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Anesthetic effect of white camphor essential oil (*Cinnamomum camphora* L. 1753) and its use for transport of bighead carp (*Hypophthalmichthys nobilis* R. 1845) stocking material

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Abstract

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The aim of the present study is to examine the efficacy of white camphor essential oil as an anesthetic for bighead carp (*Hypophthalmichthys nobilis* R. 1845) and as an sedative agent for transport of bighead carp stocking material. The fish used in the research have an average body weight (BW, g) of 35.02±16.58 g and an average total length (TL, cm) of 15.44±2.33 cm. For the experiment of the anesthetic effect of *C. camphora*, five treatments are conducted with five experimental concentrations: 0.20 ml.l^{-1}, 0.30 ml.l^{-1}, 0.40 ml.l^{-1}, 0.50 ml.l^{-1} and 0.60 ml.l^{-1}. For each concentration 10 individuals are used or a total of 50 fish. For the transport experiment, 3 concentrations are used: 0.06 ml.l^{-1}, 0.08 ml.l^{-1} and 0.10 ml.l^{-1}. The applied stocking density is 3 fish/l. For each concentration 30 fish are used or a total of 90 fish.

Based on the results it can be concluded that at concentration of 0.60 ml.l^{-1} the induction of anesthesia is the fastest (4.88±0.43 min), thus the recovery time at this concentration is the longest (9.44±1.28 min). From all tested concentrations the recovery time is the shortest at the lowest concentration (0.20 ml/l) – 2.29±0.32 min ($P \leq 0.001$). The behavior of the fish and the phases in which they enter (Phase 0 and Phase 2) change every hour in all experimental variants of the transport experiment. For transport of bighead carp stocking material it can be recommended to apply for 1 h a concentration of 0.10 ml.l^{-1}, at which the highest percentage of fish are in state of decreased locomotor activity and static position. At the lowest concentration of 0.06 ml.l^{-1} the highest percentage of bighead carp stocking material remain in active state throughout the experiment, which makes this concentration of *C. camphora* inapplicable for transport of *H. nobilis*.

Keywords: Hypophthalmichthys nobilis; bighead carp; *Cinnamomum camphora;* white camphor oil; transport; anesthesia

Introduction

The use of fish anesthesia is important to provide welfare of individuals and allow the prevention of physical injures and ease the work process during fish farming or research purposes (Zahl et al., 2012; Benovit et al., 2015). Anesthetics are used to facilitate handling during biometrics, artificial spawning, vaccination, sorting of specimens, blood collection, surgery, transportation, etc. (Maricchiolo & Genovese, 2011; Javahery et al., 2012). Appropriate anesthetics at optimal concentrations are expected to minimize the deleterious effects of stress on fish (Roohi & Imanpoor, 2015). A suitable anesthetic should rapidly immobilized the fish and result in uneventful recovery, as well as to be widely available, cost-effective, and present low or no toxity. Anesthetics should not build up in fish tissues and organs and pose prob-

lems for human or animal consumption and the excretion of the anesthetics from the fish body should be fast (Javahery et al., 2012; Azad et al., 2014; Roohi & Imanpoor, 2015).

In aquaculture there is a variety of anesthetics, both chemical (MS-222, quinaldine, benzocaine etc.) and those of natural origin, such as essential oils. The source of camphor essential oil is *Cinnamomum camphora*, an evergreen tree of the family Lauraceae. The tree is native to China, Formosa and Japan (Yoshida et al., 1969). The traditional essential oil is obtained from the wood and bark (Dung & Khien, 1991; Pandey et al., 1997), as well as from leaves, branches, trunks and roots (Wanyang et al., 1989).

Depending on which part of the tree is extracted, three types of camphor oil are obtained: white, yellow and brown. Yellow and brown camphor oils are carcinogenic and are not used in practice, because they have a very high content of the chemical safrole (Clarke, 2008). Camphor essential oil has several chemical types based on the active substances and major compounds: camphor – type, linalool-type, cineol-type, isonerolidol-type and borneol-type (Wanyang et al., 1989; Frizzo et al., 2000; Freitas Souza, 2019). Camphor oil has mycostatic effect, antimicrobial activity and strong fungistatic activity (Mishra et al., 1991; Yeh et al., 2009; Pragadheesh et al., 2013), and insecticidal activity (Guo et al., 2016). The use of camphor oil as an anesthetic is not as common as its use as an antibacterial and antifungal agent. The scientific results on the anesthetic and sedative effect of camphor oil are scarce (Aydin & Barbas, 2020). It has only been used to anesthetize clown anemonefish (*Amphiprion ocellaris*) in a comparative study with clove and mint essential oils (Pedrazzani & Neto, 2014; Ostrensky et al., 2016). The authors established that at 0.027 $ml.l^{-1}$ clove, 0.17$ml.l^{-1}$ mint and 0.50 $ml.l^{-1}$camphor essential oils surgical anaesthesia is achieved after 5.18 min, 5.2 min and 8.92 min, respectively.

Clove oil, *Eugenia caryophyllata* is the most commonly used plant-based anesthetic in aquaculture (Velisek et al., 2005; Zaikov et al., 2008, Zaikov et al., 2009; Ogretmen & Gokcek, 2013; Yildiz et al., 2013; Kamble et al., 2014; Diyaware et al., 2017; Park et al., 2018). Different studies have been conducted on the use of basil essential oil *Ocimum basilicum* (Netto et al., 2017; Khumpirapang et al., 2018), thyme *Thymus vulgaris* (Yousefi et al., 2018a), mint *Mentha piperita* (Pedrazzani & Neto, 2014; Mazandarani & Hoseini, 2017; Rezende et al., 2017), rosemary *Rosmarinus officinalis* (Ghazilou & Chenary, 2011), lavender *Lavandula angustifolia* (Metin et al., 2015; Can & Sumer, 2019) for the different fish species.

Only one experiment has been conducted with *Hypophthalmichthys nobilis* by Akbary et al. (2016). The authors studied the stress caused in the experimental individuals with body weight of 65.4 g, during anesthesia with 2-phenoxyethanol based on cortisol levels and other hematological parameters.

Live transport of fish, at each stage of their individual development, is one of the major causes of stress due to the capture, packing, high loading density, changes in water quality and the transport itself (Harmon, 2009; Sampio & Freire, 2016). Furthermore, stress by transport induce an osmotic imbalance with ion loss in freshwater fish (Garcia et al., 2015; Ostrensky et al., 2016; Teixeira et al., 2018).

Transported fish can die from shock caused by stress during transport (Bulgarian Food Safety Agency, 2011). In this regard, the addition of an anesthetic with a certain concentration in the transport tank could relieve stress and prevent mortality, with transportation of live fish being one of the main activities in fish farming (Souza et al., 2019).

Up to date no studies have been performed regarding the transport of bighead carp. Research on the use of anesthetics (essential oils or chemical agents) in the transport of live fish is very scarce (Benovit et al., 2012; Tondolo et al., 2013; Balamurugan et al., 2016; Sampaio & Freire, 2016; Zhao et al., 2017; Teixeira et al., 2018; Oliveira et al., 2019).

Zhao et al. (2017) conducted an experiment with eugenol at different doses and exposures by examining its effect on grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), with weigh of 1.5 kg, in order to determine the most appropriate concentration of eugenol for transport of grass carp. The experiment also included taking blood samples to examine the residual concentration of eugenol in the blood plasma, muscles and liver. A study by Oliveira et al. (2019) also refers to the use of eugenol in the transport of angel fish (*Pterophyllum scalare*), and the methods used are similar to Zhao et al. (2017).

Teixeira et al. (2018) evaluated the efficacy of the essential oil of *Aloysia triphylla* as stress reducer in transport of Nile tilapia juveniles, *Oreochromis niloticus* for period of 8 hours and its influence on survival, water quality and biochemical parameters (plasma cortisol, glucose, lactate).

The aim of the present research is to investigate the anesthetic effect of white camphor essential oil, as well as its application in transport of bighead carp stocking material, as up to date there is no data on the anesthesia of bighead carp with *C. camphora* oil or its application in the transport of this fish species.

Material and Methods

The study is conducted at the Institute of Fisheries and Aquaculture, Plovdiv in April 2021.

Subject of research

The subject of the experiment was *H. nobilis* stocking material hatched by artificial propagation in May 2020 and afterwards grown in the ponds of the experimental base of IFA, Plovdiv in polyculture with common carp, *Cyprinus carpio* and grass carp, *Ctenopharyngodon idella*. For the purpose of the study, the fish were caught from the experimental ponds and transfered for storage in 3 m^3 tanks. The biometric characteristics of the experimental fish are presented in Table 1.

Table 1. Body weight (BW, g) and total length (TL, cm) of the fish

Statistical value	BW, g	TL, cm
mean±SD	35.02±16.58	15.44±2.33
min-max	13.45-22.0	11.30-19.40
CV, %	47.35	15.11

Essential oil

The *C. camphora* essential oil was purchased commercially, with listed ingredients of 100% pure white camphor oil, produced in Sofia, Bulgaria by Zoya Goes Pretty®. The experimental solutions were prepared by diluting the oil in ethyl alcohol (95%) in 1:9 ratio and added to 10 l experimental tanks with vigorous stirring before treatment.

Preliminary test

Due to the lack of sufficient data, a preliminary test was performed to study different concentrations of white camphor essential oil. A treatment of 5 fish, with two repetitions, was used since the size of the fish allows observations of more than one specimen.

In order to preserve the well-being of the fish and to prevent mortality, the lowest experimental concentration was 0.02 $ml.l^{-1}$. The preliminary test was performed with 3 experimental concentrations: 0.02 $ml.l^{-1}$, 0.08 $ml.l^{-1}$ and 0.12 $ml.l^{-1}$, with an exposure of 20 min so that the effect of the oil can be monitored for a longer period of time.

Experiment of the anesthetic effect of C. camphora

Based on the preliminary test, 6 experimental concentrations were used: 0.20 $ml.l^{-1}$, 0.30 $ml.l^{-1}$, 0.40 $ml.l^{-1}$, 0.50 $ml.l^{-1}$ and 0.60 $ml.l^{-1}$. For each concentration, 10 fish (five treatments with two fish) are used or a total of 50 fish for the experiment. In order to ensure the welfare of the treated specimens, the biometric parameters, body weight (BW, g) and body length (TL, cm) are measured after exposure to the anesthetic solution (Table 1).

When preparing the solutions for anesthesia and recovery, the temperature of the water was equalized to the temperature of the water in the storage tanks. Before adding the anesthetic solution, the temperature (T˚C) and the level of dissolved oxygen (O_2, mg/l) were measured. To recover from anesthesia, the fish were transferred in tanks with the same volume of clean water (10 l) with placed microcompressors, where they were observed until complete recovery. The time required for the induction of anesthesia and subsequent recovery was measured with a stopwatch, taking into account the time of each phase. When processing the results, the data was converted into minutes according to the following formula: min = (min * 60 + sec)/60.

The behavior of the fish was described and analyzed according to the phases of anesthesia and recovery determined by Hamackova et al. (2006):

Phases of anesthesia

Phase 1. Accelleration of the opercular movements, increased respiratory activity.

Phase 2. Decreased respiratory activity accompanied by uncoordinated movements.

Phase 3. Loss of equilibrium, decreased opercular movements, the fish still react to strong external stimuli.

Phase 4. Complete immobilization, the fish lie on the bottom and do not react to handling.

Phases of recovery

Phase 1. Beginning of movements.

Phase 2. Weak, uncoordinated locomotor activity.

Phase 3. Normal position of the body. Normal locomotor activity is regained.

Transport experiment

The transport experiment was carried out in laboratory conditions according to the "Instruction for the application of the requirements for the transport of live fish" (BFSA, 2011). Prior to the beginning of the experiment, the fish were not fed for 24 hours and an assessment of their health was made.

The experiment was conducted with three concentration variants of camphor oil, based on the preliminary test: Variant 1 – 0.06 $ml.l^{-1}$, Variant 2 – 0.08 $ml.l^{-1}$ and Variant 3 – 0.10 $ml.l^{-1}$. Plastic tubs with a volume of 10 l, covered with plastic lids, were used for this purpose of the experiment. The applied density was 3 fish/l calculated in relation to the average weight of the fish and the standard of 120 kg/m^3 density without being further increased (Table 2). For each variant 30 fish were used or a total of 90 fish.

Microcompressors were placed in the water 1 hour before the introduction of the fish in the experimental variants. Hydrochemical measurements (T°C and O_2 mg/l) were performed every hour during the experiment.

Table 2. Requirements for density and duration of transport of live fish

Fish species	Density	Duration of transport, hours
Herbivorous fish		
Stocking material	70 130 kg/m³ depending on the weight	from 3 to 6
The amount of fish with an average body weight below 100 g can be increased by 60-80%		

Source: Instruction for application of the requirements for transport of live fish (BFSA, 2011).

The behavior of the fish in the three variants was observed every hour according to the table of Hamackova et al. (2006). The fish in each phase were counted and their percentage was calculated according to their total number for each variant. The following formula was used: Phase,%=(number of fish in the phase*100)/total number of fish.

At the end of the experiment, the water from the tanks was drained slowly so that the fish do not stay dry. Immediately after that, clean water of the same volume was added. After 3 to 5 minutes the fish were released into their natural environment.

Statistical analysis

The results obtained for the induction of anesthesia and the period of recovery, for each concentration and phase, were analyzed at a confidence level of P≤0.05, followed by multiple comparisons of the mean values. The results obtained from the transport experiment, from 1 h to 4 h, were analyzed at a confidence level of P≤0.05. Phase 0 and Phase 2 of each variant have been compared in vertical order as follows: Variant 1 with Variant 2; Variant 1 with Variant 3; Variant 2 with Variant 3. The correlation between the mean values, and the level of significance, are indicated with different superscripts. For the analysis of the results, from both experiments, a comparative Student T-test (paired two sample for means) is applied by using Excel – Data analysis.

Results and Discussion

Preliminary test

At all experimental concentrations in the preliminary test (0.02 ml.l^{-1}, 0.08 ml.l^{-1} and 0.14 ml.l^{-1}) the fish reach only Phase 2 of anesthesia (Figure 1).

Phase 2 occurred after 5 min at all three experimental concentrations and at the lowest concentration of 0.02 ml.l^{-1} the fish retain their normal activity for the longest period of time. In Phase 2 the fish remain static, do not move or perform very slow, uncoordinated movements. A recovery process is observed at the highest concentration of 0.14 ml.l^{-1}, but only in 40% of the fish with an average recovery period of 0.37 min. At the other two concentrations, 0.02 ml.l^{-1} and 0.08 ml.l^{-1}, no recovery process is observed.

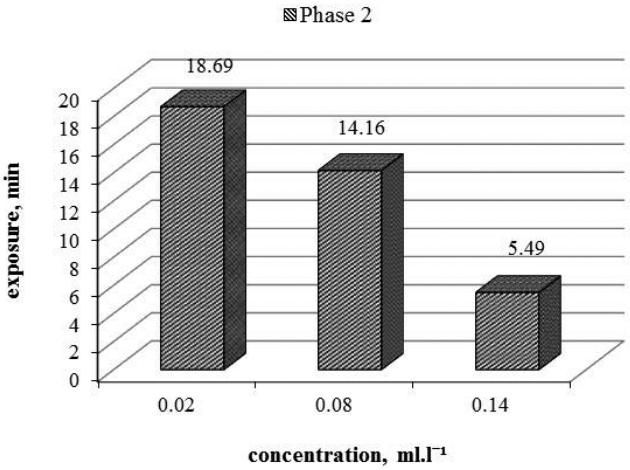

Fig. 1. Time to reach Phase 2 at different experimental concentrations from the preliminary test

Experiment of the anesthetic effect of C. camphora

The results of the anesthesia with the experimental concentrations (0.20 ml.l^{-1}, 0.30 ml.l^{-1}, 0.40 ml.l^{-1}, 0.50 ml.l^{-1}, 0.60 ml.l^{-1}) are presented in Table 3.

At 0.20 ml.l^{-1}, the bighead carp stocking material reached Phase 4 of complete anesthesia for the longest period of time compared to other concentrations (P≤0.001). The recovery period at the lowest concentration is the shortest (P≤0.001), and no statistical differences are established between the experimental groups in Phase 1 of recovery. Initially, the fish swim normally, with Phase 2 occurring with very strong, accelerated movements that gradually subside, and throughout Phase 2 the fish remain static and uncoordinated, until they enter Phase 3. This behavior is observed at all concentrations – from 0.20 ml.l^{-1} to 0.50 ml.l^{-1}. At 0.50 ml.l^{-1} Phase 3 of loss of balance occurs in 40% of fish, the rest enter directly from Phase 2 to Phase 4 of complete anesthesia. At the highest concentration of white camphor oil (0.60 ml.l^{-1}) Phase 3 is not observed and from static position the anesthetized fish fall directly at the bottom of the tank.

Phase 4 of complete anesthesia occurs most slowly at the lowest concentration of 0.20 ml.l^{-1} and fastest at the highest concentration of 0.60 ml.l^{-1} (P≤0.001). The obtained results show that with the increasing of the concentration the time required for anesthesia decreases.

Fish of all experimental concentrations go through all phases of the recovery process. The higher anesthetic con-

Table 3. Duration (min) of the phases of anesthesia and recovery of bighead carp stocking material (T1)

	Concentration, ml.l^{-1}				
	0.20	0.30	0.40	0.50	0.60
A2	0.17±0.04^{b*}	0.22±0.05^{a*c**d**e*}	0.30±0.06^{b**d***}	0.18±0.03^{b**c***}	0.15±0.03^{b*}
A3	12.74±2.88^{b***}	9.97±1.81^{a***}	7.62±1.44^{ns}	5.90±0.85 40%	–
A4	12.90±2.87^{b***}	10.25±1.91^{a***d***}	8.0±1.48^{d**}	6.05±0.59^{b***c**e***}	4.88±0.43^{d***}
R1	1.58±0.31^{ns}	1.66±0.27^{ns}	3.21±0.50^{ns}	4.95±0.61^{ns}	5.07±0.51^{ns}
R2	1.78±0.32^{c***}	1.84±0.27^{c***}	4.20±1.36^{a***b***d*}	5.39±0.71^{c*e***}	7.87±1.09^{d***}
R3	2.29±0.32^{c***}	2.38±0.28^{c***}	4.60±1.45^{a***b***d**}	6.37±0.67^{c**e***}	9.44±1.28^{d***}

Note: At 0.50 ml.l^{-1} 40% of the fish reach Phase 3 of anesthesia. At 0.60 ml.l^{-1} Phase 3 of anesthesia is not observed.
Values connected by different superscripts are significantly different ($P \leq 0.05$)
***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns – non significant;
A2 – Phase 2 of anesthesia a – 0.20 ml.l^{-1}
A3 – Phase 3 of anesthesia b – 0.30 ml.l^{-1}
A4 – Phase 4 of anesthesia c – 0.40 ml.l^{-1}
R1 – Phase 1 of recovery d – 0.50 ml.l^{-1}
R2 – Phase 2 of recovery e – 0.60 ml.l^{-1}
R3 – Phase 3 of recovery

centrations prolong the time required for the occurrence of Phase 3 of full recovery ($P \leq 0.001$).

The main hydrochemical parameters of dissolved oxygen and water temperature in the anesthesia and recovery tanks, for each concentration, are presented in Table 4.

Table 4. Main hydrochemical parameters of the water in the tanks for anesthesia and recovery

Statistical value	Anesthesia bath		Recovery bath	
	O_2 mg.l^{-1}	T, °C	O_2 mg.l^{-1}	T, °C
mean±SD	3.82±0.19	20.86±0.82	4.78±0.55	21.02±0.33
min	3.60	19.60	4.20	20.60
max	4.10	21.60	5.50	21.50

The temperature in the tank for anesthesia and in the tank for recovery have close values of 20.86±0.82°C and 21.02±0.33°C, respectively. The oxygen in the tank for anesthesia has an average value of 3.82±0.19 mg.l^{-1} which is lower compared to the oxygen in the tank for recovery (4.78±0.55 mg.l^{-1}). The reason for this are the installed microcompressors which support the recovery process.

Ostrensky et al. (2016) investigate the anesthetic effect of three essential oils clove, mint and camphor on clown anemone fish (*Amphipriono cellaris*) at concentrations of 0.0025 ml.l^{-1}, 0.005 ml.l^{-1} and 0.0075 ml.l^{-1}; 0.02 ml.l^{-1}, 0.025 ml.l^{-1}and 0.030 ml.l^{-1} and 0.10 ml.l^{-1}, 0.12 ml.l^{-1} and 0.14 ml.l^{-1}, respectively. The authors state that mint and camphor essential oils have proven anesthetic effects on clownfish and can be used during animal laboratory handling. At concentration of 0.10 ml.l^{-1} of camphor essential oil the fish only enter Phase 2 of anesthesia, which is also confirmed in the present research.

Pedrazzani & Neto (2014) also examine the anesthetic effect of camphor (*Cinnamomum camphora*), clove (*Syzygium aromaticum*) and mint (*Mentha arvensis*) essential oils on clown anemonefish (*Amphipriono cellaris*) juveniles with mean weight of 0.48 g ± 0.21 g. The authors found that concentration of 0.027 ml.l^{-1} of clove, 0.07 ml.l^{-1} of mint and 0.5 ml.l^{-1} of camphor oil produce an anesthetic effect for 5.18 min, 5.2 min and 8.92 min, respectively. The obtained results show that the camphor oil, although with several times higher concentration, has the slowest anesthetic effect compared to clove and mint oil. When comparing the results of Pedrazzani & Neto (2014) for camphor oil with those from the current study, it can be seen that at 0.5 ml.l^{-1} the time, required for anesthesia of clown anemone fish juveniles, is almost 3 min longer than anesthesia period of bighead carp stocking material. Unlike the time required for anesthesia, recovery time of clown anenemone fish (3.8 min) is shorter than bighead carp recovery period (6.37 min). Differences in the period of anesthesia and recovery are most probably due to the different experimental fish species, differentiation of the phases of anesthesia, preparation of the anesthetic solution, water temperature, etc.

Akbary et al. (2016) found that the most effective concentration for anesthesia of bighead carp *H. nobilis* with 2-phenoxyethanol is 0.9 ml.l^{-1}, which is higher than the white camphor oil concentration established to be most effective in the current study (0.6 ml.l^{-1}). The authors also state that at 0.10 ml.l^{-1} concentration of 2-phenoxyethanol no anesthetic effect is achieved, which is established for the same concentration of camphor oil in our study.

In their research, Oliveira et al. (2019) report that the most effective concentration of eugenol for anesthesia of

Table 5. Results of the experiment for transport of bighead carp stocking material with white camphor essential oil in three experimental variants

Hour	Variant 1 (0.06 ml.l^{-1})		Variant 2 (0.08 ml.l^{-1})		Variant 3 (0.10 ml.l^{-1})	
	Phase 0	Phase 2	Phase 0	Phase 2	Phase 0	Phase 2
1	65%	35%	38%	62%	24%	76%
2	50%	50%	30%	70%	15%	85%
3	40%	60%	24%	76%	20%	80%
4	32%	68%	18%	82%	10%	90%
P-value	b**c*	b**c*	a**c*	a**c*	a*b*	a*b*

Note: The number of fish are presented in percentage
Phase 0 – normal locomotor activity; Phase 2 – uncoordinated movements (static position)
Variant 1 – a; Variant 2 – b; Variant 3 – c
Values connected by different superscripts are significantly different (P≤0.05)
***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; ns – non significant

angelfish juveniles (*Pterophyllum scalare*) is 0.053 ml.l^{-1} which is much lower effective concentration compared to the effective camphor concentration in the present research (0.60 ml.l^{-1}).

Tondolo et al. (2013) recommend concentration of 0.15 ml.l^{-1} of *Nectandramega potamica* on fat snook (*Centropomus parallelus*), which induce complete anesthesia in 5.6 to 8 min, with recovery period of 1 to 10 min. When comparing the results from the current study with those established by Tondolo et al. (2013) it can be stated that white camphor oil is an effective anesthetic at much higher concentrations compared to *Nectandra megapotamica*.

Transport experiment

During the transport experiment, the behavior of the fish varied between normal motor activity and Phase 2 of anesthesia, expressed in very slow uncoordinated movements and static position. Throughout the experiment, Phase 3 is not observed in any of the experimental variants. Upon external stimuli the fish immediately regain their normal body position (Table 5). Survival rate at the end of the experiment is 100%.

Throughout the experiment, in all experimental variants, the percentage of fish in Phase 0 decreases every hour (P ≤ 0.01), which is in relation to the increasing percentage of fish in Phase 2 (P ≤ 0.01). Thus, as the transport time increases, the number of fish in Phase 0 decreases in inversely proportional correlation to the fish entering Phase 2 of anesthesia. The results of the statistical analysis for Phase 0 and Phase 2 of the different variants show are similar. The number of fish in Phase 0, for the whole duration of the transport experiment, are statistically significant with those from Variant 2 (P ≤ 0.01) and from Variant 3 (P ≤ 0.05).

The hydrochemical measurements during transport experiment are presented in Figure 2 and Figure 3.

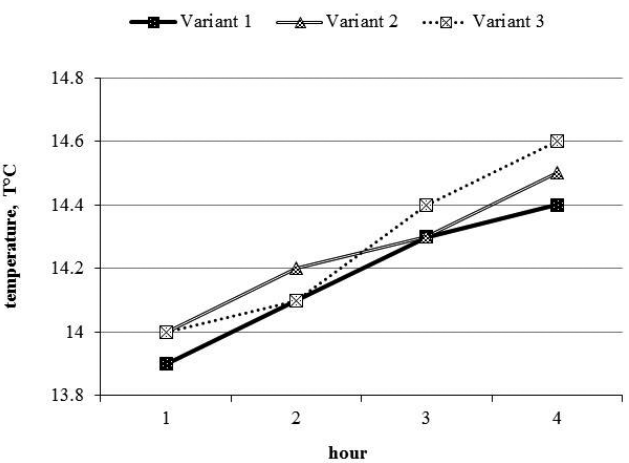

Fig. 2. Measured temperature per hour in the three experimental variants

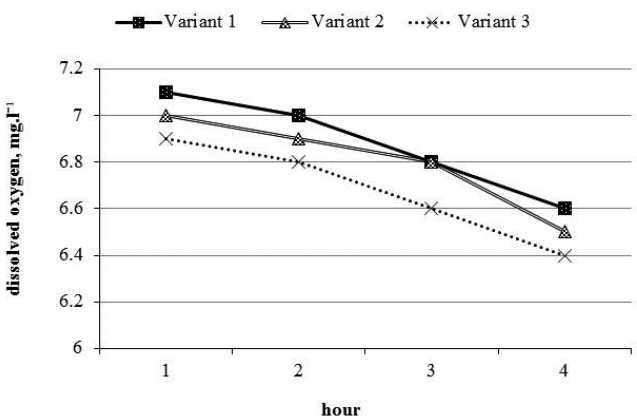

Fig. 3. Amount of dissolved oxygen per hour in the three experimental variants

The measured temperature varies from 13.9ºC to 14.6ºC. According to the official instructions of Bulgarian Food Safety Agency (BFSA), the temperature should not exceed 21ºC. The amount of dissolved oxygen is in the range of 6.4-7.1 mg.l^{-1} which is within the permissible values according to BFSA.

Zhao et al. (2016) apply eugenol on grass carp (*C. idella*) with body weight of 1.5±0.1 kg in simulated transport conditions at different concentrations (0.005, 0.01, 0.015, 0.02, 0.03 and 0.04 ml.l^{-1}) for 8 h. The authors found that the most effective concentration is 0.01 ml.l^{-1} eugenol, at which, during the whole duration of the experiment, the fish maintain their balance, slow breathing and decreased activity, and then recover in a few minutes. The authors do not recommend the application of concentrations higher than 0.04 ml.l^{-1} as it cause mortality.

Oliveira et al. (2019) reporte that the most optimal concentration of eugenol for transport of angelfish juveniles (*Pterophyllum scalare*) for 7 hours is 0.02 ml.l^{-1}, at which the fish are in a state of sedation.

When comparing the results of Zhao et al. (2016) and Oliveira et al. (2019) with the results from our study we can conclude that white camphor oil is safer anesthetic than eugenol, although it is used at much higher concentrations.

Teixeira et al. (2018) evaluated the efficacy of *Aloysia triphylla* as stress reducer in transport of Nile tilapia juveniles, *Oreochromis niloticus* for period of 8 hours. The authors established that most effective concentration of *Aloysia triphylla* is 0.03 ml.l^{-1}.

In another study, Tondolo et al. (2013) established that *Nectandra megapotamica* essential oil is not able to prevent the stress of anesthesia during transport, based on the results obtained from the parameters glucose, lactate, and Na+ and K+ plasma levels.

Ostrensky et al. (2016) investigate the effect of three essential oils clove, mint and camphor for transport of clown anemonefish for period of 6h, 12h and 24h and their influence on water quality. The applied concentrations of camphor oil are 0.10 ml.l^{-1}, 0.12 ml.l^{-1} and 0.14 ml.l^{-1} and density of 5 fish/l. The obtained results show that the lowest concentration of camphor oil (0.10 ml.l^{-1}) is sufficient to induce only Phase 2 at 6 h, while 0.12 ml.l^{-1} and 0.14 ml.l^{-1} induce Phase 4 at 6 h and led to Phase 2 and 4, respectively, after 12 h. In the current study, at 0.10 ml.l^{-1}, 32% of the fish retain their normal locomotor activity at 4 h. Ostrensky et al. (2016) established that the highest concentration of camphor oil (0.14 ml.l^{-1}) cause death in 37% of the fish, which is observed between 12 h and 24 h. The authors report that 0.12 ml.l^{-1} is the most suitable concentration for use in confinement transportation of *A. ocellaris*, although it induced Phase 3 of anesthesia (total loss of balance) during the first hours of transportation, but it presents no deleterious effects to the fish. At this concentration the fish remained in Phase 2 for most of the confinement transport period. Ostrensky et al. (2016) emphasize that Phase 2 is desirable for confinement transport of live fish. In the current study, the purpose of the applied camphor concentrations in the transport experiment is also to achieve and maintain Phase 2 of anesthesia.

Conclusion

Based on the results of the experiment, it can be concluded that at concentration of 0.20 ml.l^{-1} the fish reach Phase 4 of complete anesthesia for the longest period compared to the other concentrations ($P \leq 0.001$). As the concentration increases, the time required for anesthesia decreases, with the fastest induction time and the slowest period of recovery being observed at the highest concentrations of 0.50ml.l^{-1} and 0.60 ml.l^{-1}.

The choice of a specific concentration of white camphor oil for anesthesia of bighead carp stocking material depends on the targeted phase of anesthesia (Phase 3 or Phase 4). Concentration of 0.40 ml.l^{-1} can be used to achieve Phase 3 (sedative effect) for an average period of 7.62 min. Concentrations of 0.50 ml.l^{-1} and 0.60 ml.l^{-1} can be applied to achieve Phase 4 (complete anesthesia) within 4.88-6.05 min. At 0.60 ml.l^{-1} anesthesia is achieved for an average of 4.88 min, which is only 1.17 min slower compared to the time required for induction of anesthesia at 0.50 ml.l^{-1}, with higher oil consumption needed at the highest concentration.

For transport of bighead carp stocking material, for 1 h, the optimal concentration of white camphor essential oil is 0.10 ml.l^{-1}, at which the highest percentage of fish are in state of decreased locomotor activity and static position. For a transport with a duration of 2 h, 3 h and 4h, the concentrations of 0.08 ml.l^{-1} and 0.10 ml.l^{-1} can be applied, preferably the higher concentration at which higher percentage (90%) of the individuals are in Phase 2 expressed in slow uncoordinated movements. At the lowest concentration of 0.06 ml.l^{-1} the highest percentage of fish remain in an active state throughout the experiment, which makes this concentration unsuitable for usage in transport of bighead carp stocking material.

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