INTRODUCTION
Anaesthetics play an important role in both fisheries research and aquaculture, being used to facilitate various handling procedures, such as weighing, sorting, collection of spawning material, tagging, or veterinary treatment (Summerfelt and Smith 1990, Kazuñ, Siwicki 2001).

Anaesthetics act with various intensity, driving fish into general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduced skeletal muscle tone (McFarland 1960). Regardless of the agent, the process of anaesthesia in fish, develops in a similar way and runs in a progressive pattern (McFarland 1959). Overdosing an anaesthetic or retaining the fish in an anaesthetic bath for too long leads to the fading of ventilation, hypoxia, and finally—respiratory and cardiac collapse (Tytler and Hawkins 1981). The fading of ventilation is an important warning sign suggesting that the exposure should be terminated (Hajek and Klęszko 2004, Dziaman et al. 2005).

THE ANAESTHETIC EFFECT OF CLOVE OIL ON COMMON CARP, CYPRINUS CARPIO L.

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Background. Clove oil, containing the active ingredient eugenol, has been reported to be an inexpensive and effective fish anaesthetic. The objective of the presently reported study was to establish the lowest effective concentration of clove oil for the common carp, Cyprinus carpio L., and safe working ranges for the anaesthetic.

Materials and Methods. Carp were exposed to the concentrations of 10, 20, 30, 40, 50, 100, 150, and 200 mg · L⁻¹ of clove oil. The onsets of individual phases of anaesthesia and recovery rates were studied. In experiment 1 the fish were held in the anaesthetic bath until the lack of responses to handling was observed and in experiment 2—until the fading of ventilation.

Results. Clove oil at the concentrations ranging from 30 to 200 mg · L⁻¹ induced general anaesthesia. The lowest concentration causing general anaesthesia with an average induction time below 3 min was 40 mg · L⁻¹. Recovery was concentration-independent and lasted for about 4 min (experiment 1). An increase in the concentration shortened the time of ventilation during anaesthesia and prolonged the recovery (experiment 2).

Conclusion. Clove oil is a potent anaesthetic for carp, the safest and most effective at the concentrations of 30–50 mg · L⁻¹. Therefore those solutions should be used, in the aquaculture practice, when the procedure requires more than 5 min of the exposure to the anaesthetic.

Keywords: clove oil, eugenol, anaesthesia, common carp, Cyprinus carpio, fish

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Anaesthetics act with various intensity, driving fish into general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduced skeletal muscle tone (McFarland 1960). Regardless of the agent, the process of anaesthesia in fish, develops in a similar way and runs in a progressive pattern (McFarland 1959). Overdosing an anaesthetic or retaining the fish in an anaesthetic bath for too long leads to the fading of ventilation, hypoxia, and finally—respiratory and cardiac collapse (Tytler and Hawkins 1981). The fading of ventilation is an important warning sign suggesting that the exposure should be terminated (Hajek and Klęszko 2004, Dziaman et al. 2005).

The most widely used anaesthetics include MS-222 (tricaine methanesulphonate), benzocaine, etomidate, methomidate, 2-phenoxyethanol, quinaldine, and quinaldine sulphate. Also clove oil has recently been pointed out as a potential fish anaesthetic. Clove oil is derived from the stem, leaves, and buds of the clove tree, Eugenia caryophyllata, and it contains the active ingredient eugenol. It has been reported to be effective on several species of fish (Soto and Burhanuddin 1995, Anderson et al. 1997, Mun-day and Wilson 1997, Keene et al. 1998, Peake 1998, Iversen et al. 2003, Wagner et al. 2003). Its main advantages lie in its low cost, and its relative safety to both fish and humans (Keene et al. 1998).

There has been only one attempt to study the effects of clove oil on common carp, performed by Velišek at al. 2005. Those authors investigated acute toxicity (LC) of clove oil using 10-min and 96-h exposure time with a survival as the main criterion. The objective of the presently reported study was to establish the lowest effective con-
cetration (defined by Gilderhus 1990) and safe working
ranges based on the criterion of the regularity of ventilation.

MATERIAL AND METHODS

The experiments were carried out using 169 common
carp with average weight of 32.6 ± 8.2 g ( ̅x ± s). The fish
were obtained from a cage culture facility at Gryfino near
Szczecin, Poland. Those used in the first experiment 1
were collected in September 2005, while those used in
experiment 2 were obtained about one month later. The
fish were acclimated for 14 days in a 1000-L common tank
prior to trial, in aerated tap water at 20 ± 1°C.

The clove oil (80.8% eugenol) obtained from the “Avi-
cenna–oil” company, Wroclaw, Poland, was first dissolved
in 95% ethanol at the ratio of 1 : 2 (clove oil : ethanol) and
then diluted by shaking with a small amount of water. The
solution was added to the experimental tank 30 min before
the introduction of fish. The induction and recovery were
carried out in two 30-

L aquaria with aerated tap water at 20 ± 1°C. After the
experiments the fish were transferred to a 1000-L tank and
observed for 10 days.

The “lowest effective concentration” is the concentra-
tion that produces general anaesthesia within 3 min and
allows the recovery within 10 min (Gilderhus1990, Weyl
et al. 1996).

The term “safe exposure time” denotes the part of ex-
posure when the fish breathe regularly, without signs of
oxygen deficiency.

This experimental protocol was approved by the Local
Ethical Committee for Experiments on Animals (Agricul-
tural University of Szczecin, Poland)

Experimental protocol

Experiment 1 (Determining the lowest effective con-
centration of clove oil). The fish were individually placed
in the test aquarium with an anaesthetic solution and held
there until the lack of responses to handling. To assess the
depth of anaesthesia the fish were taken by hand and re-
moved from the water. If there was a response, the expo-
sure was continued, if there was no reaction, the fish were
weighed and transferred to the recovery tank and observ-
ed until the lack of responses to handling. To assess the
induction and recovery was measured (in seconds) and
changes in the behaviour noted. The concentrations of
20, 30, 40, 50, 100, 150, and 200 mg·L–1 of clove oil were
used.

Experiment 2 (Determining safe exposure time of the
to effective concentrations of clove oil). The procedure for
this experiment was the same as for the first one, except
for the duration of the exposure. The fish were held in the
anaesthetic bath until the fading of the ventilation was
observed. The exposure was terminated when the inter-
vals between individual respiratory movements extended
to 4–5 s. The concentrations of 30, 40, 50, 100, 150, and
200 mg·L–1 of clove oil were used.

To describe the process of anaesthesia we used a modi-
ﬁed classification of anaesthesia stages adopted from
Schoettger and Julin (1967):

1. Sedation—reduced mobility
2. Partial loss of equilibrium—lateral (side) inclination
3. Total loss of equilibrium—almost horizontal position
   of the fish on the bottom of the tank; weak body move-
   ments; removal from the water evoking agitation
4. General anaesthesia—no body movement except for
   regular ventilation, no reaction to being caught and re-
   moved from the water
5. Medullary collapse—respiratory movement ceases

Statistical analyses were performed using General Linear
Model:

\[ Y_{ijk} = \mu + K_i + b_j(x_j - m) + e_{ijk} \]

where:
- \( Y_{ijk} \) response time [s]
- \( \mu \) overall mean
- \( K_i \) the effect of a particular concentration
- \( b_j \) regression coefficient of fish weight
- \( x_j \) individual fish weight
- \( m \) mean weight
- \( e_{ijk} \) random error

The Tukey Test was used to compare the means and
Sjostov–Stoline test for samples of unequal size.

The data of “recovery” and “general anaesthesia” from
the experiment 1 and the data of “fading ventilation”, “re-
gular ventilation”, “regaining equilibrium”, and “recovery”
from experiment 2 were transformed logarithmically to
restore the normality in the distribution and reduce the
variance. The transformed data were analyzed but the
original values are presented in Tables 1 and 2.

RESULTS

Experiment 1. The clove oil administered at the concen-
trations ranging from 30 to 200 mg·L–1 resulted in
progressive anaesthesia. After the transfer to a tank with
clean water all the fish recovered. Anaesthesia symptoms
faded in a reverse order. The mean times of the duration
of anaesthesia and the recovery symptoms are presented in
Table 1.

The clove oil at the concentration of 10 mg·L–1 result-
ed in sedation only. The concentration of 20 mg·L–1
caused the equilibrium disturbances in all fish and gen-
eral anaesthesia of one fish within 10 min. The increase in
the concentration resulted in the shortening of induction.
The lowest concentration of clove oil resulting in general
anaesthesia in less than 3 min was the concentration of 40
mg·L–1.

In the recovery process, the longest period of regaining
equilibrium was observed at the highest concentration, but
full recovery did not depend on the concentration and last-
ed for about 4 min.

Experiment 2. In this experiment the exposure con-
tinued until fading of ventilation was observed. The trans-
fer of the fish to the second aquarium resulted in a recov-
ery which began with regaining regular ventilation.
The mean times of the duration of anaesthesia and recovery symptoms are presented in Table 2. The induction time decreased along with the increase of the concentration from 2 min 28 s at the concentration of 30 mg·L⁻¹ to 53 s at the concentration of 200 mg·L⁻¹. Clove oil at all solutions resulted in the fading of ventilation. The longest safe exposure time (almost 15 min) was associated with the solutions of 30 and 40 mg·L⁻¹. An increase in the concentration shortened the time of ventilation during anaesthesia.

All the fish recovered from anaesthesia. The significant differences in the recovery times were independent of the anaesthetic solution. No mortalities were recorded within 10 days post experimental.

**DISCUSSION**

The presently reported results confirm the anaesthetic properties of clove oil for common carp. In experiment 1, the lowest effective concentration causing general anaesthesia with average induction time below 3 min was 40 mg·L⁻¹. The induction at the solution of 30 mg·L⁻¹, exceeded the time limit of 3 min. In experiment 2, the induction time at both concentrations was similar and did not exceed 3 min. We do not know why the carp reached the general anaesthesia faster in experiment 2 at 30 mg·L⁻¹. Perhaps it was affected by the fact, that the fish were purchased one month later. We can conclude, that according to our criterion of effectiveness, the concentration of 30 mg·L⁻¹ of clove oil is a threshold concentration for carp. Also, the experiments conducted on carp (Velišek et al. 2005b); rainbow trout, *Oncorhynchus mykiss* (cf. Keene et al. 2004; Prince and Powell 2000, Velišek et al. 2005a); perch, *Perca fluviatilis*; and tench, *Tinca tinca* (cf. Hamáčková et al. 2001, 2004) indicate that the concentration of 30 mg·L⁻¹ of clove oil is not strong enough to induce anaesthesia in less than 3 min. However, the solution of 20 mg·L⁻¹ was effective in that manner on sockeye salmon, *Oncorhynchus nerka* (cf. Woody et al. 2002) and the solution of 25 mg·L⁻¹ on juvenile longarm mullet, *Valamugil cunnesius* and silver moony, *Monodactylus argenteus* (cf. Durville and Collet 2001).

In our experiments the exposure time was not predefined but it was individually adjusted for the reaction of each fish, depending on the anaesthesia level. We observed that if the exposure was prolonged until the fish become anaesthetized, the recovery was concentration-independent and lasted for about 4 min. Similar observations were made by Inoue et al. (2003) on juveniles of matrixá, *Brycon cephalus*. The recovery in the experiment with a prolonged exposure was longer at all concentrations, but with the solutions of 30 and 40 mg·L⁻¹ it was still below 10 min.

The results of experiment 2 showed that lower effective concentrations (30–50 mg·L⁻¹) translated into longer duration of general anaesthesia with the ventilation maintained. Therefore, in the aquaculture practice, those solu-
tions should be used when application requires more than 5 min of the exposure to the anaesthetic.

**CONCLUSION**

Concentrations of 30–50 mg·L⁻¹ of clove oil are the safest and the most effective ones when applied to carp, and the solution of 40 mg·L⁻¹ is the lowest concentration that induces anaesthesia in less than 3 min. If the exposure is prolonged until the fish become anaesthetized, the recovery is concentration-independent and lasts for about 4 min.

**REFERENCES**


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**Table 2**

Timing [s] of anaesthesia- and recovery phases in carp exposed to various clove oil concentrations until the fading of ventilation; mean values ± standard error of the mean (in parentheses)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Concentration [ mg·L⁻¹ ] (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Sedation</td>
<td>26</td>
</tr>
<tr>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>Partial loss of</td>
<td>42A</td>
</tr>
<tr>
<td>equilibrium</td>
<td>(3)</td>
</tr>
<tr>
<td>Total loss of</td>
<td>82AB</td>
</tr>
<tr>
<td>equilibrium</td>
<td>(8)</td>
</tr>
<tr>
<td>General anaesthesia</td>
<td>148ABC</td>
</tr>
<tr>
<td>Fading</td>
<td>(16)</td>
</tr>
<tr>
<td>Ventilation</td>
<td>770abcABC</td>
</tr>
<tr>
<td>Fading</td>
<td>(46)</td>
</tr>
<tr>
<td>Regular ventilation</td>
<td>300</td>
</tr>
<tr>
<td>(129)</td>
<td>(38)</td>
</tr>
<tr>
<td>Regaining equilibrium</td>
<td>474</td>
</tr>
<tr>
<td>(149)</td>
<td>(51)</td>
</tr>
<tr>
<td>Recovery</td>
<td>535AB</td>
</tr>
<tr>
<td>(152)</td>
<td>(65)</td>
</tr>
</tbody>
</table>

Mean values having the same superscript (capital) letter in the same row are significantly different at *P* ≤ 0.01; mean values having the same superscript (lower-case) letter in the same row are significantly different at *P* ≤ 0.05.


