# Efficacy of different anaesthetics for pikeperch (*Sander lucioperca* L.) in relation to water temperature

**Jiri Kristan [1], Alzbeta Stara [1], Miroslav Polgesek [2], Alexandru Drasovean [3], Jitka Kolarova [1], Josef Priborsky [1], Miroslav Blecha [1], Petr Svacina [1], Tomas Policar [1], Josef Velisek [1]**

1 University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic
2 West Pomeranian University of Technology Szczecin, Faculty of Food Sciences and Fisheries, Szczecin, Poland
3 University de Stiinte Agricole si Medicina Veterinara and Banatului Timisoara, Timişoara, jud. Timiş, Romania

*Correspondence to:* Dipl.-Ing. Jiri Kristan, PhD.
University of South Bohemia in Ceske Budejovice,
Faculty of Fisheries and Protection of Waters,
South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology
Zatisi 728/II 389 25 Vodnany, Czech Republic.
TEL: +420 387 774 610; E-MAIL: kristj01@frov.jcu.cz

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**Abstract**

**OBJECTIVES:** The objectives of the study were to compare the different doses of clove oil, Propiscin, and tricaine methane sulphonate (MS 222) in relation to water temperature in pikeperch aquaculture.
**DESIGN:** For assessment of this experiment 168 fish (10.77±0.59 cm total body length and 7.88±1.74 g body weight) were used. Three different anaesthetic treatments (Propiscin, clove oil and MS 222) were used. Three doses of each anaesthetic treatment (Propiscin: 0.5; 1; 1.5 ml.L$^{-1}$, clove oil: 15; 30; 60 mg.L$^{-1}$, MS 222: 50; 100; 150 mg.L$^{-1}$) were compared at three different temperatures 9.5; 15.5 and 23 °C.
**RESULTS:** In comparison of these doses of anaesthetic in different temperature, the significantly shortest time to attain phase A7 (total complete anaesthesia) was observed for Propiscin (1.5 ml.L$^{-1}$) 0:31±0:04 min (23 °C) to 0:33±0:25 min (9.5 °C) compared to MS 222 (150 mg.L$^{-1}$) 1:04±0:21 min (23 °C) to 1:54±0:32 min (9.5 °C) and clove oil (60 mg.L$^{-1}$) 1:05±0:17 min (23 °C) to 3:05±0:31 min (9.5 °C). On the other hand, the longest time of anaesthesia recovery was attained using Propiscin (1.5 ml.L$^{-1}$) 10:35±1:40 min (23 °C) to 32:30±1:10 min (9.5 °C) compared to clove oil (60 mg.L$^{-1}$) 2:39±0:50 min (23 °C) to 9:36±2:34 min (60 mg.L$^{-1}$, 9.5 °C) and MS 222 (150 mg.L$^{-1}$) 2:26±1:27 min (23 °C) to 4:59±0:39 min (9.5 °C).
**CONCLUSIONS:** The results from this study showed that the optimal and sufficient doses in all tested temperatures for pikeperch are 30 mg.L$^{-1}$ of clove oil, 100 mg.L$^{-1}$ of MS 222 and 0.5 ml.L$^{-1}$ of Propiscin.



ORIGINAL ARTICLE

**Abbreviations:**

| | |
|---|---|
| $ANC_{4.5}$ | - acid neutralization capacity |
| ANOVA | - analysis of variance |
| $COD_{Mn}$ | - chemical oxygen demand |
| MS 222 | - tricaine methane sulphonate |
| RAS | - recirculating aquaculture system |

## INTRODUCTION

Anaesthetics are required to aquaculture practises and are commonly used in routine aquaculture procedures to reduce movability and stress factor in fish. Anaesthetics are also widely administered in cases of measuring, photographing, injection, tagging, blood sampling and mainly for the artificial stripping of fish (Cooke *et al.* 2004; Macova *et al.* 2008; Gullian & Villanueva 2009; Velisek *et al.* 2009, 2011; Kristan *et al.* 2012). Anti-stress agents have a wide chemical variety (Iwama & Ackerman 1994; Kazun & Siwicki 2001; Velisek & Svobodova 2004; Velisek *et al.* 2005; Altun *et al.* 2009).

At the time, the commonly used anaesthetics in pikeperch (*Sander lucioperca*) are clove oil (Kristan *et al.* 2013), tricaine methane sulphonate (MS 222) (Zarski *et al.* 2012) and Propiscin (Zakes & Demska-Zakes 2005). Clove oil is a dark brown liquid which is distilled from natural leaves of the clove trees *Eugenia aromatica* and *Eugenia caryophyllata* (Sato & Burhanuddin 1995; Keene *et al.* 1998). Eugenol (4-allyl-2-methoxyphenol) is the main ingredient of clove oil and makes up about 90–95% of the oil weight (Davis *et al.* 2013).

MS 222 is an isomer of benzocaine with an additional sulphonate radical, making it more soluble but also more acidic in solution (Congleton 2006). It is the most commonly used anaesthetic for fish (Marking & Meyer 1985). Propiscin was developed at the Inland Fisheries Institute in Poland and is routinely used for immobilization of fish in Poland fisheries (Szkudlarek & Zakes 1996). The active substance of Propiscin is etomidate [etomidate (1)-ethyl 1-(a-methylbenzyl) imidazole-5-carboxylate] (Kazun & Siwicki 2001).

To our knowledge, no recommended and examined doses at different water temperatures in pikeperch anaesthetized with clove oil, Propiscin or MS 222 in the available literature. The purpose of this study was to provide practical information on the lowest effective doses of the most commonly used anaesthetics in relation to water temperature.

**Tab. 1.** Description of the anaesthesia phases and recovery, modified according to Park *et al.* (2008).

| Phase | Characteristic behaviour |
|---|---|
| **Anaesthesia** | |
| A1 | Regular respiratory motion, normal locomotor activity and opercular movement. |
| A2 | Irregular respiratory motion, fast swimming, swimming erratic. |
| A3 | Partial loss of equilibrium, rolling from side to side. |
| A4 | Complete loss of equilibrium, swimming turned out, pectoral fin, pelvic fin and dorsal fin movement stop. |
| A5 | Anal fin and tail fin movement stop, little sedation. |
| A6 | Only opercular movement, perfect sedation, acoustic reflex. |
| A7 | Opercular movement and acoustic reflex stop. |
| **Anaesthesia recovery** | |
| R1 | Opercular movement, acoustic reflex. |
| R2 | Tail and anal fin movement. |
| R3 | Pectoral fin, pelvic fin and dorsal fin movement. |
| R4 | Swimming turned out, rolling from side to side. |
| R5 | Regular respiration, swimming erratic. |
| R6 | Normal locomotor activity, normal swimming, responsiveness to visual stimuli. |

## MATERIALS AND METHODS

### Anaesthetics

MS 222 was purchased from Sigma-Aldrich Chemicals Ltd., Propiscin was supplied by the Division of Fish Pathology and Immunology at Zabieniec (Inland Fisheries Institute in Olsztyn, Poland) and clove oil (eugenol concentration 78%) was from the Kulich Company (Jan Kulich, Hradec Kralove/Ricany, Czech Republic.

### Fish

The fish (n=168, TL=10.77±0.59 cm, W=7.88±1.74 g) were obtained from recirculating aquaculture system (RAS) at the University of South Bohemia, Faculty of Fisheries and Protection of Waters. To maintain growth, the fish were fed with food (Inicio Plus, BioMar). All fish were starved for 24 h before experiments were conducted.

### Experimental procedure

Eight fishes were used for each temperature and each dose of anaesthetics. The experiments were carried out under laboratory conditions into 40 L tanks containing 20 L water. Before start of experiments, the following parameters: dissolved oxygen, >90%; pH, 7.5; acid neutralization capacity ($ANC_{4.5}$), 0.98 $mmol.L^{-1}$; chemical oxygen demand ($COD_{Mn}$), 1.15 $mg.L^{-1}$; total ammonia, 0.01 $mg.L^{-1}$; $NO_3^-$, 6.75 $mg.L^{-1}$; $NO_2^-$, <0.02 $mg.L^{-1}$; were checked.

All experiments were performed in triplicated. The anaesthetics effect of MS 222, Propiscin and clove oil was investigated at three concentrations. Anaesthetics were dosed as followed: MS 222 50; 100; 150 $mg.L^{-1}$, Propiscin 0.5; 1; 1.5 $ml.L^{-1}$ and clove oil 15; 30; 60 $mg.L^{-1}$.

The experiments were carried out under the following three different water temperatures 9.5, 15.5 and 23 °C after previous one month long adaptation of fish to the given temperatures. The anaesthesia levels and recovery times of fish were measured using stopwatch. An exposure to anaesthesia was chosen in advance to be max 15 min and the phases of anaesthesia (Table 1) were studied individually during this period. Anaesthesia time was determined individually from the time when the fish was stocked in anaesthetics water to the time of the phase A7. Then, the fish were placed to clean aerated water of the same temperature and recovery time was observed.

*Statistical analysis*

Statistical analysis was performed using program Statistica 9.0 for Windows (StatSoft, INc., Czech Republic). Data were first tested for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance (Bartlett's test). If those conditions were satisfied, Tukey's multiple comparison test was applied to identify which treatments were significantly different.

## RESULTS AND DISCUSSION

Anaesthesia is necessary for many hatchery procedures involving fish. All of the anaesthetics tested in this study are used in aquaculture (King *et al.* 2005; Velisek *et al.* 2009; Weber *et al.* 2009; Kristan *et al.* 2012). However, no recommended doses are in literature for pikeperch. Optimum anaesthetic concentrations are usually expected to induce anaesthesia within 3 min and recovery within 10 min (Son *et al.* 2001; Park *et al.* 2008). The optimum concentrations of anaesthetics can be influenced by species, water temperature, and other ambient physical and chemical characteristics (Hikasa *et al.* 1986; Son *et al.* 2001).

The anaesthesia time at different concentration of clove oil, Propiscin and MS 222 are presented in Figure 1 and the recovery time is demonstrated in Figure 2. The concentration of clove oil (0.015 mg.L$^{-1}$) was not sufficient at the lowest temperature (9.5 °C). Similar results with this concentration presented King *et al.* (2005), who also not achieved phase A7 (opercular movement and acoustic reflex stop) in black sea bass (*Centropristis striata* L.). At 15.5 °C, the anaesthesia time was 10.37±1.07 min and 8.46±2.15 min at 23 °C. The recovery time ranged from 3.35±0.36 min at 15.5 °C to 2.54±1.04 min at 23 °C (Figure 2C). At concentration of clove oil (30 mg.L$^{-1}$), the anaesthesia time was 3.10±1.01 min at 9.5 °C, 3.50±1.18 min at 15.5 °C and 2.16±0.31 min at 23 °C. The recovery time was 9.36±2.34 min at 9.5 °C, 3.49±0.37 min at 15.5 °C and 2.17±0.43 min at 23 °C. This concentration studied Hamackova *et al.* (2001) for perch (*Perca fluviatilis*) at different water temperatures and reported longer anaesthesia and recovery time than in this study. On the other hand, Weber *et al.* (2009) observed similar results (induction 3.16±0.40 and recovery time 3.76±1.01 min) in the Senegalese sole (*Solea senegalensis* Kaup 1858). At concentration of clove oil (60 mg.L$^{-1}$), the anaesthesia time was 3.05±0.31 min at 9.5 °C, 1.53±0.16 min at 15.5 °C and 1.05±0.17 min at 23 °C. The recovery time was 7.37±1.31 min at 9.5 °C, 5.09±0.45 min at 15.5 °C and 2.39±0.50 min at 23 °C. Similar results of anaesthesia time with dose 50 mg.L$^{-1}$ presented Park *et al.* (2008) in kelp grouper (*Epinephelus bruneus*), but the recovery time are not in agreement with this study. In case of temperatures 15.5 and 23 °C, the anaesthesia times significantly ($p<0.05$) decreased with increasing doses of the clove oil. In contrast, the recovery time with increasing doses was insignificant. However, the recovery time significantly ($p<0.05$) decreased with increased water temperature.

The Propiscin at concentration of 0.5 ml.L$^{-1}$, the anaesthesia time was 1.58±0.39 min at 9.5 °C, 3.35±0.21 min at 15.5 °C and 1.33±0.16 min at 23 °C. The recovery time was 18.03±1.59 min at 9.5 °C, 11.25±2.12 min at 15.5 °C and 6.07±0.13 min at 23 °C. At concentration of 1 ml.L$^{-1}$ Propiscin, the anaesthesia time was 1.18±0.40 min at 9.5 °C, 1.37±0.16 min at 15.5 °C and 0.49±0.18 min at 23 °C. The recovery time was 32.37±4.07 min at 9.5 °C, 16.31±1.37 min at 15.5 °C and 9.35±1.43 min at 23 °C. At concentration of 1.5 ml.L$^{-1}$ Propiscin, the anaesthesia time was 0.33±0.24 min at 9.5 °C, 0.31±0.06 min at 15.5 °C and 0.31±0.04 min at 23 °C. The recovery time was 32.30±1.10 min at 9.5 °C, 20.20±0.57 min at 15.5 °C and 10.35±1.40 min at 23 °C.

In all tested temperatures, the anaesthesia times significiantly ($p<0.05$) decreased with increasing doses of the Propiscin. These data also demonstrate that low

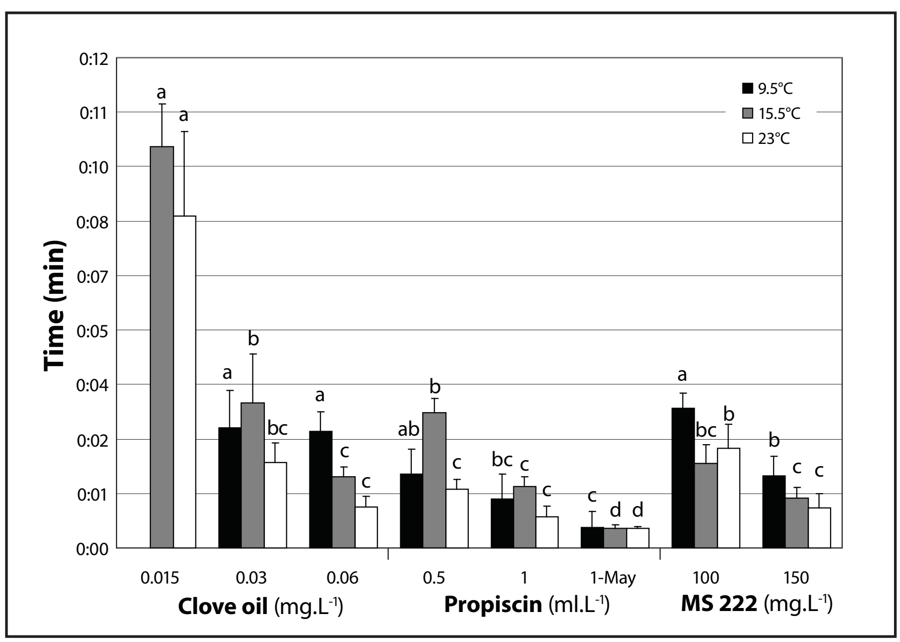


**Fig. 1.** Effect of anaesthesia dose on anaesthesia time in pikeperch at 9.5, 15.5 and 23°C. Different letters on the bars are significantly different ($p<0.05$).

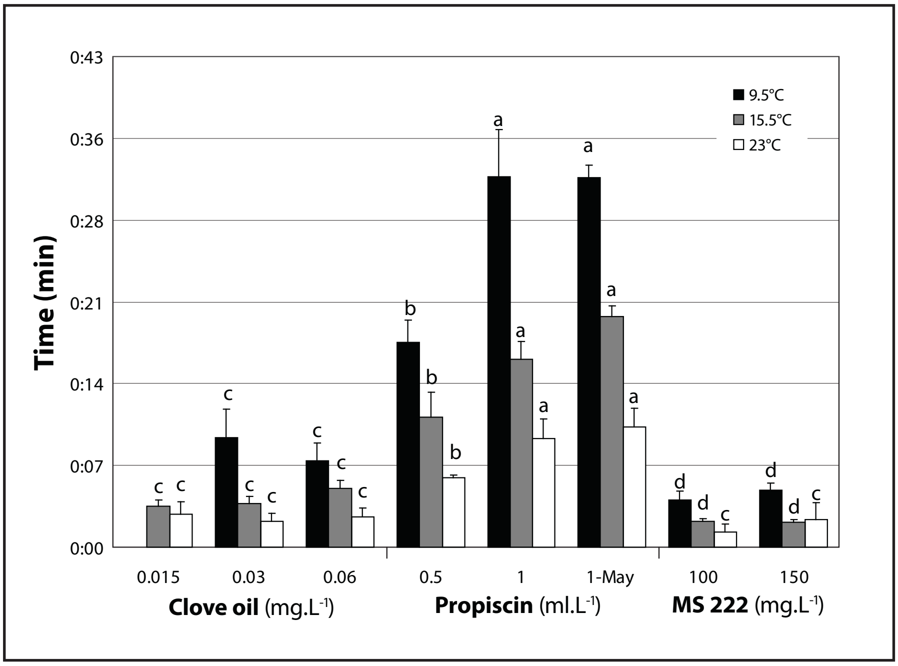


**Fig. 2.** Effect of anaesthesia dose on recovery time in pikeperch at 9.5, 15.5 and 23°C. Different letters on the bars are significantly different ($p<0.05$).

1.18±0.18 min at 15.5 °C and 1.04±0.21 min at 23 °C. The recovery time was 4.59±0.39 min at 9.5 °C, 2.13±0.13 min at 15.5 °C and 2.26±1.27 min at 23 °C. The recovery time with this dose was prolonged and according to Gullian & Villanueva (2009) is 120 mg.L$^{-1}$ MS 222 toxically.

The interaction of water temperature and dose on recovery time was significiantly ($p<0.05$) decreased anaesthesia time with increasing of water temperature. In agreement with Keene *et al.* (1998) and Gullian & Villanueva (2009) the difference in the recovery time between clove oil Propiscin and MS 222 is based on a metabolic constraint in three anaesthetics.

temperature significiantly ($p<0.05$) prolonged recovery times. The Propiscin concentration studied only Kazun & Siwicki (2001), who used this anaesthetics for many fish species: common carp (*Cyprinus carpio* L.), grass carp (*Ctenopharyngodon idella* Val.), catfish (*Clarias gariepinus*, Burchell), burbot (*Lota lota* L.), rainbow trout (*Oncorhynchus mykiss*, Valbaum), huchen (*Hucho hucho*, L.), brown trout (*Salmo trutta m. fario*, L.) and grayling (*Thymallus thymallus*, L.). This study used doses of 0.5 and 1 ml.L$^{-1}$. For all these fish, the anaesthesia time was 0.5–3 min and recovery time was 20–120 min. In our study, the pikeperch achieved similar results with rainbow trout. The Propiscin is probably slightly toxic because the recovery time was deemed so slow.

The concentration of MS 222 (50 mg.L$^{-1}$) was not efficient at all tested temperatures (9.5, 15.5 and 23 °C). Son *et al.* (2001) also not achieved anaesthesia rime with this concentration in juvenile black rockfish (*Sebastess schlegeli*). Satterfield & Flickinger (1995) established an efficient concentration of MS 222 for anaesthesia at 99 mg.L$^{-1}$ with anaesthesia time 211 seconds for walley (*Stizostedion witreum*). They also found the lower concentrations of 66 mg.L$^{-1}$ worked but it was deemed so slow. At concentration of 100 mg.L$^{-1}$ MS 222, the anaesthesia time was 3.41±0.25 min at 9.5 °C, 2.14±0.29 min at 15.5 °C and 2.38±0.38 min at 23°C. The recovery time was 4.10±0.44 min at 9.5 °C, 2.16±0.12 min at 15.5 °C and 1.22±0.39 min at 23 °C. These results explain that this concentration worked very well. Similar results with 100 mg.L$^{-1}$ MS 222 were obtained by Gullian & Villanueva (2009), who reported anaesthesia time (1.29 min) and recovery time (1.46 min) in juvenile cobia (*Rachycentron canadum*). At concentration of 150 mg.L$^{-1}$ MS 222, the anaesthesia time was 1.54±0.32 min at 9.5 °C,

## CONCLUSION

In conclusion, the results from this study showed that the optimal and sufficient doses in all tested temperatures for pikeperch are 30 mg.L$^{-1}$ of clove oil, 100 mg.L$^{-1}$ of MS 222 and 0.5 ml.L$^{-1}$ of Propiscin. Nevertheless, Propiscin has very long recovery time and price of MS 222 in comparison with clove oil is more expensive.

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