Öz

Amaç: Araştırmamanın amacı, balıkların sedasyonuna yönelik en uygun protokoller belirlemek için, propofol ve karanfil yağının taşıma sırasında metabolik etkinliğini azaltmakda etkinliğini incelemektir.

Gereç ve Yöntem: Araştırmaoda 126 Dempsey balığı kullanıldı. Kontrol (n:42), propofol (n:42) ve karanfil yağ grubları (n:42) litre su başına üç litre oksijenle dolduruldu ve 0.1 ml/L propofol ve karanfil yağ ile takviye edilmiş kapalı plastik torbaları yerleştirildi. Soluman oranları, indükşyon ve işleme süreleri, yem alım ve renk değişirmesi süreleri kaydedildi.

Bulgular: Elde edilen bulgular, propofol grubunun solunum hızında azalmayan (67.26 ± 6.3, 50.26 ± 9.4, 36.52 ± 4.6, 11.74 ± 3.5, 4.50 ± 2.2, 3.69 ± 1.5) ve müdahale süreleri (dakika başına frekans) (80.12 ± 1.84) kontrol ve karanfil yağ gruplarından istatistiksel olarak farklı (P < 0.05) olduğu ortaya koyuldu. Duyarlılık kontrolleri bakımından, propofol grubunun işığa, titreşime ve dokunmaya daha az duyarlı olduğu tespit edildi.

Öneri: Düşük maliyetli ve kolay bulunabilirliği göz önüne alındığında, propofol, karanfil yağda daha uygun bulunuyor ve karanfil yağın daha etkili bir ajan olduğu tespit edildi. Bu nedenle akvaryum balığı taşımacılığında propofolun önemiği olarak kullanılması önerilebilir.

Anahtar kelimeler: Akvaryum balıkları, solunum sayısı, sedasyon

Abstract

Aim: The aim of this research was to examine the efficacies of propofol and clove oil to decrease the metabolic activity during transportation in order to determine optimal protocols for sedation of fish.

Materials and Methods: 126 Jack Dempsey fish were used in this research. Control (n:42), propofol (n:42) and clove oil (n:42) groups placed into closed clear plastic fish bags filled with three litres of oxygen per litre of water and supplemented with 0.1 ml/L of propofol and clove oil. Respiratory rates, induction and recovery times, feed intake and color-changing times were measured while reactions to light, vibration and touch were scored for sensitivity controls.

Results: According to the results, the decrease of respiratory rates per minute (67.26 ± 6.3, 50.26 ± 9.4, 36.52 ± 4.6, 11.74 ± 3.5, 4.50 ± 2.2, 3.69 ± 1.5) and recovery time respiratory rates (frequency per minute) of the propofol group (80.12 ± 1.84) differed from the control and clove oil groups (p < 0.05). Regarding the sensitivity controls, propofol group was less sensitive to light, vibration and touch.

Conclusion: Considering its low cost and easy availability, since propofol has been found to be more suitable and clove oil was more ineffective than propofol, therefore propofol can be recommended as a priority in the transport of aquarium fish.

Keywords: Aquarium fish, respiratory rate, sedation
Introduction

Aquarium fish commerce is growing around the world (Allen et al 2017), but there are still serious losses during collection, packing, storage and transportation. Therefore, handling procedures should be strictly followed to reduce injury and mortalities. In this context, appropriate density, temperature and sedation are some of the main requirements of a healthy transportation. On the other hand, physical activity, oxygen carrying property of the water and oxygen consumption of the fish are influenced by the temperature changes. Therefore temperatures lower than the raising conditions should be provided to reduce physical activity and prevent deaths or injuries. For instance, one part of fish filled with ten part of water (2 kg/20 L) in a large oxygen filled space polythene bag can provide safe transportation at 10°C for 5 hours (Belema et al 2017).

Sedation is also useful in reducing physical activity, oxygen consumption, and excretion of metabolic products during long distance fish transportation. In this respect, low-dose anaesthetics can be used for sedation and reduce metabolic rates (Hoskonen and Pirhonen 2004; Ross and Ross 2008). Some of the changes observed during induction are balance in swimming, posture, behaviour; gill ventilation rate, eye motion, reflex responses and heart rate (Sneddon, 2012). Ambient conditions, body weight, physiological stress are the main factors, which affect the dose of the anaesthetic agent. The most common anaesthetic drugs used in fish transportation are MS-222 (Tricaine methanesulfonate), benzocaine, isoeugenol, etomidate, 2-phenoxyethanol, and quinaldine (Sneddon 2012). Clove oil (Eugenol) is also an effective, local and natural anaesthetic/sedative drug. Clove oil is commonly used to immobilize fish for handling, sorting, tagging, artificial reproduction procedures and surgery and to suppress sensory systems during invasive procedures with low intoxication and mortality risks (Javahery et al 2012, Soto and Burhanuddin 1995) compared to other agents but few studies have examined the use of low concentrations to achieve sedation for fish handling and transport (Cookea et al 2004). After administration of clove oil into the bath of the fish, it directly affects the fish systematically. Once clove oil absorbed through the gills and skin, anaesthetic agent enters the bloodstream and is distributed throughout the body. Clove oil penetrates rapidly into the gill epithelium and is absorbed by body tissues. On the other hand, similar to the clove oil, propofol, as a sedative agent, produces significant reduction in the respiratory and heart rates in fish (Fleming et al 2003, Javahery et al 2012, Mitchell et al 2009). Anaesthetic or sedative effect of these drugs depends on the dose used. In terms of easy accessibility, low cost and limited harmful effects propofol and clove oil could be a good alternative to MS-222 and other drugs.

Low dose of propofol decreases the metabolic activity by its sedation effect better than the same dose of clove oil in Jack Dempsey fish during transportation. This study was conducted to compare the use of propofol and clove oil in sedation of Jack Dempsey fish during transportation.

Material and Methods

Material

Male and female Jack Dempsey fish (Rocio octofasciata, n:126) were randomly selected from aquarium, allocated to three groups (control, propofol and dove oil, n:42 each) and placed into fresh water. Fish in each group were then randomly allocated to six subgroups (n:7) to represent the repetitive experiments.

Experimental room and equipment

Temperature of the experimental room was controlled by electrical heaters located on the walls. Size of the aquariums in which the fish were kept until the start of the trial was 100*45*35 cm3. Propofol (Propofol 1% MCT Fresenius, Fresenius Kabi AB, SE-751 74 Uppsala, Sweden) was obtained from a commercial firm (Cevizlibağ Pharmacy, Zeytinburnu, İstanbul) and clove extract oil (Clove Extract Oil Soluble, 100 g, Alfasol®) was obtained from a commercial firm (Kimbiotek Kimyevi Maddeler san. Tic. A.Ş.).

Method

After being taken to 70*45*35 cm3 aquariums and fasted for 24 hours, the weight and length of the fish were measured. Room temperature and humidity, bath temperature, pH, TDS (Total Dissolved Solids) and salinity were recorded by water proof ExStil® II pH/conductivity meter, EC500, Etech. Each subgroup was placed into transparent plastic bags (51.5-30 cm) filled with 2 litres of water and oxygen (three parts oxygen to one part water), relevant agent (0.1 ml L-1 propofol, 0.1 ml L-1 clove oil) and kept for 24 hours at room temperature (22-23°C) to simulate transportation conditions. No agent was added into the bath of the control group. Applying 0.1 ml/L into the bath was chosen as a sedation dose for the current study, aiming the best effective dose for longer distances of the fish transport with minimum loss and stress. These dosages had been determined during the preliminary trials to produce a long-term effective immobilization without any harmful effect. Injectable solution of 10 mg/ml propofol was directly supplemented to the bath whereas dove oil was first dissolved in 95% ethanol (since it does not dissolve in water) at 1:10 ratio before supplementing.
Measurements

Sedation start time (induction), respiratory rates, ambient parameters were recorded before, during and after the sedation period. Respiratory rate was measured by counting opercular movements per minute. Behavioural reactions such as sensitivity to light, vibration and touch were examined on the group base by exposing the fish to a sudden light, tapping an object on the surface of the table and touching the fish over the plastic bags by a pencil at regular intervals. Some fish swam away when exposed to sudden light, fish reacted vibration by tapping an object on the surface of the table with sudden short sharp movements and touching fish over the plastic bags by a pencil at regular intervals caused moving away or no reaction. The responses to physical stimulations were analysed using a scoring method from least to most movement (ranging from 1 to 5) based on Likert scale. After 24 hours, fish were taken out of the bags and placed into fresh water for recovery. Finally, fish were put into a conventional aquarium for feed intake and color changing time observations. Color changes were observed by a color scale.

It is well known that ambient conditions affects activity, oxygen consumption and survival of fish. For this reason, cooling the fish has frequently been used to calm the fish during transport. Therefore, all fish groups in the study were kept under approximately 10°C lower temperature and 10% higher humidity (Table 1) than the routine conditions they kept. Control group was added to the table to compare the differences from the other groups but this does not mean control group was also sedated.

Statistical analysis

SPSS Version 22.0 was used to perform statistical analysis. The group means except induction were compared using ANOVA test where significance was tested by Tukey post hoc test. Induction time was compared with an independent samples t-test while the sensitivity differences were tested by Wilcoxon signed rank test. The changes within the groups were tested by General Linear Model (GLM) Repeated Measures test and Bonferroni test was used for determining the significance (p < 0.05).

| Ambient conditions                     | Control (n=6) x ± SD | Propofol (n=6) x ± SD | Clove Oil (n=6) x ± SD |
|----------------------------------------|---------------------|----------------------|----------------------|
| Pre-sedation room temperature (°C)     | 32.9 ± 0.16         | 30.8 ± 0.38          | 31.4 ± 0.16          |
| Sedation room temperature (°C)         | 22.5 ± 0.05         | 21.9 ± 0.11          | 23.2 ± 0.77          |
| Post-sedation room temperature (°C)    | 32.2 ± 0.27         | 31.6 ± 0.44          | 31.4 ± 0.05          |
| Pre-sedation room humidity (%)         | 41.5 ± 0.54         | 35.5 ± 1.64          | 37.5 ± 2.74          |
| Sedation room humidity (%)             | 52.0 ± 2.19         | 47.0 ± 1.09          | 55.5 ± 1.64          |
| Post-sedation room humidity (%)        | 39.5 ± 0.55         | 39.0 ± 2.19          | 38.0 ± 1.09          |
| Pre-sedation water temperature (°C)    | 28.8 ± 0.22         | 27.9 ± 0.38          | 27.9 ± 0.87          |
| Sedation water temperature (°C)        | 23.1 ± 0.11         | 23.0 ± 0.27          | 22.95 ± 0.27         |
| Post-sedation water temperature (°C)   | 27.2 ± 0.05         | 27.1 ± 0.11          | 27.0 ± 0.10          |
| Pre-sedation TDS (ppm)                 | 363.0 ± 13.14       | 370.5 ± 1.09         | 371.6 ± 5.48         |
| Sedation TDS (ppm)                     | 358.0 ± 18.62       | 373.5 ± 2.74         | 366.5 ± 8.61         |
| Post-sedation TDS (ppm)                | 462.0 ± 10.95       | 438.5 ± 10.41        | 428.5 ± 12.59        |
| Pre-sedation conductivity (ppm)        | 454.0 ± 15.88       | 456.0 ± 20.81        | 467.0 ± 4.38         |
| Sedation conductivity (ppm)            | 449.5 ± 20.26       | 457.7 ± 25.41        | 458.1 ± 7.81         |
| Post-sedation conductivity (ppm)       | 580.0 ± 19.71       | 549.0 ± 13.15        | 561.5 ± 34.51        |
| Pre-sedation salinity (ppm)            | 226.5 ± 8.22        | 256.0 ± 21.36        | 232.0 ± 4.38         |
| Sedation salinity (ppm)                | 235.5 ± 1.64        | 238.0 ± 2.19         | 227.0 ± 2.19         |
| Post-sedation salinity (ppm)           | 289.0 ± 8.76        | 272.5 ± 4.93         | 269.0 ± 7.67         |
| Pre-sedation water pH                  | 8.1 ± 0.09          | 8.1 ± 0.09           | 8.1 ± 0.09           |
| Sedation water pH                      | 7.7 ± 0.12          | 7.6 ± 0.16           | 7.4 ± 0.13           |
| Post-sedation water pH                 | 8.1 ± 0.08          | 8.1 ± 0.05           | 8.1 ± 0.08           |
Ethical approval

This study was approved by the Animal Experiments Local Ethics Committee of Tekirdağ Namık Kemal University with the reference number T2018-6 17/05/2018.

Results

A decreasing trend was observed in respiratory rates just after the beginning of the sedation process until the end of the sedation period (Table 2). Although decreasing trends appeared in all groups, the changes in groups demonstrated significant (p < 0.05) differences. Contrary to this trend, a sudden increase was seen in the first 10 min of the post-sedation period in all groups, however the differences between the propofol and other groups were obvious (p < 0.05).

Sensitivity measurements provided additional information for the effect of propofol and clove oil during the sedation period (Table 3). Treatment groups were significantly differed (p < 0.05) with the control group in response to light in all stages of the sedation period while propofol and clove oil groups only differed at the 24 h (p < 0.05). Regarding the touch and vibration tests, all groups differed (p < 0.05) until the 24 h while propofol and clove oil groups started to give similar reactions at the 24 h.

Repeated measures test revealed that respiratory rates in the control group showed a gradual decrease with little drops during the sedation period and a sudden increase up to the beginning level just after the sedation period (Table 4).

Discussion

In the current study, signs of sedation started after an initial period of excitation (fast and circular swim, frequent respiration) within seconds during the preliminary trials using 0.1-0.3 mL/L of the agent in the bath. The second stage of sedation consisted in a sudden stop of swimming activity and consequent sinking to the bottom while the third stage was characterized by an evident decrease of respiratory rate and the fish restarted swimming in a slower rhythm. An obvious calming effect was therefore observed by loss of mobility and reduction of respiratory rates. All sedated fish were calmer than the control fish at the beginning and remained calm until the end of the experiment.

Swimming activity disappeared in propofol and clove oil groups one hour after exposure to the treatment, whereas the control group was active during the whole experiment with slight changes in movements. Propofol group showed obvious responses at all stages of the sedation period (Table 4).

Table 2. Comparison of the average live weight, body length, induction time and respiratory rates (frequency per minute) between groups

| Measured Parameters | Control (n=42) | Propofol (n=42) | Clove Oil (n=42) | p
|---------------------|---------------|----------------|----------------|---
| Live weight (g)     | 3.01 ± 0.09a  | 2.72 ± 0.08a   | 2.96 ± 0.12a   | 0.106
| Body length (cm)    | 5.39 ± 0.07a  | 5.27 ± 0.06a   | 5.30 ± 0.08a   | 0.457
| Induction time* (min)| -             | 2.79 ± 0.10    | 2.90 ± 0.05    | 0.345
| Respiratory rates/min |              |                |                |
| Pre-sedation        | 140.88 ± 2.45a| 145.29 ± 1.49a | 139.00 ± 1.76a | 0.068
| Sedation 10 min     | 109.88 ± 1.74a| 67.26 ± 0.97a  | 84.48 ± 1.02a  | 0.000
| Sedation 20 min     | 108.98 ± 2.25a| 50.26 ± 1.45a  | 74.33 ± 1.00a  | 0.000
| Sedation 30 min     | 97.60 ± 2.05a | 36.52 ± 0.71a  | 65.88 ± 1.12a  | 0.000
| Sedation 1 hour     | 89.33 ± 2.40a | 11.74 ± 0.74a  | 48.81 ± 1.96a  | 0.000
| Sedation 3 hours    | 81.29 ± 2.46a | 7.10 ± 0.54a   | 14.90 ± 0.78a  | 0.000
| Sedation 5 hours    | 59.40 ± 2.56a | 4.50 ± 0.33a   | 10.55 ± 0.54a  | 0.000
| Sedation 7 hours    | 12.79 ± 0.56a | 3.69 ± 0.24a   | 5.05 ± 0.29a   | 0.000
| Sedation 24 hours   | 9.38 ± 0.30a  | 4.10 ± 0.24a   | 4.74 ± 0.26a   | 0.000
| Post-sedation 10 min| 114.62 ± 2.27a| 80.12 ± 1.84a  | 119.40 ± 1.59a | 0.000

p: ANOVA and *Independent Samples T-Test. Means within rows with different superscripts differ from each other (p < 0.05)
| Parameter | Time | Groups   | Mean Rank | df | \( \chi^2 \) | \( p \) |
|-----------|------|----------|-----------|----|------------|--------|
| Light     | 10 min | Control | 53.00\(^a\) | 2  | 51.065     | 0.000  |
|           |       | Propofol | 18.50\(^a\) |    |            |        |
|           |       | Clove oil | 24.50\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
|           | 20 min | Propofol | 19.00\(^a\) | 2  | 51.527     | 0.000  |
|           |       | Clove oil | 24.00\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Light     | 30 min | Propofol | 19.00\(^a\) | 2  | 51.495     | 0.000  |
|           |       | Clove oil | 24.00\(^a\) |    |            |        |
|           |       | Control   | 51.86\(^a\) |    |            |        |
|           | 1 h    | Propofol | 19.69\(^a\) | 2  | 47.125     | 0.000  |
|           |       | Clove oil | 24.45\(^a\) |    |            |        |
|           |       | Control   | 52.52\(^a\) |    |            |        |
| Light     | 24 h   | Propofol | 17.67\(^a\) | 2  | 44.391     | 0.000  |
|           |       | Clove oil | 25.81\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Touch     | 10 min | Propofol | 14.21\(^a\) | 2  | 51.794     | 0.000  |
|           |       | Clove oil | 28.79\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Touch     | 20 min | Propofol | 11.36\(^a\) | 2  | 57.561     | 0.000  |
|           |       | Clove oil | 31.64\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Touch     | 30 min | Propofol | 11.33\(^a\) | 2  | 57.480     | 0.000  |
|           |       | Clove oil | 31.67\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Touch     | 1 h    | Propofol | 11.40\(^a\) | 2  | 57.853     | 0.000  |
|           |       | Clove oil | 31.60\(^a\) |    |            |        |
|           |       | Control   | 52.52\(^a\) |    |            |        |
| Vibration | 24 h   | Propofol | 21.98\(^a\) | 2  | 41.395     | 0.000  |
|           |       | Clove oil | 21.50\(^a\) |    |            |        |
| Vibration | 10 min | Propofol | 12.14\(^a\) | 2  | 54.926     | 0.000  |
|           |       | Clove oil | 30.86\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Vibration | 20 min | Propofol | 13.00\(^a\) | 2  | 53.389     | 0.000  |
|           |       | Clove oil | 30.00\(^a\) |    |            |        |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Vibration | 30 min | Propofol | 12.90\(^a\) |    |            |        |
|           |       | Clove oil | 30.10\(^a\) | 2  | 53.979     | 0.000  |
|           |       | Control   | 53.00\(^a\) |    |            |        |
| Vibration | 1 h    | Control   | 53.00\(^a\) |    |            |        |
|           | Propofol | 11.93\(^a\) | 2  | 55.623     | 0.000  |
|           | Clove oil | 31.07\(^a\) |    |            |        |
|           | Control   | 43.38\(^a\) |    |            |        |
| Vibration | 24 h   | Propofol | 26.31\(^a\) | 2  | 13.426     | 0.001  |
|           | Clove oil | 26.31\(^a\) |    |            |        |

p:Wilcoxon Signed Rank Test; p:Tamhane test; p < 0.05
A distinct gradual decrease was seen in the respiratory rates in this group, which indicates the quick effect of propofol. Clove oil group also showed signs of sedation with significant decrease of respiratory rates. Respiratory rates were similar in the propofol and clove oil groups at 24th hour of the sedation period. Similar results were determined by several researchers indicating the sedative effects of propofol and clove oil with different dosages. Adel et al. (2016) used 1-5 mg/L of propofol and 25-100 mg/L clove oil into the bath of A. persicus juveniles to observe the sedative effects of drugs and main behavioural changes of fish. They stated that anaesthesia induction time was decreased by increasing anaesthetic concentration and resulted in loss of balance, body movements and some response to external stimulation in fish. Similarly, Hikasa et al. (1986) indicated that clove oil decreased respiratory rates based on the inhibition of respiratory centre in the medulla oblongata, as part of generalised depression of the central nervous system. Anderson et al. (1997) found that the efficacy of clove oil was similar to MS-222 for anaesthetising rainbow trout and noted that swimming speed after anaesthesia was not affected. Fleming et al. (2003) compared the sedative and anaesthetic effects of propofol and medetomidine-ketamine on Mexico sturgeon fish and stated that propofol resulted in mild bradycardia and apparent respiratory depression within 5 min of drug administration. They found greater depression of opercular movements in the propofol group, with the rate decreasing from 79 ± 5 bpm to 47 ± 4 bpm within 5 min of exposure. At 60 min, fish in the propofol group continued to show significantly depressed respiratory rates (48 ± 8 bpm). It could be said that appropriate sedative dosage of propofol or clove oil depends on the fish breed, transportation time, distance and environmental conditions result in obvious calming effect during transportation.

Regarding the sensitivity controls, propofol group gave fewer reactions in all sensitivity tests while control group was the most reacted group (Table 3). This indicated that the effect of propofol reduced the perception sense to light, vibration and touch, which also means reduced stress reactions during transportation. Since propofol group was less sensitive compared to control and clove oil groups in all measurement periods, this could be interpreted as one of the obvious results of the sedative impact of propofol as mentioned in the report of McFarland (1959) sedation for fish transport is characterized by a deep sedation, loss of reactivity to external stimuli, and reduction in metabolic rate. Similarly, Gholipour and Ahadizadeh (2013) stated that propofol can induce reliable anaesthesia in gold fish with lower Hb and MCHC.

For feed intake behaviour and color-changing time, experimental groups started to show normal feed intake behaviour after 24 hours, and fish color turned into normal approximately 4-5 hours in post-sedation period. On the other hand, control group showed normal feed intake behaviour immediately, but their color-change was similar to the experimental groups, which took 24 hours to become normal. Hoskøken and Pirhonen (2004) reported that they did not determine the duration of colour change, but they thought that it was probable that fish regain their colour changing ability as soon as they recover from the sedation. Thus, this study clarified the effect of clove oil sedation on the color changing ability of the fish.

No matter medical supplementation was applied or not, apparent sedation and decreased activity to the least level was seen in all groups 7 hours after bagging the fish (Table 4). Propofol group reflected the most obvious impact as a response to the sedative agent (propofol) supplementation to the bath.
Following a sharp decrease in 10 min of the sedation period, respiratory rates continued a decreasing trend for 5 hours. Then it became stable. However, fish in this group did not show a quick recovery during the 10 min of the post-sedation period. Respiratory rates in clove oil group also decreased sharply with little higher values compared to propofol group. The decreasing trend kept on for 5 hours and then became stable. Reverting back to the normal situation was conspicuously seen in this group 10 min just after the sedation period. During the recovery period, respiration increased, muscle tone returned, fin movements resumed, and the fish gradually corrected its swim until it regained full equilibrium similar to the behaviours mentioned by Neiffer and Stamper (2009). Yet, respiratory rate was lower in propofol group compared to both control and clove oil groups during the recovery period. Group II reflected the most obvious impact as a response to the sedative agent (propofol) supplementation to the bath. Following a sharp decrease in 10 min of the sedation period, respiratory rates continued a decreasing trend for 5 hours. Then it became stable. However, fish in this group did not show a quick recovery during the 10 min of the post-sedation period. Respiratory rates in Group III also decreased sharply with little higher values compared to Group II. The decreasing trend kept on for 5 hours and then became stable. Reverting back to the normal situation was conspicuously seen in this group 10 min just after the sedation period. No mortalities were observed during the experiment.

**Conclusion**

As a result, supplementing a sedative agent in oxygenated bath of Jack Dempsey aquarium fish for 24 hours had no adverse effect. Therefore using a sedative agent is strongly recommended in order to reduce the adverse effects of transport on aquarium fish.

Since low-cost and easy availability is important for field, propofol and clove oil seem as good alternatives for other drugs while same sedative dose of clove oil is more ineffective than propofol (0.1 ml/L) as a sedative agent in transportation of Jack Dempsey fish.

**Conflict of Interest**

The authors did not report any conflict of interest or financial support.

**Funding**

During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study, or no moral support.

**References**

Adel M., Sadegh AB, Yeganeh S, Movafagh AN, et al., 2016. Anesthetic efficacy of Clove Oil, Propofol, 2-Phenoxyethanol, and Ketamine Hydrochloride on Persian Sturgeon, Acipenser persicus. J World Aquacult Soc, 47 (6), 812-819.

Allen PE, Barquero MD, Bermúdez E, Calderón JC, et al., 2017. Calling for more ameliorate information in aquarium trade: analysis of live-fish import permits in Costa Rica. Manag Biol Invasion, 8 (4), 533-542.

Anderson WG, McKinley RS, Colavecchia M, 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. N Am J Fish Manage, 17(2), 301-307.

Belema M, Idowu KO, Aghogho KD, Ndubuisi A, et al., 2017. Handling and packaging of ornamental fishes for successful transport. Int J Fish Aquat Stud, 5 (5), 263-265.

Cookea SJ, Suskib CD, Ostranda KG, Tuftsib BL, et al., 2004. Behavioral and physiological assessment of low concentrations of clove oil anesthetic for handling and transporting largemouth bass (Micropterus salmoides). Aquaculture, 239 (1-4), 509-529.

Fleming GJ, Heard DJ, Floyd RF, Riggs A. 2003. Evaluation of Propofol and Medetomidine–Ketamine for Short-Term Immobilization of Gulf of Mexico Sturgeon (Acipenser oxyrinchus de Sohi). J Zoo Wildl Med, 34 (2), 153–158.

Gholipour KH, Ahadizadeh S, 2013. Use of propofol as an anesthetic and its efficacy on some hematological values of ornamental fish Carassius auratus. SpringerPlus, 2, 76.

Hikasa Y, Takase K, Ogasawara T, Ogasawara, 1986. Anaesthesia and recovery with tricaine methanesulphonate, eugenol and thiopental sodium in the carp (Cyprinus carpio). Jpn J Vet Res, 48 (2), 341–351.

Javahery S, Nekoubin H, Moradlu AH, 2012. Effect of anaesthesia with clove oil in fish (review). Fish Physiol Biochem, 38 (6), 1545–1552.

Hoskönem P, Pirhonen J, 2004. The effect of clove oil sedation on oxygen consumption of six temperate-zone fish species. Aquacult Res, 35, 1002-1005.

McFarland WN, 1959. A study of the effects of anaesthetics on the behaviour and physiology of fishes. Publ Inst Mar Sci Univ Tex, 6, 23-55.

Mitchell MA, Riggs SM, Singleton CB, Díaz-Figueroa O, et al., 2009. Evaluating the Clinical and Cardiopulmonary Effects of Clove Oil and Propofol in Tiger Salamanders (Ambystoma tigrinum). J Exot Pet Med, 18 (1), 50-56.

Neiffer DL, Stamper MA, 2009. Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs. ILAR J, 50 (4), 343-360.

Ross LG, Ross B, 2008. Anaesthetic and Sedative Techniques for Aquatic Animals Transportation and Anaesthesia, Ed; Ross LG, Blackwell Publishing Ltd, Edinburgh, Scotland, UK, 228 pp.

Sneddon LU, 2012. Clinical anesthesia and analgesia in fish. J
Exot Pet Med, 21 (1), 32-43.
Soto CG, Burhanuddin, G, 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (Siganus lineatus). Aquaculture, 136 (1-2), 149-152.

**Author Contributions**

Motivation / Concept: Tuba Özge Yaşar, Çetin Yağcılar, Mehmet Yardımcı
Design: Mehmet Yardımcı
Control/Supervision: Tuba Özge YAŞAR, Mehmet Yardımcı
Data Collection and / or Processing: Tuba Özge Yaşar, Çetin Yağcılar, Mehmet Yardımcı
Analysis and / or Interpretation: Tuba Özge YAŞAR, Mehmet Yardımcı
Writing the Article: Tuba Özge Yaşar, Çetin Yağcılar, Mehmet Yardımcı
Critical Review: Tuba Özge Yaşar, Çetin Yağcılar, Mehmet Yardımcı