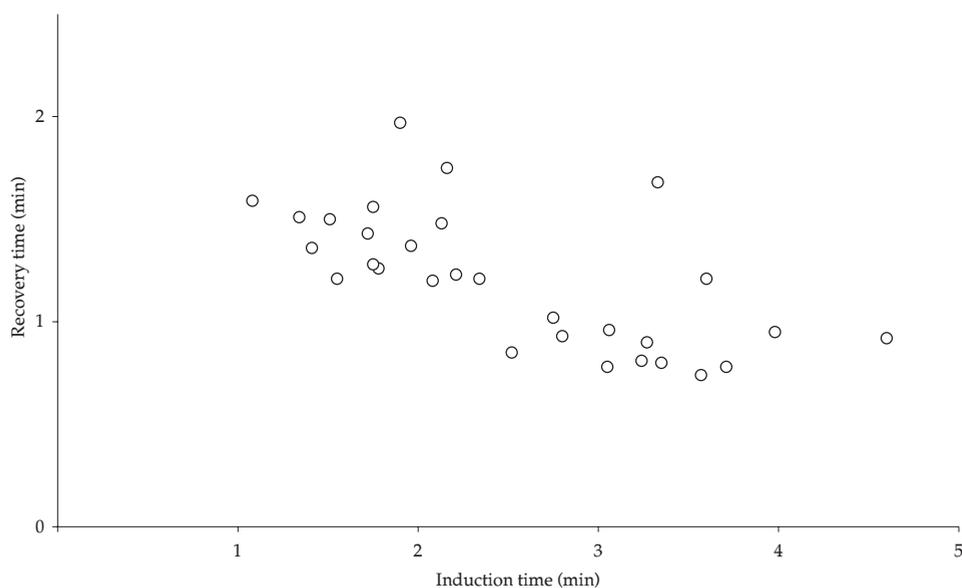


Fig. 1. General anaesthesia induction time and recovery time in 38-day-old vimba exposed to 2-phenoxyethanol at 0.40 g dm^{-3} at 25°C for 15 min.

metabolic rate. Temperature is one of the key factors that modifies metabolic rates in fish (McFarland 1960, Bonath 1977). Various authors also observed that anaesthesia induction and recovery times depend on fish size (McFarland 1960, Marking 1967, Bonath 1977, Guilderhus and Marking 1987, Weyl et al. 1996).

Fish are usually anaesthetized by immersion in an aqueous solution of an anaesthetic (Ross and Ross 1984, Guilderhus and Marking 1987). They take up the anaesthetic through the gills and skin, and then it is transported by the blood to the central nervous system (Ross and Ross 1984).

In our study, done at a constant temperature of 25°C , the tolerance of vimba to 2-phenoxyethanol increased with age. Fish of the same age did not show a significant relationship between fish size and anaesthesia induction time or fish size and recovery time. These results indicate that the reaction of vimba to 2-phenoxyethanol depends rather on age than size. Similar observations were made for tench juveniles (Myszkowski et al. 2001). The authors suggested that older fish take up and excrete the anaesthetic at a slower rate than the younger ones do. Older fish have well-developed scales, and their body surface-to-volume ratio is lower which may result in the slower skin uptake of the anaesthetic. This explains why the older individuals need more time to reach general anaesthesia.

The youngest fish showed a negative correlation between induction and recovery times (Fig. 1), which suggests that the anaesthetic effect is strongly affected by the excretion rate. The faster 2-phenoxyethanol is eliminated from the body, the longer the time needed to reach general anaesthesia at certain drug concentrations. On the other hand, the fish recover faster. The results of various studies (Kamiński et al. 2000) indicate that in fish treated with 2-phenoxyethanol the average anaesthesia induction time is longer than the average recovery time. Therefore, it seems that the uptake of the drug is slower than its excretion in clean water. It is supposed that 2-phenoxyethanol is taken up by diffusion, and eliminated using both diffusion and active transport. During exposure, the anaesthetic is excreted counter to the concentration gradient, and during recovery in accordance with it. This is probably why the recovery phase is shorter than induction.

In vimba aged over 38 days, no correlation was found between the induction and recovery time, which is difficult to explain.

According to Myszkowski et al. (2001), juvenile tench, *Tinca tinca* (L.) showed an increase of the lowest effective concentration (LEC), and a decrease of the highest safe concentration (HSC) with age. In the present study, the youngest vimba had the highest LEC and HSC values. Older fish showed longer average induction and recovery times, therefore it seems that LEC should also be higher. It also seems that HSC should be lower in older fish in comparison to younger ones. However, vimba at the age of 63-179 days showed no differences in LEC and HSC. Further studies are needed to explain these discrepancies.

In both vimba and tench (Myszkowski et al. 2001), the highest changes in LEC and HSC occurred at the earliest life stages. This could result from scale formation and development. According to Ferreira et al. (1984), anaesthetic uptake efficiency by fish skin may depend on scale thickness and size.

Our data indicate that 2-phenoxyethanol is an effective and safe anaesthetic for juvenile vimba. The short time of anaesthesia induction (up to about 6 min) and the even shorter recovery time (up to 3 min) is a considerable advantage. Moreover, a wide range (about 0.1 g dm^{-3}) between the lowest effective concentration and the highest safe concentration reduces the risk of 2-phenoxyethanol overdose.

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STRESZCZENIE

REAKCJA MŁODOCIANEJ CERTY, *VIMBA VIMBA* (L.), NA DZIAŁANIE 2-FENOKSYETANOLU

Celem badań było określenie reakcji młodocianej certy, *Vimba vimba* (L.), w wieku 38-179 dni od wyklucia na 2-fenoksyetanolu, powszechnie stosowany w akwakulturze preparat do znieczulenia ryb. Badania przeprowadzono w temperaturze 25°C. Określono stężenia tego anestetyku zapewniające indukcję pełnej anestezji w czasie nie dłuższym niż 10 min (stężenia skuteczne) oraz stężenia nie powodujące śmiertelności po 15 min ekspozycji (stężenia bezpieczne). Najniższe stężenie skuteczne (NSS) wynosiło 0,35 g dm⁻³ u ryb w wieku 38 dni, a 0,33 g dm⁻³ u ryb starszych (63-179 dni od wyklucia). Najwyższe stężenie bezpieczne (NSB) wynosiło 0,48 g dm⁻³ u certy w wieku 38 dni, a u ryb starszych 0,43 g dm⁻³.

W roztworze 2-fenoksyetanolu o stężeniu 0,40 g dm⁻³, średni czas indukcji pełnej anestezji i średni czas wybudzania były najkrótsze u ryb najmłodszych (odpowiednio 2,53 i 1,21 min). Maksymalny obserwowany u certy czas indukcji pełnej anestezji wynosił 6,22 min, a najdłuższy czas wybudzania 3,00 min.

Zakres stężeń między najniższym stężeniem skutecznym i najwyższym stężeniem bezpiecznym 2-fenoksyetanolu jest stosunkowo szeroki (nie mniej niż 0,1 g dm⁻³). W praktyce oznacza to, że zastosowanie stężenia nieznacznie wyższego od NSS pozostawia margines bezpieczeństwa utrudniający przypadkowe przedawkowanie. Wyniki badań dowiodły przydatności 2-fenoksyetanolu do skutecznej i bezpiecznej anestezji młodocianej certy.

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