The efficacy of various anaesthetics in tench (*Tinca tinca* L.) related to water temperature

J. Hamackova[1], A. Lepicova[1], P. Kozak[1], Z. Stupka[2], J. Kouril[1], P. Lepic[1]

[1]University of South Bohemia Ceské Budejovice, Research Institute of Fish Culture and Hydrobiology at Vodňany, Czech Republic

[2]University of South Bohemia Ceské Budejovice, Faculty of Agriculture in Ceské Budejovice, Czech Republic

ABSTRACT: The effect of three different anaesthetics, 2-phenoxyethanol (0.6 ml/l), Propiscin (0.75 ml/l) and clove oil (0.033 ml/l), on adult tench (*Tinca tinca* L.) of mean body weight 260 g (66–583 g), of both sexes was tested at four different water temperatures (17.9; 20.4; 22.6 and 25.1°C). The time periods necessary for the induction of particular characteristic phases of anaesthetisation and recovery were evaluated. At all temperatures, the statistically longest induction of anaesthesia ($P < 0.05$) and longest recovery ($P < 0.01$) were registered with Propiscin. With clove oil and 2-phenoxyethanol, the time period necessary for induction of phase II b anaesthesia statistically declined ($P < 0.05$) with rising temperature, however, this phenomenon was not seen with Propiscin.

Keywords: phase anaesthesia; recovery; 2-phenoxyethanol; Propiscin; clove oil

Anaesthetics are used to produce moderate sedation to reduce stress, and to immobilise fish so that they can be captured when establishing experiments, or for periodic observations, artificial reproduction, measurements or marking and others. Ranges of anaesthetics are used for fish to aid their capture, handling and transport. Anaesthetics are used routinely during commercial fish culture and also by fishermen and biologists during experiments in the field or laboratory (Mundey and Wilson, 1997).

The impact of stress induced by manipulation and anaesthesia on some physiological and biochemical blood parameters of common carp were studied by Jirasek et al. (1988). The impact of anaesthetics upon oxygen consumption in tench was described by Jirasek et al. (1978).

The properties required of an anaesthetic will vary with the research objectives. However, quick induction of anaesthesia is desirable in most cases (Marking and Meyer, 1985). These authors defined criteria for an ideal anaesthetic. Their criteria were as follows: rapid immobility, quick recovery, and non-toxicity to fish, low tissue residues and low cost.

Despite the wide range of chemicals available for anaesthetising fish and calls for studies on the comparative efficacy of these chemicals (Marking and Meyer, 1985), few rigorous comparative studies have been conducted. Gilderhus and Marking (1987) compared the efficacy of anaesthetics commonly used in rainbow trout, *Oncorhynchus mykiss* (Walbaum), and determined that MS-222, quinaldine, benzocaine and 2-phenoxyethanol were the most effective anaesthetics for this species. Kazun et al. (1999) recommended 0.5–4.0 ml/l of Propiscin depending on the fish species and size. The time of anaesthesia induction is from 1 to 3 min depend-

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project MSM No. 126100001) and the Ministry of Agriculture of the Czech Republic (Project MZe QF 3029).

ing on the water temperature. Comparative tests of some of these chemicals were conducted on commercially cultured marine fishes (Mattson and Riple, 1989). However, comprehensive comparative studies of all these chemicals in species commonly studied by marine biologists are missing. In addition, clove oil has recently been shown to be an effective fish anaesthetic (Soto and Burhanuddin, 1995) but its comparative efficacy has not been determined. Therefore, the efficacy of clove oil and other chemicals (quinaldine, MS-222, benzocaine, 2-phenoxyethanol) commonly used by marine biologists to anaesthetise fish were compared. Mundey and Wilson (1997) compared the time necessary to elicit a response across their effective concentrations, behavioural response of fishes to anaesthesia, recovery times, and survival rates. The anaesthetic properties of clove oil were tested on chinook salmon *Oncorhynchus tshawytscha*, coho salmon *O. kisutch*, rainbow trout *O. mykiss*, and white sturgeon *Acipenser transmontanus* (Taylor and Roberts, 1999). The authors found the median lethal concentrations for a 10-min exposure of 62 mg/l for chinook salmon, 96 mg/l for coho salmon, 250 mg/l for rainbow trout, and 526 mg/l for white sturgeon. A dosage of 25 mg/l was effective in anaesthetising all species for 120 min without mortality.

The aim of our experiments was to compare the efficacy of three anaesthetics in adult tench (*Tinca tinca* L.) at different water temperatures.

MATERIAL AND METHODS

Experiments were carried out under laboratory conditions. The effect of three anaesthetics on tench (*Tinca tinca* L.) was evaluated at four different water temperatures: 17.9 ± 0.04; 20.4 ± 0.09; 22.6 ± 0.12 and 25.1 ± 0.05°C. Before the experimental observations, fish were adapted to the particular temperatures. Tench originating from pond culture with a sex ratio 1 : 1 were used. For further details see Table 1. The following three anaesthetics were applied: 2-phenoxyethanol (France, lot No. S 36456 223) 0.6 ml/l, Propiscin (Poland, pharmacological preparation exclusively adjusted for use in fishery in immersion form; lot of the 2001/01 IRS-ZpiIR Zabieniec series) 0.75 ml/l and clove oil (Czech Republic, lot No. 171910) 0.033 ml/l. We used the concentrations recommended by Kouril et al. (2001).

One tench male and one female were introduced into 100-litre aquarium provided with constant aeration. Eight replications were run with each anaesthetic and water temperature, thus 192 tench specimens were used in total. The duration of particular anaesthesia phases (Table 2) was recorded for 10 minutes and then fish were transferred into clear aerated water and duration of recovery from anaesthesia (Table 2) was recorded. This study was concentrated upon the registration of anaesthesia phase II b (as induced in all fish), phase III (induced in only some cases) and observations of recovery from anaesthesia. Water temperature and dissolved oxygen concentration were currently monitored. The data were processed using analyses of variance (ANOVA) and two-sample *t*-test.

RESULTS

The anaesthetic 2-phenoxyethanol was the most efficient of the three anaesthetics we tested, that is to say induction of phase II b anaesthesia (Table 3) and time of recovery (Table 4) were quickest at all temperatures (the only exception was a quicker induction of anaesthesia at 22.6°C with clove oil). The time of induction of phase II b anaesthesia and recovery time period lasted from 2.48 ± 0.87 min to 3.98 ± 0.87 min and 5.48 ± 1.50 min to 11.77 ± 0.97 min, respectively, in dependence on the temperature. Propiscin (using a recommended dose) was the least efficient anaesthetic, which required the longest times for induction of, and recovery from, phase II b anaesthesia.

Among the three anaesthetics tested, there were no consistent patterns of changes in the induction time with temperature (Table 3). For 2-phenoxyethanol, induction times to phase II b were significantly faster ($P < 0.01$) at 25.1°C. For clove oil, there was also a decrease in the induction time with increasing temperature: induction times were significantly faster ($P < 0.01$) at 22.6 and 25.1 than at 17.9 and 20.4°C. However, with Propiscin, there were no significant differences in the time of induction with temperature.

Each of the three anaesthetics tested showed decreased recovery times with increasing temperature. For clove oil and Propiscin, recovery times at 22.6 and 25.1 were significantly faster ($P < 0.01$) than at 17.9 and 20.4. For 2-phenoxyethanol, recovery times at each temperature were significantly faster ($P < 0.01$) than those at all lower temperatures.
Anaesthesia of phase III, which resulted in cessation of breathing, was induced with 2-phenoxyethanol at

Table 1. Weight, total length and body length of experimental fish (n = 192). Values are means ± SD (n = 16) above, and min – max below

Anaesthetics	Temperature (°C)	Weight (g)	Total length (mm)	Body length (mm)
Clove oil	17.9	271.2 ± 133.3	263.5 ± 43.3	208.8 ± 35.2
		111–583		
	20.4	229.4 ± 70.4	253.44 ± 23.8	203.8 ± 21.7
		132–353		
	22.5	266.6 ± 111.8	278.0 ± 45.8	230.9 ± 36.9
		80–491		
	25.1	305.1 ± 143.3	284.1 ± 51.9	234.7 ± 42.2
		109–491		
2-phenoxyethanol	17.9	273.6 ± 97.6	269.4 ± 36.3	216.7 ± 28.2
		103–424		
	20.4	225.3 ± 116.5	245.3 ± 37.8	194.8 ± 28.4
		66–536		
	22.6	267.3 ± 137.2	270.1 ± 50.1	226.3 ± 42.6
		84–557		
	25.1	271.9 ± 98.63	270.9 ± 38.4	228.0 ± 32.1
		120–560		
Propiscin	17.9	270.2 ± 103.6	271.6 ± 37.7	216.4 ± 28.4
		146–513		
	20.4	243.9 ± 119.2	255.4 ± 37.5	202.9 ± 27.1
		86–560		
	22.8	279.1 ± 131.5	279.6 ± 51.0	235.5 ± 40.3
		111–468		
	25.0	226.9 ± 90.1	259.4 ± 33.9	216.1 ± 30.4
		99–411		

Table 2. Phases of physiological responses caused by anaesthetics. Data from Trzebiatowski et al. (1996) modified by the authors

	Phase	Characteristics
Anaesthesia induction	0	physiological position; normal locomotor activity
	I	physiological position; increased locomotor activity
	II a	decreased locomotor activity; slight tilting on the flank
	II b	flank position; immobilization
	III	breathing stopped
Recovery from anaesthesia	II b	flank position; immobilization
	II a	uncoordinated locomotion; signs of physiological position
	I	physiological position; decreased locomotor activity
	0	physiological position; normal locomotor activity

Table 3. Time of induction of anaesthesia (phase II b) in minutes (mean ± SD, *n* = 16) by three anaesthetics at four water temperatures

Anaesthetics	17.9°C	20.4°C	22.6°C	25.1°C
Clove oil	4.15 ± 1.53	4.03 ± 1.48	2.95 ± 0.80	2.95 ± 0.97
2-phenoxyethanol	3.55 ± 0.88	3.98 ± 0.87	3.05 ± 0.77	2.48 ± 0.87
Propiscin	4.85 ± 1.88	5.47 ± 1.52	4.27 ± 2.05	4.67 ± 1.32

Table 4. Time of recovery from anaesthesia in minutes (mean ± SD, *n* = 16) for three anaesthetics at four water temperatures

Anaesthetics	17.9°C	20.4°C	22.6°C	25.1°C
Clove oil	17.30 ± 4.68	16.77 ± 3.73	10.65 ± 2.35	8.53 ± 3.58
2-phenoxyethanol	11.77 ± 0.97	10.38 ± 0.50	6.88 ± 1.62	5.48 ± 1.50
Propiscin	26.60 ± 4.77	29.95 ± 3.70	14.18 ± 3.60	15.03 ± 3.30

Table 5. Time of inducing the anaesthesia (phase II b) in minutes (mean ± SD) for the respective anaesthetics at various water temperatures related to sex

Sex	Anaesthetics	17.9°C	20.4°C	22.6°C	25.1°C
Females	Clove oil	4.30 ± 1.60	4.27 ± 1.79	2.96 ± 0.77	2.89 ± 1.03
	2-phenoxyethanol	3.68 ± 0.57	3.93 ± 0.80	3.12 ± 0.84	2.54 ± 1.10
	Propiscin	5.21 ± 1.40	6.45 ± 1.19	5.46 ± 2.33	5.42 ± 1.29
Males	Clove oil	3.99 ± 1.46	3.83 ± 1.12	2.94 ± 0.84	3.00 ± 0.91
	2-phenoxyethanol	3.42 ± 1.10	4.05 ± 0.92	2.98 ± 0.70	2.41 ± 0.51
	Propiscin	4.48 ± 2.21	4.49 ± 1.11	3.23 ± 0.90	3.90 ± 0.81

Table 6. Time of recovery from anaesthesia in minutes (mean ± SD, *n* = 16) for three anaesthetics at four water temperatures according to sex

Sex	Anaesthetics	17.9°C	20.4°C	22.6°C	25.1°C
Females	Clove oil	16.45 ± 4.65	15.55 ± 4.72	9.83 ± 1.05	8.12 ± 3.75
	2-phenoxyethanol	11.96 ± 0.86	10.22 ± 0.36	6.82 ± 1.75	5.26 ± 1.49
	Propiscin	27.98 ± 6.31	29.22 ± 4.19	12.70 ± 3.02	13.95 ± 3.13
Males	Clove oil	18.15 ± 4.55	17.98 ± 1.62	11.48 ± 2.94	8.94 ± 3.34
	2-phenoxyethanol	11.58 ± 1.03	10.54 ± 0.57	6.96 ± 1.45	5.71 ± 1.47
	Propiscin	25.23 ± 1.32	30.69 ± 2.98	15.66 ± 3.52	16.12 ± 3.11

all temperatures, but with clove oil only at 22.6 and 25.1°C; phase III was not reached with Propiscin at any temperature. When evaluating the effect of sex upon phase II b, we observed that anaesthesia was induced faster in males than in females at almost all temperatures with all the three anaesthetics without any statistically differences except clove oil at the higher temperature (22.6, 25.1°C; $P < 0.05$) (Table 5). On the contrary, fish recovery from anaesthesia phase II b was quicker in females in comparison with males at temperatures of 20.4, 22.6 and 25.1°C but also without any statistically differences (Table 6). Only at the lowest temperature 17.9°C, anaesthesia recovery was faster in males than females (for 2-phenoxyethanol and Propiscin, but not for clove oil).

DISCUSSION

The tested concentration of Propiscin (0.75 ml/l) and the time required for anaesthesia induction in tench were in a good agreement with the results of Kazun et al. (1999). They studied the dependence of Propiscin anaesthetic concentration on fish weight and recommend concentrations 1.0–4 ml/l and 0.5–1.0 ml/l for common carp (*Cyprinus carpio*) and grass carp, respectively, with regard to fish size. They stated that anaesthesia appeared within 3 min at higher doses.

For the induction of anaesthesia and recovery in our experiment, we used similar criteria to those used by Trzebiatowski et al. (1996), who studied the application of Propiscin in European catfish (*Silurus glanis*) in concentrations of 0.5 and 0.75 ml/l at three different temperatures 16, 20 and 25°C. They found the negative relationship between the time of induction of, and recovery from, anaesthesia, and water temperature. In our experiments, we did not observe a direct relationship between the times for anaesthesia induction and water temperature only when using Propiscin. It could be caused by a low dose of Propiscin for tench of this weight category.

With perch (Hamackova et al., 2001), phase III of anaesthesia was reached in all cases using the given anaesthetics at various water temperatures contrary to tench, where this phase was reached only with 2-phenoxyethanol at all studied temperatures and with clove oil only at two higher temperature levels (22.6 and 25.1°C). No relationship was found between the duration of anaesthesia recovery and water temperature for tench, compared to perch (Hamackova et al., 2001).

In their study, Weyl et al. (1996) estimated the efficiency of 2-phenoxyethanol anaesthetic in two size groups of goldfish (*Carassius auratus* L.) at temperatures of 20, 25 and 30°C. In agreement with these authors, we demonstrated with shorter times being of anaesthesia induction time required at higher water temperatures.

The efficacy of clove oil as an anaesthetic was previously reported in a variety of fish including rainbow trout juveniles and adults (Anderson et al., 1997), *Siganus lineatus* (Soto and Burhanuddin, 1995), and non-salmonid fishes (walleyes *Stizostedion vitreum*, small-mouth bass *Micropterus dolomieu*, northern pike *Esox lucius*, and lake sturgeon *Acipenser fulvescens*) (Peake, 1998). Anderson et al. (1997) indicated clove oil as a suitable anaesthetic for rainbow trout juveniles and adults. The test with clove oil was performed also by Soto and Burhanuddin (1995) in *Siganus lineatus* (an aquarium fish species under our conditions), who reported it as a very efficient anaesthetic. Peake (1998) presented the results of clove oil application in non-salmonid fishes. The anaesthesia and recovery were achieved within 4.3 and 10.9 min, respectively, with a concentration of 60 mg/l at 10°C. The author indicated that exceeding the time of anaesthesia over 5 min had a considerable influence on the time of recovery. After conversion of our concentration (48.2 mg/l) our times of anaesthesia from 2.95 to 4.15 min with clove oil are in a good agreement with Peake (1998), taking into account the higher water temperature in our experiments.

Based on our results of the evaluated anaesthetics, 2-phenoxyethanol and possibly clove oil can be recommended as the most efficient for tench. According to Taylor and Roberts (1999) clove oil is an efficient and relatively safe anaesthetic.

CONCLUSION

The anaesthetic 2-phenoxyethanol was the most efficient for tench regardless of sex. Anaesthesia was induced and disappeared in the shortest time at all temperatures.

With Propiscin, anaesthesia was always induced after the longest period.

With the increasing temperature, the time for anaesthesia induction and recovery became shorter for both clove oil and 2-phenoxyethanol. No such temperature effect was proved for Propiscin.

Anaesthesia was induced more quickly in males nearly at all temperatures by means of all three anaesthetics.

In females, anaesthesia disappeared in a shorter period in comparison with males at the temperatures of 20.4, 22.6 and 25.1°C.

Phase III (breathing stopped) was reached with 2-phenoxyethanol at all temperatures, whilst with clove oil only at 22.6 and 25.1°C. When using Propiscin, this phase did not appear during the observed period at all.

REFERENCES

Anderson W.G., McKinley R.S.,Veccia M.C. (1997): The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management, 17, 301–307.

Guilderhus P.A., Marking L.L. (1987): Comparative efficacy of 17 anaesthetic chemicals on rainbow trout. North American Journal of Fisheries Management, 7, 288–292.

Hamackova J., Sedova M.A., Pjanova S.V., Lepicova A. (2001): The effect 2-Phenoxyethanol, clove oil and Propiscin anaesthetics on perch (*Perca fluviatilis)* in relation to water temperature. Czech Journal of Animal Science, 46, 469–473.

Jirasek J., Adamek Z., Giang R.M. (1978): The effect of the anaesthetics MS 222 (Sandoz) and R 7464 (Propoxate) on oxygen consumption in the tench (*Tinca tinca* L.) (in Czech). Zivocisna Vyroba, 23, 835–840.

Jirasek J., Spurny P., Mares J. (1988): Effects of manipulation stress and anaesthesia with Propoxat on some physiological and biochemical parameters of blood in carp fry (in Czech). Acta Universitatis Agriculture (Brno), XXXVI, 149–157.

Kazun K., Siwicki,A.K., Glabski E. (1999): The observations of Propiscin efficiency for total anaesthetisation of cyprinids (in Poland). In: IV. Krajowa konferencija hodowców karpia, Kiekrz 24.–26.02.1999, Wydawnictwo IRS, Olsztyn, 57–60.

Kouril J., Stupka Z., Hamackova J., Lepicova A. (2001): The preliminary results of effect of three anaesthetics on various fish (in Czech). In: Kolarova J. (ed.): Health Protection of Fish. VURH JU, Vodnany, Czech Republic. 131–136.

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

Marking L.L., Meyer F.P. (1985): Are better anaesthetics needed in fisheries? Fisheries (Bull. A.F.S.) 10, 1–5.

Mundey P.L., Wilson S.K. (1997): Comparative efficacy of clove oil and other chemicals in anaesthetisation of *Pomacentrus amboinensis,* a coral reef fish. Journal of Fish Biology, 51, 931–938.

Peake S. (1998): Sodium bicarbonate and clove oil as potential anaesthetics for nonsalmonid fishes. North American Journal of Fisheries Management, 18, 919–924.

Soto C.G., Burhanuddin C.G. (1995): Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish *(Siganus lineatus).* Aquaculture, 136, 149–152.

Trzebiatowski R., Stepanowska K., Siwicki A.K., Kazun K. (1996): The observations of Propiscin efficiency for total anaesthetisation of wels (*Silurus glanis*) (in Poland). Komunikaty Rybackie, 1, 14–18.

Taylor P.W., Roberts S. D. (1999): Clove oil: An alternative anaesthetic for aquaculture. North American Journal of Aquaculture, 61, 150–155.

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

Received: 02–12–11
Accepted after corrections: 04–11–29

Corresponding Author:

Ing. Jitka Hamackova, University of South Bohemia Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology at Vodnany, Zatisi 728/II, 389 25 Vodnany, Czech Republic
Tel. +420 383 382 402, fax +420 383 382 396, e-mail: hamackova@vurh.jcu.cz