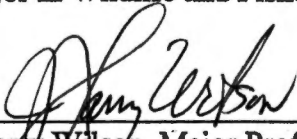


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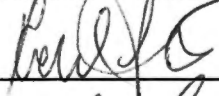
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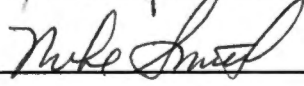


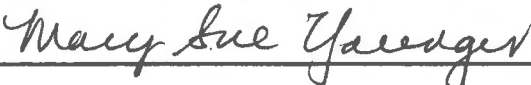
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Accepted for the Council:



Interim Vice Provost and
Dean of The Graduate School

**EFFECTIVE CLOVE OIL CONCENTRATIONS FOR
ANESTHETIZING SELECTED HATCHERY REARED
SPECIES**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Randall Todd Cooper
May 2001

AG-VET-MED.

Thesis

2001

.C69

DEDICATION

This thesis is dedicated to my wife, Kim, for her patience and understanding throughout this whole process, and to my parents, Billy and Nancy Cooper, for their everlasting support and help.

ACKNOWLEDGMENTS

Without the assistance of numerous people I would never have been able to complete this endeavor. I am particularly grateful to my major professor, Dr. Larry Wilson, who has assisted and advised me and been a great friend since the time I set foot on the campus of the University of Tennessee eight years ago. Special thanks also must go out to the rest of my thesis committee, Dr. Richard Strange, Dr. Mary Sue Younger, and Mike Smith for reviewing the manuscript and making invaluable suggestions.

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ABSTRACT

The use of traditional anesthetics for fish, such as MS-222, is currently restricted in the United States. Clove oil has been proposed recently as a substitute for these traditional chemicals, but effective concentrations of this substance are not known for many fish, other than nonsalmonids. In this study, five species of nonsalmonid, hatchery reared species were exposed to clove oil at varying concentrations to attempt to determine an effective dose. Hybrid striped bass (*Morone chrysops* x *M. saxatilis*) were found to have an effective dose of 40 mg/L. At this dosage, anesthesia induction and recovery times were 1.57 ± 0.11 (SE) and 1.23 ± 0.09 min, respectively, with an LCT (lethal concentration time) of 57.18 min for hybrids at 21.1° C. Grass carp (*Ctenopharyngodon idella*) and largemouth bass (*Micropterus salmoides*) were found to have an effective dose of 60 mg/L. Anesthesia induction and recovery times at that concentration were 1.63 ± 0.07 and 4.12 ± 0.27 min, respectively, for age-0 grass carp at 14.4° C; 1.45 ± 0.07 and 1.84 ± 0.08 for age-0 grass carp at 25.6° C; 2.48 ± 0.14 and 5.85 ± 0.22 min, respectively, for yearling grass carp at 18.9° C; 2.75 ± 0.12 and 8.60 ± 0.66 min, respectively, for brood grass carp at 18.9° C; and 1.46 ± 0.06 and 2.57 ± 0.13 min, respectively, for largemouth bass at 22.2° C. The LCT value for age-0 grass carp was 93.05 min at 14.4° C and 114.05 min at 20.0° C. The LCT for largemouth bass was not calculated, but fish survived up to 40 min of exposure with no mortalities. Striped bass (*M. saxatilis*) and black crappie (*Pomoxis nigromaculatus*) were found to have an effective dose of 80 mg/L. Anesthesia induction and recovery times at that concentration were 0.69 ± 0.03 and 1.66 ± 0.13 min, respectively, for striped bass at 25.6° C, and

3.46 ± 0.17 and 4.82 ± 0.42 min, respectively, for black crappie at 18.9°C . Striped bass had an LCT of 8.51 min, and black crappie had an LCT of 35.25 min. These effective concentrations are recommended for the grass carp, hybrid striped bass, largemouth bass, and black crappie; due to its very low LCT, no effective concentration was determined for striped bass at 25.6°C .

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CHAPTER I

INTRODUCTION

Anesthetics have long been used in fisheries and aquaculture as a way to handle fish while doing as little damage as possible. They are used to mitigate the stress from processes such as handling, transporting, and certain tagging and surgical procedures to prevent injury, death, or disease outbreak due to a lowered disease resistance.

Anesthetics are chosen on the basis of such factors as their cost, efficacy, and safety (to both the fish and the user).

The most common anesthetic used today is tricaine methanesulfonate (tricaine, or MS-222). MS-222 is one of only two anesthetics legally registered for use in the United States by the U.S. Food and Drug Administration (FDA) (Taylor and Roberts 1999). One of the main problems with MS-222 is that the FDA requires a 21-d withdrawal period before the fish can be released or consumed by humans. The cost of tricaine can also be a factor to many of its users. The other anesthetic used is carbon dioxide (CO₂). While it is a substance that is generally regarded as safe, it is known to produce relatively shallow anesthesia and fish have a tendency to react violently after application (Taylor and Roberts 1999). Many other anesthetics have been used in fisheries research, but due to various factors, such as being carcinogenic, none of them are registered for use. Researchers are in the process of trying to find an anesthetic that does not have the drawbacks of MS-222 and CO₂, but is also safe and effective and preferably more cost efficient.

Studies have been done to show the efficacy, safety, and cost efficiency of clove

oil as an alternative anesthetic (Soto and Burhanuddin 1995, Anderson et al. 1997, Munday and Wilson 1997, Keene et al. 1998, Peake 1998, Taylor and Roberts 1999, Waterstrat 1999, and Cho and Heath 2000). Due to its efficacy and history of use in human applications, attempts are being made to register it as a fish anesthetic. While much work is yet to be done, these earlier studies suggest clove oil can be a promising substitute for tricaine and all of the other anesthetics. However, safe recommendations for its usage are not known for many nonsalmonid species.

The objectives of this study were: (1) to determine effective concentrations of clove oil for use on five nonsalmonid species of fish: grass carp (*Ctenopharyngodon idella*), striped bass (*Morone saxatilis*), largemouth bass (*Micropterus salmoides*), hybrid striped bass (*M. chrysops* x *M. saxatilis*), and black crappie (*Pomoxis nigromaculatus*), (2) to determine the effects that length, temperature, and different brands of clove oil have on the induction and recovery times for grass carp, and (3) to determine the limits of prolonged exposure to clove oil for each of the five species.

CHAPTER II

LITERATURE REVIEW

Anesthesia is a biological state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical means (Summerfelt and Smith 1990). Many procedures used by fishery workers are more easily accomplished if the fish are anesthetized. The principal use of anesthetics is to minimize the stress associated with procedures such as handling and transporting, because of the physiological and behavioral effects in unanesthetized fish (Muench 1958). If stress levels are not controlled or minimized, fish can die from osmoregulatory dysfunction or a disease outbreak resulting from lowered disease resistance (Harrell 1992).

Marking and Meyer (1985) proposed that the ideal anesthetic should allow for a reasonable duration of exposure, produce anesthesia within three minutes or less, allow recovery in five minutes or less, cause no mortality in fish at treatment levels, present no mammalian safety problems, have low residual in tissue within one hour of withdrawal, and lastly, be reasonable in cost. When necessary, overdose of an anesthetic can be an acceptable means of euthanasia (Harms and Bakal 1994).

Levels of Anesthesia

There are five recognized stages of induction, ranging from sedation, narcosis or loss of equilibrium, to deep anesthesia, and five stages of recovery (Table 1). An excitement phase, which can have negative effects when dealing with larger fish, occasionally occurs between sedation and narcosis (Harms and Bakal 1994). Sedation

Table 1. Stages of fish anesthesia induction and recovery.

Stage of anesthesia ¹	Description
1	<i>Sedation</i> : Partial or total lack of reaction to external stimuli.
2	<i>Subtranquility</i> : Partial loss of equilibrium, uncoordinated movement followed by active, erratic swimming.
3	<i>Tranquility</i> : Inverted swimming with intermittent resting on bottom, but react to vibrational stimuli.
4	<i>Anesthesia</i> : Loss of reflex activity with slow erratic opercular movement.
5	<i>Medullary collapse</i> : Respiratory movement ceases. Fish are pale. Death.
Stage of recovery ²	Description
1	Reappearance of opercular movement.
2	Partial recovery of equilibrium with partial recovery of swimming motion.
3	Total recovery of equilibrium.
4	Reappearance of avoidance swimming motion and reaction in response to external stimuli, but still behavioral response is stolid.
5	Total behavioral recovery; normal swimming.

¹ Source: Plumb, J.A., T.E. Schwedler, and C. Limsuan (1983)

² Source: Cho, G.K., and D.D. Heath (2000)

may be quite adequate for transportation and other procedures such as blood collecting and weighing, while anesthesia (Stage 4) is usually required for immobilization and to reduce the reflex responses so that fish are not traumatized by handling procedures. Although sedated fish (Stage 1) have a reduced response to stressors, it should be noted that they are not fully anesthetized and therefore may still feel pain and undergo some stress (Burka et al. 1997). However, Carmichael et al. (1984) using largemouth bass, and Harrell (1992) using striped bass, found that both species had lower stress and mortality when transported sedated in a combination of salt and a mild anesthetic.

Considerations of Use

Water quality is an important consideration in the use of anesthetics. The parameters to be considered include temperature, dissolved oxygen, salinity, pH, and hardness (Harms and Bakal 1994). Lemm (1993) reported that lower concentrations were needed at higher temperatures for induction of striped bass with a variety of anesthetics and that water temperature and induction time had an inverse relationship. Marking (1967) found that rainbow trout (*Oncorhynchus mykiss*) were slightly more resistant at lower temperatures when testing for median lethal concentrations (LC50, the concentration lethal to 50% of test animals). Adequate aeration is mandatory, particularly when many fish are to be anesthetized in the same water. Since some anesthetics drastically affect pH, buffers must be added to avoid the stress associated with rapid changes in pH (Harms and Bakal 1994).

Salts and chemicals in the water can affect the uptake of some anesthetics in the water. Salts have the capacity to buffer the anesthetic and minerals in the water can affect

uptake across the gills (Burka et al. 1997). For water hardness, Marking (1967) reported that higher concentrations of anesthetics were needed to anesthetize rainbow trout in soft water (hardness 10 mg/L); Schoettger and Julin (1967) found that rainbow trout in soft water (hardness 10 mg/L) needed a much longer exposure time to be anesthetized than fish in hard water (hardness 180 mg/L). Another factor in anesthetizing fish is the size of the fish. Gilderhus (1989) found that the size of rainbow trout was positively correlated with effective concentration.

Registered Anesthetics

There are only two anesthetics registered for aquacultural use by the FDA: tricaine methanesulfonate (tricaine, or MS-222) and carbon dioxide (CO₂) (Taylor and Roberts 1998). The major drawbacks for tricaine are the requirement of a 21-d withdrawal period after exposure and the relatively high cost of using the drug (Marking and Meyer 1985). Significant studies have been done with tricaine on salmonid and nonsalmonid fishes. Schoettger et al. (1967) reported that concentrations of 100-140 mg/L of MS-222 were effective for rapid loss of equilibrium in channel catfish (*Ictalurus punctatus*) within two minutes, and they were able to tolerate exposure for 4 to 11 minutes. Concentrations of 70 mg/L produced moderately rapid anesthesia for at least 30 minutes and 20-40 mg/L maintained sedation for six hours. Schoettger and Julin (1967) stated that concentrations of 80-135 mg/L of tricaine were effective for rapid anesthetizing rainbow trout within three minutes, with a toleration of exposure from 4-12 minutes, while concentrations of 50-60 mg/L induced a moderate rate of anesthesia which could be maintained for approximately 30 minutes, and sedation was maintained for 5-6 hours at 15-30 mg/L.

Gilderhus and Marking (1987) found that four minutes at 60 mg/L were needed to induce adequate anesthesia in rainbow trout. Lemm (1993) reported that 150 mg/L were an effective concentration for striped bass from 13-23° C, while Bills et al. (1993) discovered the LC50 at 24 hours for juvenile striped bass to be 49 mg/L. Mazik and Simco (1994) documented a decrease in mortality in Phase I striped bass during hauling in water consisting of 0.5 and 1.0% CaCl₂. Masee et al. (1995) reported the lowest effective concentration of tricaine was 55 mg/L and 75 mg/L for larval red drum (*Sciaenops ocellatus*) and goldfish (*Carassius auratus*), respectively.

Limitations of tricaine have occasionally caused researchers to use CO₂ anesthesia. Carbon dioxide is allowed under a GRAS (generally regarded as safe) declaration by the FDA and is introduced into the water either directly through an air stone or indirectly by addition of sodium bicarbonate (NaHCO₃) and an acid (Peake 1998). In a survey of federal, state, and private fishery workers, Marking and Meyer (1985) reported that the biggest drawbacks for using CO₂ were its partial effectiveness, slow action, lethality for repeat exposure, and production of stress in fish. Also, fish have a tendency to exhibit an extreme hyperactive response when first immersed in water containing high concentrations of carbon dioxide. However, MacKinlay et al. (1994) attributed most of the stress to handling rather than the anesthetic.

Peake (1998) found that 2.66 g/L of NaHCO₃ was an effective concentration for lake sturgeon (*Acipenser fulvescens*), smallmouth bass (*M. dolomieu*), walleye (*Stizostedion vitreum*), and northern pike (*Esox lucius*) to reach Stage 4 anesthesia. Post (1979) recommended carbon dioxide anesthesia not be used at levels high enough to

cause loss of reflex and opercular activity. Booke et al. (1978) reported a combination of pH 6.5 and 642 mg/L of NaHCO₃ was the most effective treatment for meeting Stage 2 anesthesia in rainbow trout, brook trout (*Salvelinus fontinalis*), and carp (*Cyprinus carpio*). Gilderhus and Marking (1987) found that for adult rainbow trout at 900 mg/L NaHCO₃, induction time was marginal and recovery times exceeded criteria by 60% when using the criteria of three minutes for induction and ten minutes for recovery after 15 minutes of exposure. In addition to the two approved chemicals, electric shock produces short term loss of equilibrium, and the only restrictions on its use are related to safety (Marking and Meyer 1985).

Unregistered Anesthetics

Examples of anesthetics that have been used but are not registered with the FDA are benzocaine, etomidate, metomidate, quinaldine sulfate, and 2-phenoxyethanol. Benzocaine was suggested as an acceptable substitute for MS-222 by McErlean and Kennedy (1968) because it acted more rapidly and at lower concentrations, and was less than 1/10 the cost; the primary disadvantage was that it was less soluble in water. Gilderhus (1989) reported that 25-45 mg/L of benzocaine was an effective concentration range at 7-17° C for juvenile chinook salmon (*O. tshawytscha*) and rainbow trout. Gilderhus (1990) also confirmed that 25-30 mg/L was an effective concentration for spawning-phase chinook salmon at 10-13° C as well. Gilderhus et al. (1991) recorded that an effective concentration of benzocaine for subadult striped bass was 60 and 55 mg/L at 18 and 22° C, respectively. Lemm (1993) obtained similar results with the same size fish at concentrations of 70 and 55 mg/L at 18 and 23° C; he also recorded an

effective sedative dose of 25 mg/L at 13-23° C.

Metomidate and its analog, etomidate, have also been researched extensively. Lemm (1993) found that subadult striped bass could be effectively anesthetized with concentrations of 7.5, 10.0, and 10.0 mg/L at 13, 18 and 23° C, respectively, and also sedated with a dose of 0.5 mg/L across that range of temperatures. However, Massee et al. (1995) proved metomidate to be an ineffective anesthetic for both red drum and goldfish larvae, with mortality occurring in red drum larvae in all tested concentrations (3.0-4.5 mg/L). Plumb et al. (1983) reported that channel catfish could be effectively anesthetized at 0.8-1.2 mg/L of etomidate and could withstand concentrations ranging from 0.4 to 3.6 mg/L for up to 80 minutes with very low mortality. Davis et al. (1982) tested levels of plasma corticosteroids (a test of stress) in yearling striped bass exposed to 25 mg/L of tricaine, 2.5 mg/L of quinaldine, or 0.1 mg/L of etomidate, alone or in combination with salt; their investigations indicated that etomidate alone or combined with salt limited the increase in plasma corticosteroids during exposure and confinement much more than all of the other anesthetics and anesthetic/salt combinations. However, in tests done by Gilderhus and Marking (1987) on rainbow trout, metomidate and etomidate both failed to meet their efficacy criteria. They were rejected because their recovery times exceeded criteria by 80 and 100%, respectively.

For quinaldine sulfate, effective concentrations of 55, 25, and 25 mg/L at 13, 18 and 23° C, respectively, for anesthesia, and 5 mg/L for sedation for subadult striped bass were proposed by Lemm (1993). Juvenile striped bass were also found to have an LC50 of 26.8 mg/L at 24 hours and 22.4 mg/L at 96 hours exposure (Bills et al. 1993).

Gilderhus and Marking (1987) reported that 40 mg/L at 12° C met their criteria of efficacy, although after five minutes exposure, the fish reacted strongly and were not manageable. Massee et al. (1995) reported the lowest effective concentrations of quinaldine sulfate to be 35 mg/L at 26° C for larval red drum and 60 mg/L at 24° C for larval goldfish. For 2-phenoxyethanol, McCarter (1992) found that grass carp and silver carp (*Hypophthalmichthys molitrix*) could be sedated at a concentration of 0.2 mL/L in 2-3 minutes and anesthetized at 0.4 mL/L in 5-10 minutes.

Clove Oil

As a result of the drawbacks of each of these anesthetics, clove oil has been introduced as an alternative. Clove oil has already been generally recognized as safe by the FDA, and its chief advantages include low cost, potential for limited regulatory restrictions or withdrawal times, and safety to both fish and humans (Waterstrat 1999). It is a distilled product of the clove tree (*Eugenia aromatica*), and its active ingredient is eugenol (Taylor and Roberts 1998). Keene et al. (1998) concluded that clove oil met seven of the eight criteria established for an ideal anesthetic (Marking and Meyer 1985).

Studies of the efficacy of clove oil are fairly limited but diverse. Taylor and Roberts (1998) reported that the median lethal concentrations for a 10 minutes exposure were 62 mg/L for chinook salmon, 96 mg/L for coho salmon (*O. kisutch*), 250 mg/L for rainbow trout, and 526 mg/L for white sturgeon (*A. transmontanus*); a dosage of 25 mg/L was effective in anesthetizing all four species for 120 minutes with no mortality. Anderson et al. (1997) compared the efficacy of clove oil to MS-222 and found that it was as effective as MS-222 at 40 and 120 mg/L in inducing anesthesia for both juvenile

and adult rainbow trout, and that exposure to clove oil was not detrimental to critical swimming speed of either age group. Furthermore, Cho and Heath (2000) concluded that there appeared to be no adverse physiological repercussions in choosing clove oil over tricaine to immobilize juvenile chinook salmon.

In nonsalmonid fish studies, Peake (1998) suggested an optimal concentration of 60 mg/L of clove oil which yielded a Stage 4 anesthesia in 4.3 minutes and recovery in 10.9 minutes for walleye, and found the same concentration to be effective for lake sturgeon, smallmouth bass, and northern pike. Waterstrat (1999) stated that channel catfish can be safely maintained in a concentration of 100 mg/L of clove oil for periods up to 15 minutes before encountering prolonged recovery times and mortality. For marine fishes, Soto and Burhanuddin (1995) concluded that clove oil was highly effective for measuring length and weight of juvenile rabbitfish (*Siganus lineatus*), and Munday and Wilson (1997) found that clove oil was as effective as quinaldine, benzocaine, MS-222, and 2-phenoxyethanol for anesthetizing a coral reef fish, *Pomacentrus amboinensis*.

CHAPTER III

METHODS

Tests were conducted over a nine-month period from April 2000 to December 2000 at the University of Tennessee, Knoxville, Joe E. Johnson Animal Research and Teaching Unit. During this time, water temperatures in the lab fluctuated between 14 and 26° C. Experiments were run at temperatures established by seasonal weather patterns, as water temperature could not be controlled throughout most of the experimental period. Fish were held at a low density in large circular tanks (800-1000 L), in a flow-through system with a water flow rate of at least 10 L/min. Fish were fed a diet of 2% of body weight per day. All fish to be used in experiments were kept off feed for at least 24 hours prior to testing.

Sources for Anesthetics

Clove oil was obtained from two different sources: Sigma Chemical Company, St. Louis, Missouri, and NOW Foods, Glendale Heights, Illinois. The Sigma brand was ordered directly from Sigma, and the NOW brand was bought locally from Nature's Pantry Health Store. Sigma clove oil was the main brand used in the experiments, with the NOW brand used in a single test to compare the two brands. Hereafter, any reference to clove oil solution can be assumed to be the Sigma brand unless directly referred to as being the NOW brand.

Due to its insolubility in water, clove oil was first put into solution with ethanol at a ratio of 1 : 9 (clove oil : ethanol). Anderson et al. (1997) concluded that the ethanol itself did not have an anesthetic effect on test fish. The ethanol was also acquired from

the Sigma Chemical Company. The solution was prepared for each test individually, instead of using a prepared stock solution, to ensure the proper ratio was used for each experiment.

Source of Fish

All fish used were obtained from the Tennessee Wildlife Resource Agency at Eagle Bend Hatchery in Clinton, TN. All of the fish, with the exception of the crappie, were removed from the ponds with nets; the crappie were captured by angling. Fish were transported directly (<40 kilometers) to the lab in water treated with NaCl to reduce stress. All fish were allowed to acclimate to the lab for at least one week before any tests were run. Age-0 grass carp (81-142 mm) were received on 2 March 2000, and tested at temperatures from 14.4-25.6° C. Yearling grass carp (390-460 mm) were received 2 October 2000, and acclimated and tested at 18.9° C. Brood grass carp (550-750 mm) were received 20 May 2000 and were also acclimated and tested at 18.9° C. Largemouth bass (138-204 mm) and hybrid striped bass (89-174 mm) were both acquired 2 October 2000 and tested at 22.2 and 21.1° C, respectively. The black crappie (131-240 mm) were acquired last on 1 December 2000, and acclimated and tested at 18.9° C.

Test Conditions

All juvenile fish were tested in small circular tanks (18 cm x 26 cm) which contained 100 L of water. The adult and brood fish were tested in larger circular tanks (48 cm x 36 cm) which were filled with 300 L of water. In all cases, the appropriate dosage was added to the water in the holding container and stirred vigorously to ensure proper mixing before fish were added. Fish were then transported by net from the

holding tanks and introduced to the anesthetic tank and then into an identical tank with untreated flowing water for recovery. Oxygen levels were monitored constantly. After each experiment, fish were held for 48 hours and any deaths were noted. Preliminary trials were first conducted in all cases to determine a suitable range of clove oil concentrations for each species.

Criteria of Efficacy

The criteria of efficacy to find the lowest effective concentration of clove oil were modeled after Marking and Meyer's (1985) criteria for the ideal anesthetic: (1) induction of anesthesia in 3 min or less, (2) recovery in 5 min or less, and (3) no mortality of fish following 15 min of exposure to the effective concentration. The third criterion was tested afterwards during the survival tests. Additionally, the criteria for the age-0 fish were lowered to 2.0 and 4.0 min for induction and recovery, respectively, to compensate for the faster induction and recovery of the juvenile fish. All three criteria were not always met in every case.

Grass Carp

Seven experiments (GC-1, GC-2, GC-3, GC-4, GC-5, GC-6, GC-7 and GC-8) were conducted with age-0, yearling, or brood grass carp. In experiment GC-1, two replicates of 10 age-0 grass carp were individually exposed to 40, 60, 80, or 100 mg/L of clove oil at 14.4° C and the times for induction and recovery were recorded. Induction time was defined as the time it took until immobilization (Stage 4). After induction, the fish were carefully removed and quickly measured before being placed in the recovery tank. Recovery was defined as the time it took to regain normal swimming motion, as

opposed to just regaining equilibrium. The criteria of efficacy were used to determine the lowest effective concentration of clove oil.

In experiment GC-2, two replicates of 10 age-0 fish were exposed to 40, 60, or 80 mg/L of the NOW brand clove oil at 14.4° C, with all procedures the same as above.

Criteria of efficacy were used to determine the lowest effective concentration of clove oil with the NOW brand. These results were then compared to the results of the fish tested at 40, 60, or 80 mg/L of clove oil from GC-1 to compare the effectiveness of the different brands (experiment GC-3).

In experiment GC-4, two replicates of 10 age-0 fish were tested at 60 mg/L of clove oil following the same procedures as above in a water temperature of 25.6° C. The results were then compared to the results of the fish tested at 60 mg/L of clove oil from GC-1 to compare the effects at markedly different temperatures.

In experiment GC-5, ten yearling grass carp were individually exposed to 40, 60, 80, or 100 mg/L of clove oil at 18.9° C and the time until induction and recovery was recorded using the procedures as described above. Criteria of efficacy were then used to determine the lowest effective concentration of clove oil. In experiment GC-6, eleven of the brood grass carp were individually exposed to 60 mg/L of clove oil at 18.9° C and times to induction and recovery recorded, using the same procedures as above. Results from these fish were compared to the results from the fish exposed to 60 mg/L of clove oil from GC-5 to test for a difference in the size of fish and to test the efficacy of 60 mg/L of clove oil on fish of this length.

In experiment GC-7, ten groups of 10 age-0 grass carp were exposed to the lowest

effective concentration (60 mg/L) of clove oil for 10, 20, 30, 45, 60, 75, 90, 105, 120, or 135 min at 14.4° C or 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, or 165 min at 20.0° C and then placed into recovery tanks to test the effects of prolonged anesthetic exposure. In the recovery tanks, the fish were kept for 48 hours to monitor survival. Median lethal concentration times (LCT, the time at which 50% of the test animals had died) were then determined for each of the temperatures and compared to one another.

In experiment GC-8, five groups of 8 yearling grass carp were exposed to the lowest effective concentration (60 mg/L) of clove oil for 30, 60, 120, 150, or 180 min at 18.9° C following the same procedure as GC-7. The LCT was determined and compared to the LCT's in GC-7.

Striped Bass

Two experiments (SB-1 and SB-2) were conducted on striped bass. In experiment SB-1, two replicates of 10 fish were individually exposed to 40, 60, 80, or 100 mg/L at 25.6° C in the same manner as described above. The criteria of efficacy were used to determine the lowest effective concentration of clove oil. In experiment SB-2, four groups of 10 fish were exposed to the lowest effective concentration (80 mg/L) of clove oil for 10, 20, 30, or 40 min at 25.6 ° C and then placed into recovery tanks to test prolonged anesthetic exposure. In the recovery tanks, the fish were kept for 48 hours to monitor survival. The LCT was determined for striped bass at that temperature.

Largemouth Bass

Two experiments (LB-1 and LB-2) were conducted on largemouth bass. In experiment LB-1, two replicates of 10 fish were individually exposed to 40, 60, 80, or

100 mg/L at 22.2° C in the same manner as described above. The criteria of efficacy were used to determine the lowest effective concentration of clove oil. In experiment LB-2, groups of ten fish were exposed to the lowest effective concentration (60 mg/L) of clove oil for 10, 20, 30, or 40 min at 22.2° C and then placed into recovery tanks to test prolonged anesthetic exposure. In the recovery tanks, the fish were kept for 48 hours to monitor survival. The LCT was determined for largemouth bass at that temperature.

Hybrid Striped Bass

Two experiments (HB-1 and HB-2) were conducted on hybrid striped bass. In experiment HB-1, two replicates of 10 fish were individually exposed to 30, 40, 60, or 80 mg/L at 21.1° C in the same manner as described above. The criteria of efficacy were used to determine the lowest effective concentration of clove oil. In experiment HB-2, eight groups of 10 fish were exposed to the lowest effective concentration (40 mg/L) of clove oil for 10, 20, 30, 40, 50, 60, 70, or 80 min at 21.1° C and then placed into recovery tanks to test prolonged anesthetic exposure. In the recovery tanks, the fish were kept for 48 hours to monitor survival. The LCT was determined for hybrid striped bass at that temperature.

Black Crappie

Two experiments (BC-1 and BC-2) were conducted on black crappie. In experiment BC-1, two replicates of 10 fish were individually exposed to 60, 80, or 100 mg/L at 18.9° C in the same manner as described above. The criteria of efficacy were used to determine the lowest effective concentration of clove oil. In experiment BC-2, six groups of 10 fish were exposed to the lowest effective concentration (60 mg/L) of

clove oil for 10, 20, 30, 40, 50, or 60 min at 18.9° C and then placed into recovery tanks to test prolonged anesthetic exposure. In the recovery tanks, the fish were kept for 48 hours to monitor survival. The LCT was determined for black crappie at that temperature.

Statistical Analysis

Analysis of variance (ANOVA) tests were used to determine differences among treatment groups after data were tested for normality (Proc UNIVARIATE; SAS Institute 1988). When *F*-values were significant, a Bonferroni multiple-comparison test was used to compare each treatment group (Proc GLM; SAS Institute 1988). Length was taken into account as a covariate in the model; if it did not have a significant effect, it was dropped from the model. Proc PROBIT (SAS Institute 1988) was used to obtain LCT's and 95% confidence limits. In all cases, $P < 0.05$ was considered to be significant.

CHAPTER IV

RESULTS

During experiments, induction time was defined as the point when fish were rendered immobile. Recovery time was defined as the point at which fish were upright and had retained a normal swimming motion.

Grass Carp

Age-0 grass carp in all trials remained calm until loss of equilibrium. At that point, the fish turned over and began to swim upside down in a steady, but not frenzied, manner. The yearling and brood grass carp followed a similar pattern, but did not do a great deal of swimming after losing equilibrium. Very few fish became agitated in any manner; only one yearling fish actually jumped out of the water.

Experiment GC-1. The means and standard errors for induction and recovery times for age-0 fish exposed to four different concentrations of clove oil at 14.4° C are presented in Figure 1 and Table 2. Fish exposed to 40 mg/L required significantly more time to reach induction than those at 60 mg/L, and both were significantly longer than at 80 and 100 mg/L. No significant differences among mean induction times were found between the 80 and 100 mg/L solutions. Fish exposed to 40 mg/L had a significantly shorter recovery time than fish exposed to 60, 80, or 100 mg/L levels. No significant differences were found among those three concentrations. Length, considered as a covariate, did not have a significant relationship to time in this size range for either induction ($P=0.1412$) or recovery ($P=0.9669$), and was therefore taken out of the model. Concentrations of 60, 80, and 100 mg/L met the criteria for induction, but only 40 mg/L

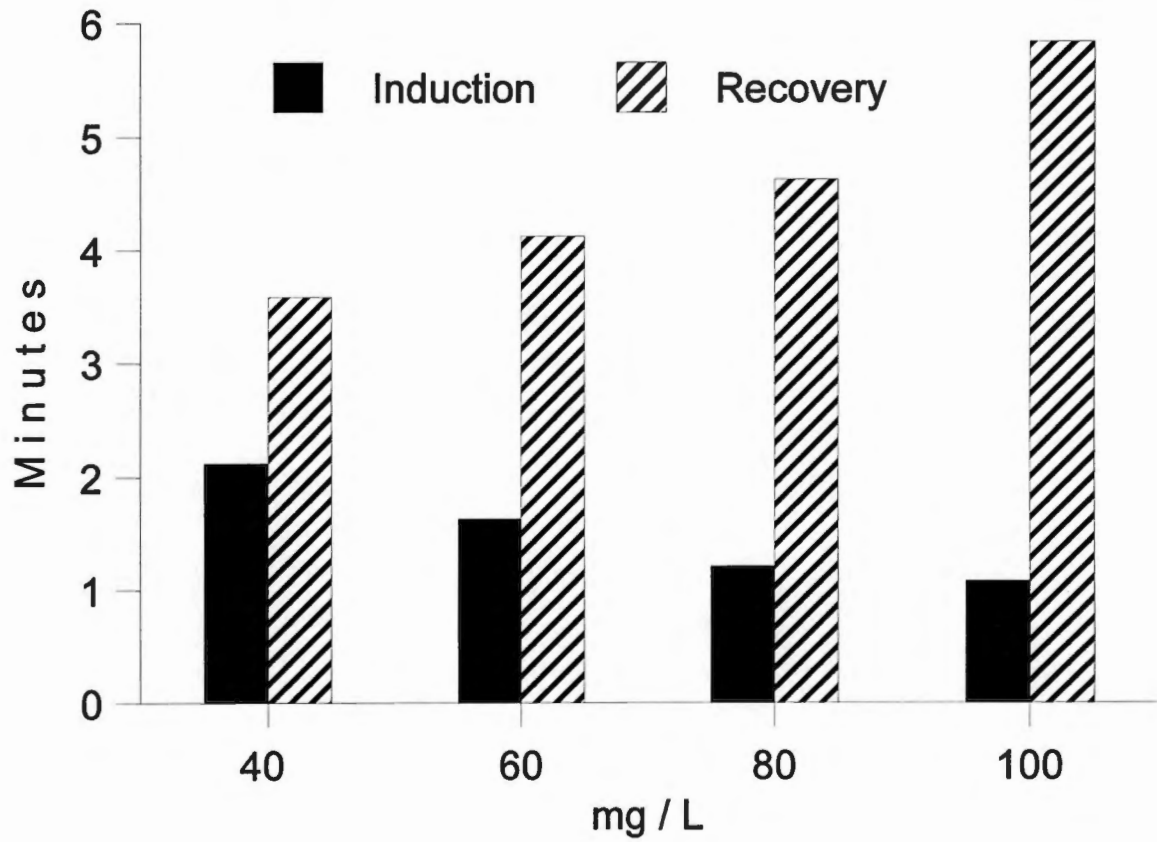


Figure 1. Experiment GC-1: Mean induction and recovery times for age-0 grass carp (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 14.4° C.

Table 2. Experiment GC-1: Mean ($\pm$ SE) induction and recovery times (min) for age-0 grass carp (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 14.4° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
40	2.12 a	0.09	3.58 a	0.27
60	1.63 b	0.07	4.12 b	0.27
80	1.21 c	0.05	4.62 b	0.27
100	1.08 c	0.06	5.84 b	0.42

did so for recovery. A dosage of 60 mg/L was chosen as the lowest effective concentration because its recovery time was not significantly different than the recovery time for 40 mg/L, which was below 4 minutes. No mortalities were recorded in this experiment.

Experiment GC-2. The means and standard errors for induction and recovery for age-0 fish exposed to three different concentrations of NOW clove oil at 14.4° C are shown in Figure 2 and Table 3. Induction time for 40 mg/L was significantly longer than 60 mg/L, which was itself significantly longer than 80 mg/L. All three concentrations were significantly different for recovery times as well. Length was not considered in this experiment, as the range of fish was exactly the same as the range in GC-1. Although no single concentration fit the criteria of efficacy for age-0, 40 mg/L would be chosen as the lowest effective concentration for NOW clove oil, due to increasingly long recovery times of 60 and 80 mg/L. No mortalities were recorded in this experiment.

Experiment GC-3. The comparison of the cumulative means and standard errors of three concentrations for each brand of clove oil is presented in Table 4. Induction times for NOW clove oil were significantly higher than the induction times for Sigma clove oil. On the average, fish exposed to NOW clove oil needed 0.18 more min (approximately 11 seconds) to reach Stage 4 anesthesia than fish exposed to Sigma clove oil at the same concentration. For induction, there was no significant interaction between brand and concentration ($P=0.2224$). For recovery, a significant interaction between brand and concentration was found ($P=0.0015$).

The means and standard errors for recovery times for all of the brand and

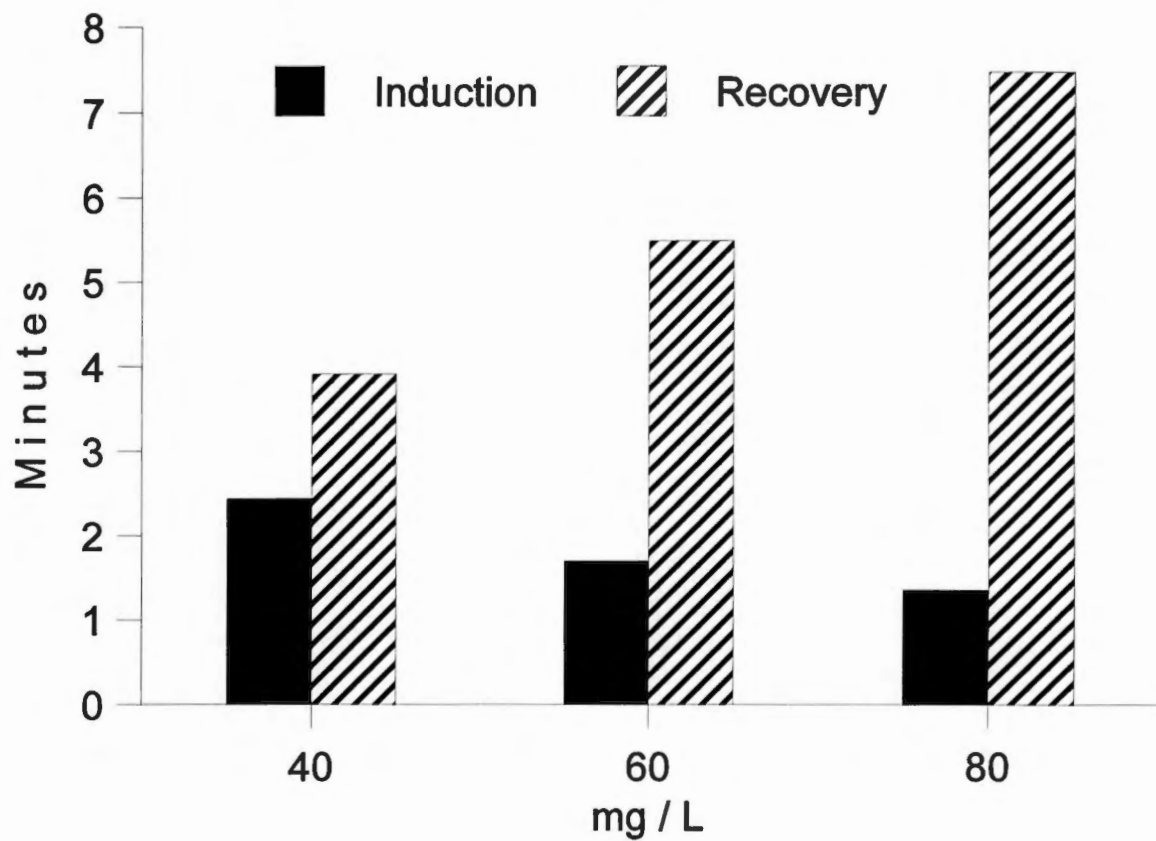


Figure 2. Experiment BC-2: Mean induction and recovery times for age-0 grass carp (20 fish per concentration) exposed to NOW clove oil at concentrations of 40, 60, and 80 mg/L at 14.4° C.

Table 3. Experiment GC-2: Mean ($\pm$ SE) induction and recovery times (min) for age-0 grass carp (20 fish per concentration) exposed to NOW clove oil at concentrations of 40, 60, and 80 mg/L at 14.4° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
40	2.44 a	0.10	3.91 a	0.20
60	1.70 b	0.07	5.49 b	0.31
80	1.36 c	0.06	7.47 c	0.59

Table 4. Experiment GC-3: Cumulative mean ($\pm$ SE) induction times (min) for age-0 grass carp (20 fish per concentration, 60 fish per brand) exposed to each brand of clove oil at concentrations of 40, 60, and 80 mg/L at 14.4° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Brand	Concentrations	Induction time	
		Mean	Standard error
NOW ¹	40, 60, 80	1.83 a	0.07
Sigma ²	40, 60, 80	1.66 b	0.06

¹ NOW Foods, Glendale Heights, Illinois

² Sigma Chemical Company, St. Louis, Missouri

concentration combinations are presented in Table 5. Recovery time at 40 mg/L of NOW was significantly shorter than 60 and 80 mg/L of NOW but not significantly different than any of the Sigma concentrations. Recovery at 60 mg/L of NOW was significantly shorter than 80 mg/L of NOW and significantly longer than 40 and 60 mg/L of Sigma, but not significantly different than the 80 mg/L Sigma dosage. Recovery at 80 mg/L of NOW was significantly longer than any of the other brand and concentration interactions. Figure 3 illustrates the interaction between recovery in the two brands and at three concentrations. No mortalities were recorded in this experiment.

Experiment GC-4. There were no significant differences in the induction times of the fish exposed to 60 mg/L of clove oil at 14.4 and 25.6° C ($P=0.0919$). However, recovery at 14.4° C took significantly longer than at 25.6° C ($P<0.0001$) (Table 6). Length did not have an effect on induction ($P=0.7566$) or recovery ($P=0.5345$) in fish exposed at 25.6° C, so it was not included in the model. No mortalities were recorded in this experiment.

Experiment GC-5. The means and standard errors for induction and recovery times, respectively, for yearling fish exposed to four different concentrations of clove oil at 18.9° C are presented in Figure 4 and Table 7. Fish exposed to 40 mg/L required significantly more time to reach induction than they did at 60 mg/L, and fish at 60 mg/L required significantly more time than fish at 80 and 100 mg/L. There was no significant difference in induction times at the 80 and 100 mg/L levels. Fish exposed to 100 mg/L required a significantly longer time to recover those at 40 and 60 mg/L, but not 80 mg/L. There were no significant differences in recovery times for the 40, 60, and 80 mg/L

Table 5. Experiment GC-3: Mean ($\pm$ SE) recovery times (min) for age-0 grass carp (20 fish for each concentration and brand combination) exposed to each brand of clove oil at concentrations of 40, 60, or 80 mg/L at 14.4 °C.

Brand	Concentrations	Recovery time	
		Mean	Standard error
Sigma ¹	40	3.58 a	0.34
NOW ²	40	3.91 a	0.34
Sigma	60	4.12 a	0.34
Sigma	80	4.62 ab	0.34
NOW	60	5.49 b	0.34
NOW	80	7.47 c	0.34

¹ NOW Foods, Glendale Heights, Illinois

² Sigma Chemical Company, St. Louis, Missouri

Table 6. Experiment GC-4: Mean ($\pm$ SE) induction and recovery times (min) for age-0 grass carp (20 fish for each temperature) exposed to clove oil at a concentration of 60 mg/L at two different temperatures. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Temperature (°C)	Induction time		Recovery time	
		Mean	Standard error	Mean	Standard error
60	14.4	1.63 a	0.07	4.12 a	0.27
60	25.6	1.45 a	0.07	1.84 b	0.08

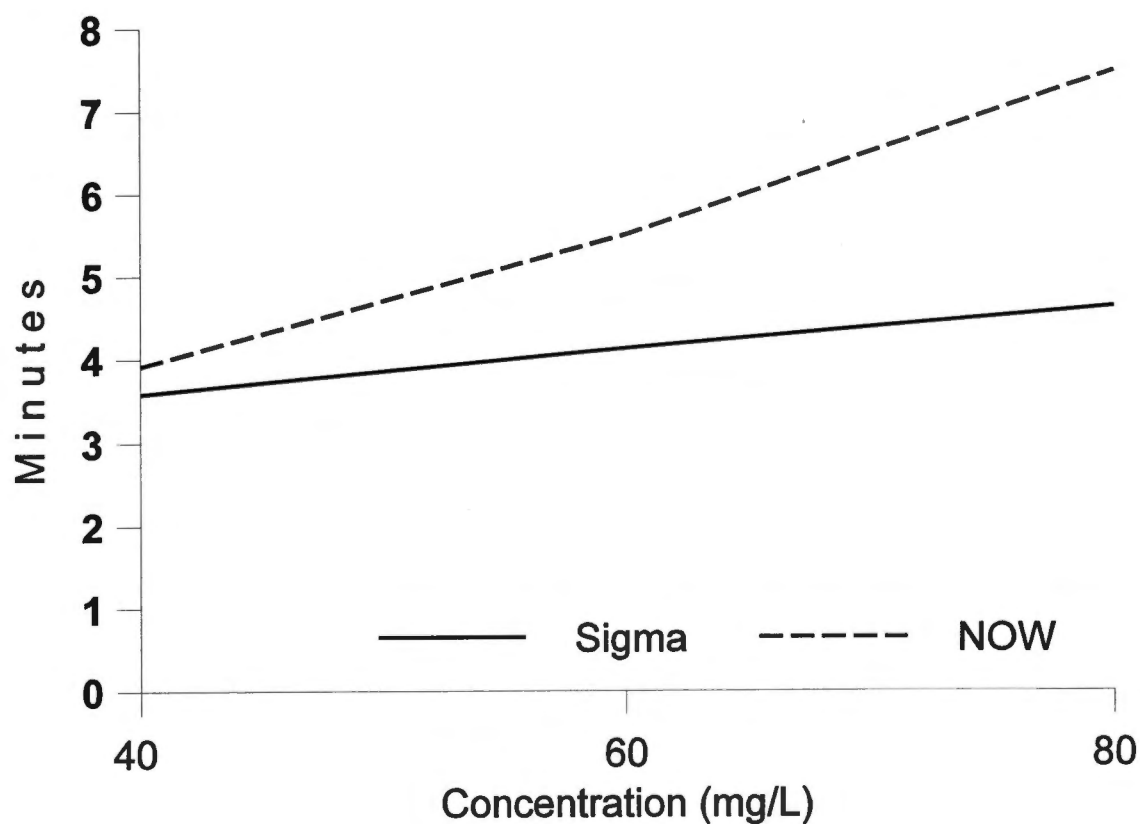


Figure 3. Experiment GC-3: Interaction of brand and concentration on recovery times of age-0 grass carp (20 fish for each concentration and brand combination) exposed to NOW or Sigma brand at 40, 60, or 80 mg/L.

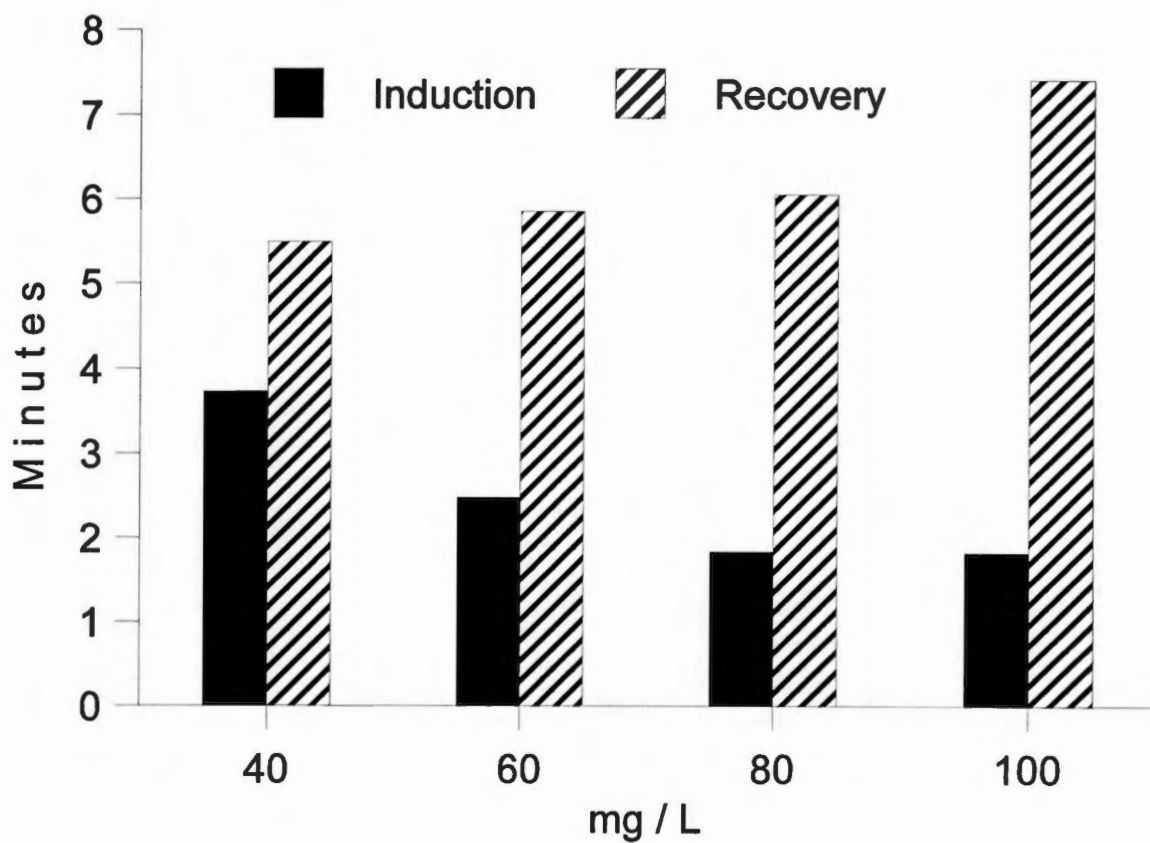


Figure 4. Experiment GC-5: Mean induction and recovery times for yearling grass carp (10 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 18.9° C.

Table 7. Experiment GC-5: Mean ($\pm$ SE) induction and recovery times (min) for yearling grass carp (10 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 18.9° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
40	3.73 a	0.20	5.49 a	0.44
60	2.48 b	0.14	5.85 ab	0.22
80	1.84 c	0.05	6.05 b	0.23
100	1.83 c	0.15	7.41 b	0.48

levels, but all had significantly faster times than 100 mg/L. Length as a covariate was found to have no effect on time for either induction ($P=0.1412$) or recovery ($P=0.7862$) within this size range, and was left out of the model. Although no concentration met the criterion for recovery, 60 mg/L was chosen as the lowest effective concentration because it was the lowest concentration that had an induction of less than 3 min and recovery time was not significantly longer than the time for 40 mg/L.

Experiment GC-6. The only opportunity to test the effect of length between different size ranges with the same concentration and the same temperature was between yearling and brood grass carp with 60 mg/L clove oil at 18.9° C. Length as a covariate had no effect on time for either induction ($P=0.1566$) or recovery ($P=0.0719$). The brood grass carp had a mean ($\pm$ SE) time of 2.75 (0.12) min for induction and 8.60 (0.66) min for recovery. While induction time falls within the criteria of efficacy, the recovery time falls well outside the criterion of five min.

Experiment GC-7. The percent mortality of age-0 grass carp exposed to the lowest effective concentration of clove oil (60 mg/L) in water temperatures of 14.4 and 20.0° C, are presented in Tables 8 and 9, respectively. For the lower temperature, exposures varied between 10 and 135 min; mortalities were first observed at 60 min. The calculated probabilities of mortality for this temperature and dosage level (Table 10) indicated an LCT value of 93.05 min. For the higher temperature, exposure ranged from 10 to 175 min; mortalities were first observed at 90 min. The calculated probabilities of mortality for this temperature and dosage level (Table 11) indicated an LCT value of 114.05 min. The predicted values of percent mortality for each temperature are illustrated

Table 8. Experiment GC-7: Percent mortality of age-0 grass carp exposed to clove oil at 60 mg/L at 14.4° C for various exposure times; N = 10 fish for each treatment.

Treatment	Number of survivors	Percent mortality
control	10	0
10 min	10	0
20 min	10	0
30 min	10	0
45 min	10	0
60 min	9	10
75 min	7	30
90 min	7	30
105 min	2	80
120 min	2	80
135 min	0	100

Table 9. Experiment GC-7: Percent mortality of age-0 grass carp exposed to clove oil at 60 mg/L at 20.0° C for various exposure times; N = 10 fish for each treatment.

Treatment	Number of survivors	Percent mortality
control	10	0
10 min	10	0
20 min	10	0
30 min	10	0
45 min	10	0
60 min	10	0
75 min	10	0
90 min	8	20
105 min	7	30
120 min	5	50
135 min	0	100
150 min	1	90
165 min	0	100

Table 10. Experiment GC-7: Probability of mortality of age-0 grass carp exposed to clove oil at 60 mg/L at 14.4° C.

Probability	Time	95% Fiducial Limits	
0.10	63.90	44.94	74.47
0.20	73.91	59.46	83.02
0.30	81.12	69.38	89.73
0.40	87.30	77.29	96.03
0.50	93.05	84.06	102.53
0.60	98.82	90.23	109.65
0.70	104.99	96.23	117.86
0.80	112.20	102.70	128.02
0.90	122.21	111.06	142.73

Table 11. Experiment GC-7: Probability of mortality of age-0 grass carp exposed to clove oil at 60 mg/L at 20.0° C.

Probability	Time	95% Fiducial Limits	
0.10	88.23	70.47	98.10
0.20	97.09	83.37	105.66
0.30	103.48	92.22	111.55
0.40	108.94	99.32	117.05
0.50	114.05	105.45	122.70
0.60	119.15	111.06	128.87
0.70	124.61	116.53	136.00
0.80	131.00	122.40	144.88
0.90	139.87	129.93	157.80

in Figure 5.

Experiment GC-8. Exposure times for yearling grass carp at the lowest effective concentration (60 mg/L) varied between 30 and 180 min at 18.9° C. No mortalities were observed until the 180 min exposure where one fish died. Therefore, it was not possible or necessary to calculate an LCT for yearling grass carp at this dose and temperature.

Striped Bass

Striped bass exposed to clove oil became very agitated. At all doses, the fish swam and thrashed violently until induction, when they very rapidly became very still. During recovery, fish came to the surface and began swimming with their heads at the surface of the water at a 45° angle before attaining a normal swimming motion.

Experiment SB-1. The means and standard errors for induction and recovery, respectively, for striped bass exposed to four different concentrations of clove oil at 25.6° C are presented in Figure 6 and Table 12. Fish exposed to 40 mg/L required significantly more time to reach induction than any of the other concentrations. No significant differences among mean induction times were found between the 60, 80, and 100 mg/L solutions. Fish exposed to 80 and 100 mg/L had a significantly longer recovery time than 40 and 60 mg/L. No significant differences were found among 80 and 100 mg/L or 40 and 60 mg/L. Length, considered as a covariate, did not have a significant relationship to time in this size range for either induction ($P=0.6582$) or recovery ($P=0.2542$), and was therefore taken out of the model. All concentrations met the criteria for both induction and recovery, but mortalities occurred in the 40 and 60 mg/L doses. All fish at those doses recovered, but eight fish died at 40 mg/L and two fish died at 60 mg/L; all

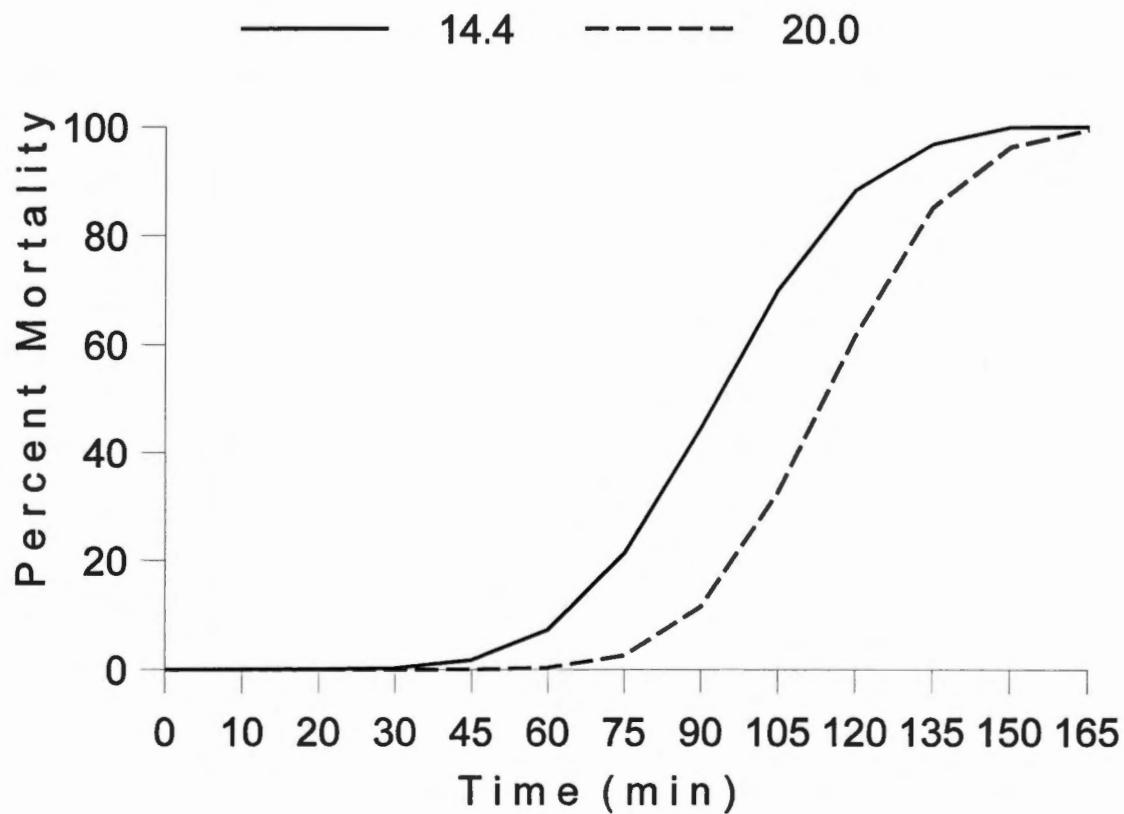


Figure 5. Experiment GC-7: Mortality curve for age-0 grass carp exposed to 60 mg/L of clove oil at 14.4 and 20.0° C.

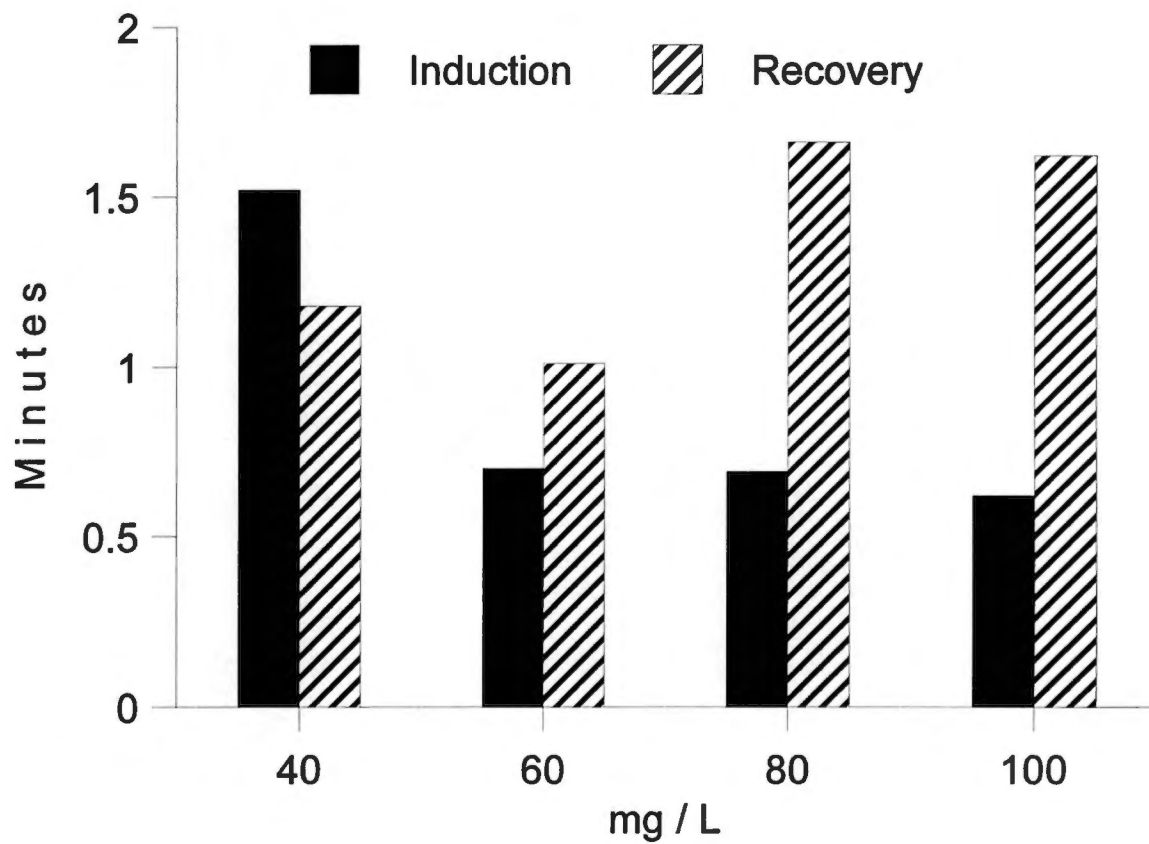


Figure 6. Experiment SB-1: Mean induction and recovery times for striped bass (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 25.6° C.

Table 12. Experiment SB-1: Mean ($\pm$ SE) induction and recovery times (min) for striped bass (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 25.6° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
40	1.52 a	0.06	1.18 a	0.09
60	0.70 b	0.03	1.01 a	0.04
80	0.69 b	0.03	1.66 b	0.13
100	0.62 b	0.03	1.62 b	0.13

mortalities occurred within 24 hours. Therefore, 80 mg/L was chosen as the lowest effective concentration because it was the lowest concentration at which no mortalities occurred.

Experiment SB-2. The percent mortality of striped bass exposed to the lowest effective concentration of clove oil (80 mg/L) in water temperatures of 25.6° C is given in Table 13. Exposures varied between 10 and 30 min with mortalities first being observed at 10 min. The LCT for a 80 mg/L dose was determined to be 8.51 min.

Largemouth Bass

These fish were one of the two species that had to be treated with formalin in the lab for gill parasites. They were allowed to acclimate for one week after the treatment before any tests were run. The largemouth bass remained fairly calm when exposed to clove oil, with less than half of the fish becoming agitated. Most fish swam around slowly towards the surface before losing equilibrium and slowly falling down to the bottom of the tank. Fish reached Stage 4 anesthesia soon after reaching the tank bottom. During recovery, fish also underwent repeated attempts at turning over before managing to right themselves. However, it took the fish a minimum of two times before they could remain upright and then begin to regain normal swimming motion.

Experiment LB-1. The means and standard errors for induction and recovery, respectively, for largemouth bass exposed to four different concentrations of clove oil at 22.2° C, are given in Figure 7 and Table 14. Fish exposed to 40 mg/L required significantly more time to reach induction than any of the other concentrations. No significant differences among mean induction times were found between the 60 and 100

Table 13. Experiment SB-2: Percent mortality of striped bass exposed to clove oil at 80 mg/L at 25.6° C for various exposure times; N = 10 fish for each treatment.

Treatment	Number of survivors	Percent mortality
control	10	0
10 min	2	80
20 min	1	90
30 min	0	100

Table 14. Experiment LB-1: Mean ($\pm$ SE) induction and recovery times (min) for largemouth bass (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 22.2° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
40	2.51 a	0.13	2.52 a	0.14
60	1.46 b	0.06	2.57 a	0.13
80	1.07 c	0.03	2.42 a	0.22
100	1.24 bc	0.05	2.85 a	0.20

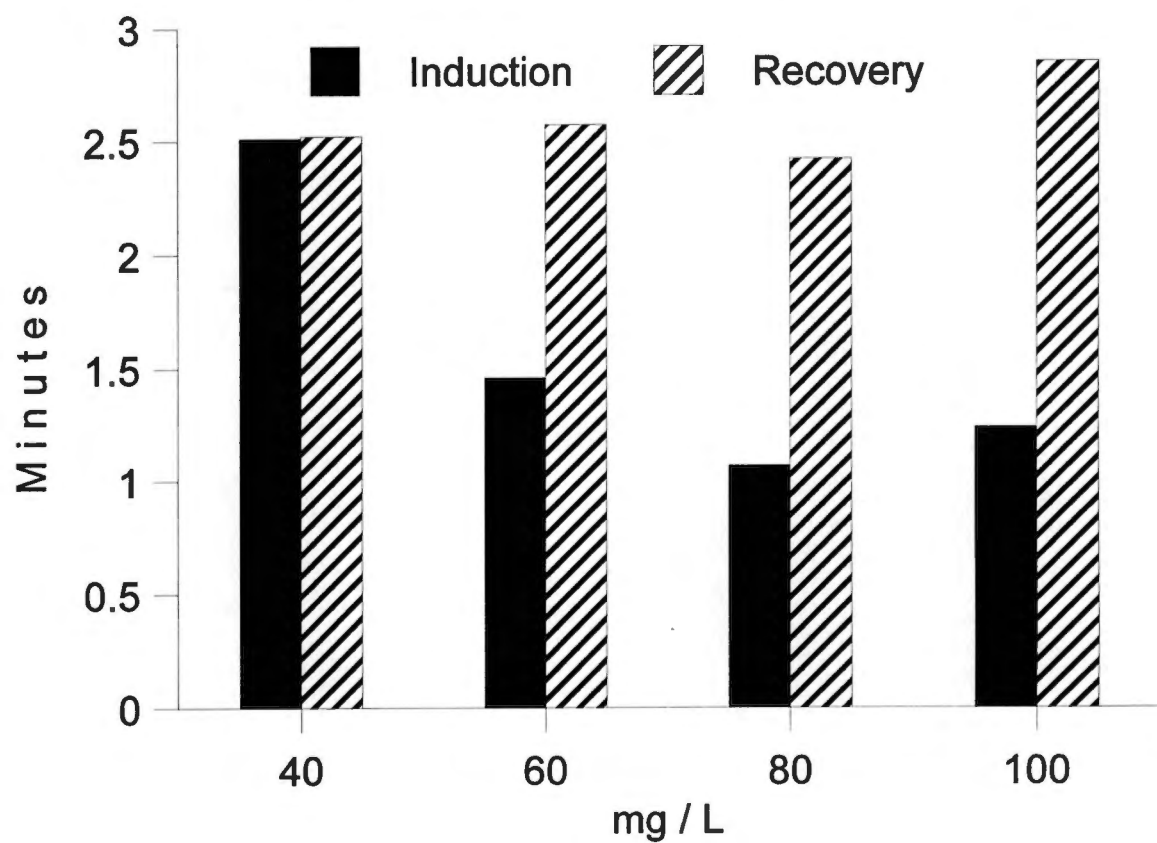


Figure 7. Experiment LB-1: Mean induction and recovery times for largemouth bass (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 22.2° C.

mg/L groups or the 80 and 100 mg/L groups. Interestingly, 60 mg/L had a significantly longer induction than 80 mg/L, but not 100 mg/L. No significant differences were found among any of the concentrations for recovery time, and all met the criterion of efficacy for recovery. Length, considered as a covariate, did not have a significant relationship to time in this size range for either induction ($P=0.1309$) or recovery ($P=0.5067$), and was therefore taken out of the model. All concentrations except for 40 mg/L met the criterion for induction; therefore, 60 mg/L was chosen as the lowest effective concentration. No mortalities were recorded in this experiment.

Experiment LB-2. Due to a shortage of fish available, a complete survival test was not run on largemouth bass. However, since part of the criteria of efficacy was no mortality of fish following 15 min of exposure to the effective concentration, four groups of 10 largemouth were exposed to 60 mg/L in times varying between 10 and 40 min. No mortalities were recorded, confirming 60 mg/L to be the lowest effective concentration.

Hybrid Striped Bass

The hybrid striped bass reacted similar to the striped bass, although whereas the striped bass became extremely agitated, the hybrids were only mildly agitated. When the hybrids reached Stage 4, they succumbed rapidly and the induction point was very easy to identify. Recovery for this species followed closely the stages of recovery as defined by Cho and Heath (2000).

Experiment HB-1. The means and standard errors for induction and recovery, respectively, for hybrid striped bass exposed to four different concentrations of clove oil at 21.1 ° C are presented in Figure 8 and Table 15. Fish exposed to 30 mg/L required

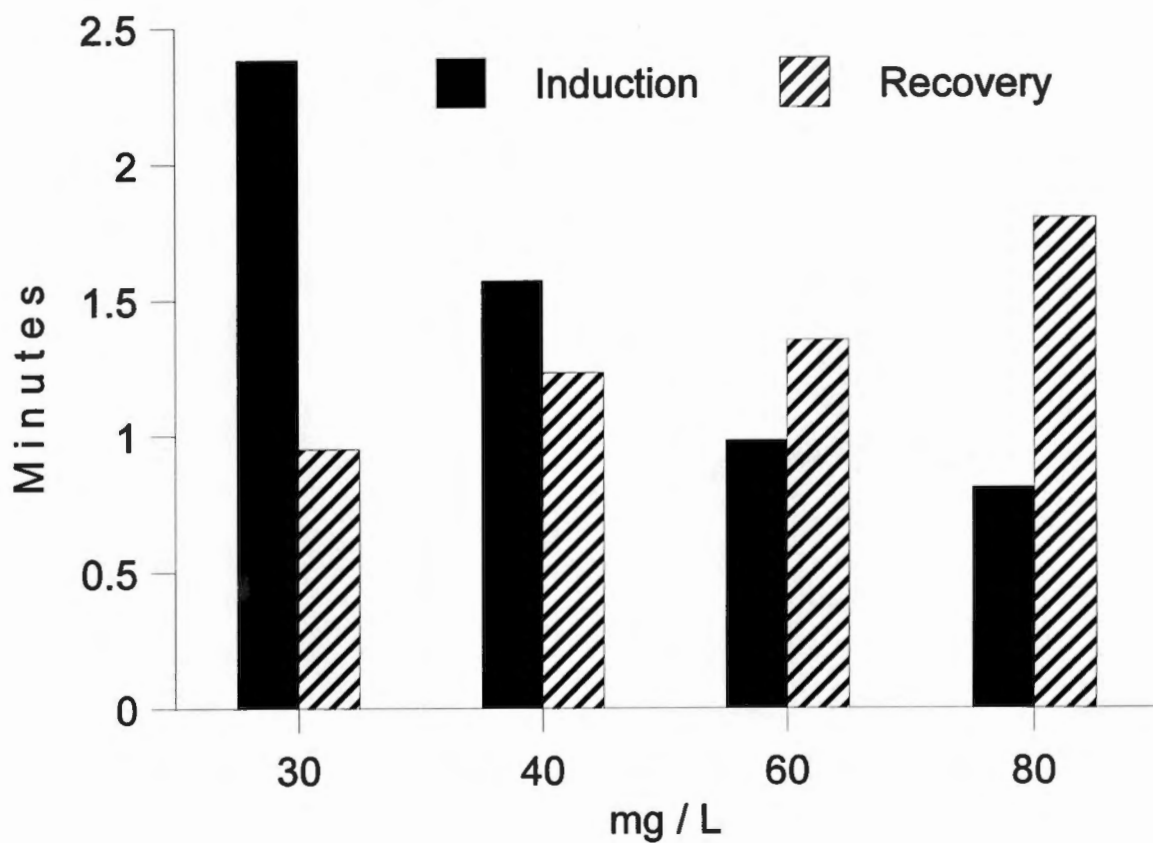


Figure 8. Experiment HB-1: Mean induction and recovery times for hybrid striped bass (20 fish per concentration) exposed to clove oil at concentrations of 30, 40, 60, and 80 mg/L at 21.1 ° C.

Table 15. Experiment HB-1: Mean ($\pm$ SE) induction and recovery times (min) for hybrid striped bass (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, 80, and 100 mg/L at 21.1 ° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
30	2.38 a	0.18	0.95 a	0.05
40	1.57 b	0.11	1.23 ab	0.09
60	0.98 c	0.03	1.35 b	0.05
80	0.81 c	0.03	1.80 c	0.13

significantly more time to reach induction than any of the other concentrations. Fish exposed to 40 mg/L required significantly more time to reach induction than those at 60 and 80 mg/L. No significant differences among mean induction times were found between the 60 and 80 mg/L solutions. Fish exposed to 80 mg/L had a significantly longer recovery time than all of the other concentrations. No significant differences were found among 40 and 60 mg/L or 30 and 40 mg/L, although recovery at 60 mg/L took significantly longer than recovery at 30 mg/L. Length, considered as a covariate, did not have a significant relationship to time in this size range for either induction ($P=0.7996$) or recovery ($P=0.9926$), and was therefore taken out of the model. All concentrations met the criterion for recovery, and all concentrations except for 30 mg/L met the criterion for induction. Therefore, 40 mg/L was chosen as the lowest effective concentration. No mortalities were recorded in this experiment.

Experiment HB-2. The percent mortality of hybrid striped bass exposed to the lowest effective concentration of clove oil (40 mg/L) in water temperatures of 21.1° C ranged from 0 to 100 percent (Table 16); exposures varied between 10 and 80 min. Mortalities first began at 50 min with the LCT for a 40 mg/L dose determined to be 57.18 min (Table 17). The predicted values of percent mortality are illustrated in Figure 9.

Black Crappie

The black crappie was the other species that required formalin treatment for gill parasites in the lab. They, too, were allowed to acclimate for one week after the treatment. The crappie remained very calm during exposure to the clove oil. However, they tended to remain upright until reaching Stage 4 anesthesia. This sometimes made it

Table 16. Experiment HB-2: Percent mortality of hybrid striped bass exposed to clove oil at 40 mg/L at 21.1° C for various exposure times; N = 10 fish for each treatment.

Treatment	Number of survivors	Percent mortality
control	10	0
10 min	10	0
20 min	10	0
30 min	10	0
40 min	10	0
50 min	5	50
60 min	5	50
70 min	2	80
80 min	0	100

Table 17. Experiment HB-2: Probability of mortality of hybrid striped bass exposed to clove oil at 40 mg/L at 21.1° C.

Probability	Time	95% Fiducial Limits	
0.10	42.20	29.78	48.35
0.20	47.34	37.95	52.65
0.30	51.05	43.53	56.05
0.40	54.22	47.96	59.29
0.50	57.18	51.73	62.69
0.60	60.14	55.09	66.50
0.70	63.31	58.30	70.97
0.80	67.01	61.67	76.59
0.90	72.15	65.93	84.77

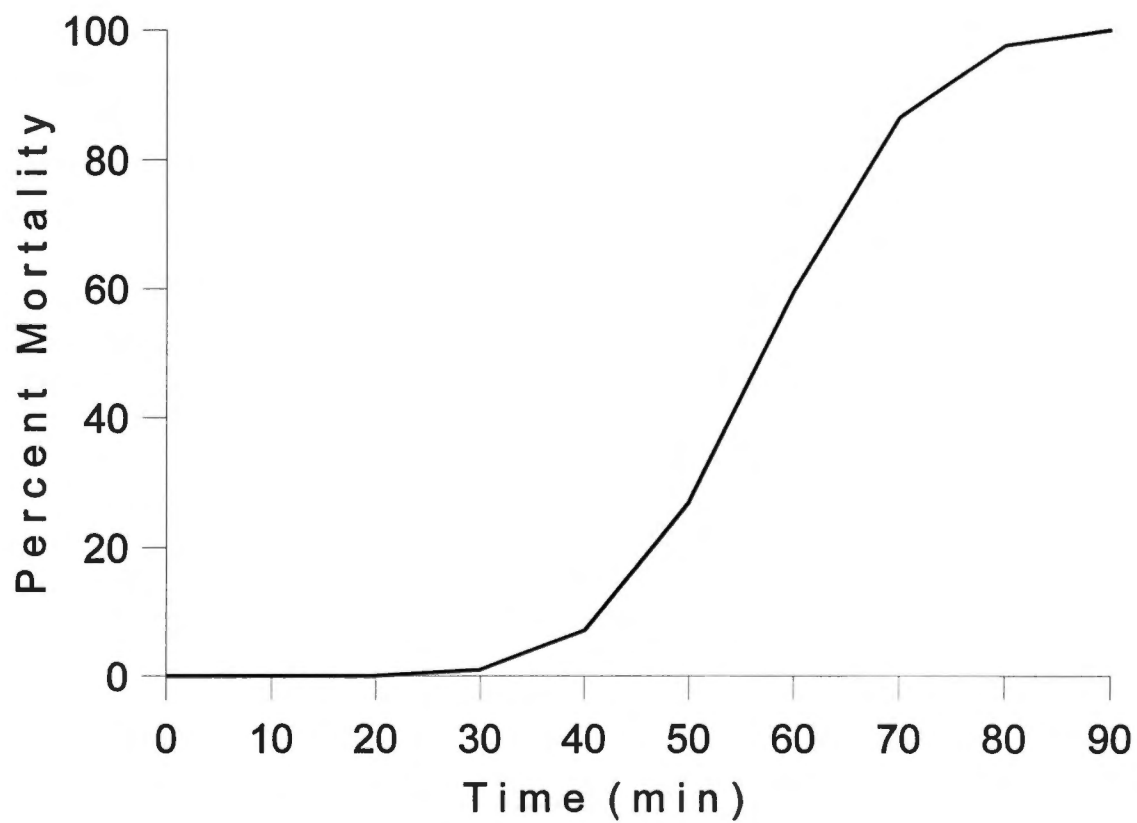


Figure 9. Experiment HB-2: Mortality curve for hybrid striped bass exposed to 40 mg/L of clove oil at 21.1° C.

very difficult to assess exact induction times. Recovery followed the normal progression, although it was not attained quickly. Fish regained equilibrium, and then after a short time, regained normal swimming motion.

Experiment BC-1. The means and standard errors for induction and recovery for black crappie exposed to three different concentrations of clove oil at 18.9° C are given in Figure 10 and Table 18. Fish exposed to 60 mg/L required significantly more time to reach induction than any of the other concentrations. No significant differences among mean induction times were found between the 80 and 100 mg/L solutions. Fish exposed to 100 mg/L had a significantly longer recovery time than those exposed at 60 mg/L. No significant differences were found among 60 and 80 mg/L or 80 and 100 mg/L. Length, considered as a covariate, did not have a significant relationship to time in this size range for induction ($P=0.1367$), and was therefore taken out of that model. However, length did have a significant relationship to time in recovery ($P=0.0408$), and was therefore left in that model. None of the concentrations met the criteria for induction or recovery. However, 80 mg/L was chosen as the lowest effective concentration because it was closer to 3 min and significantly faster in induction than 40 mg/L, and not significantly slower in recovery time. No mortalities were recorded in this experiment.

Experiment BC-2. The percent mortality of black crappie exposed to the lowest effective concentration of clove oil (80 mg/L) in a water temperature of 18.9° C is presented in Table 19; exposures varied between 10 and 60 min. Mortalities first began at 20 min, with the LCT value for a 40 mg/L dose determined to be 35.25 min (Table 20). The predicted values of percent mortality are shown in Figure 11.

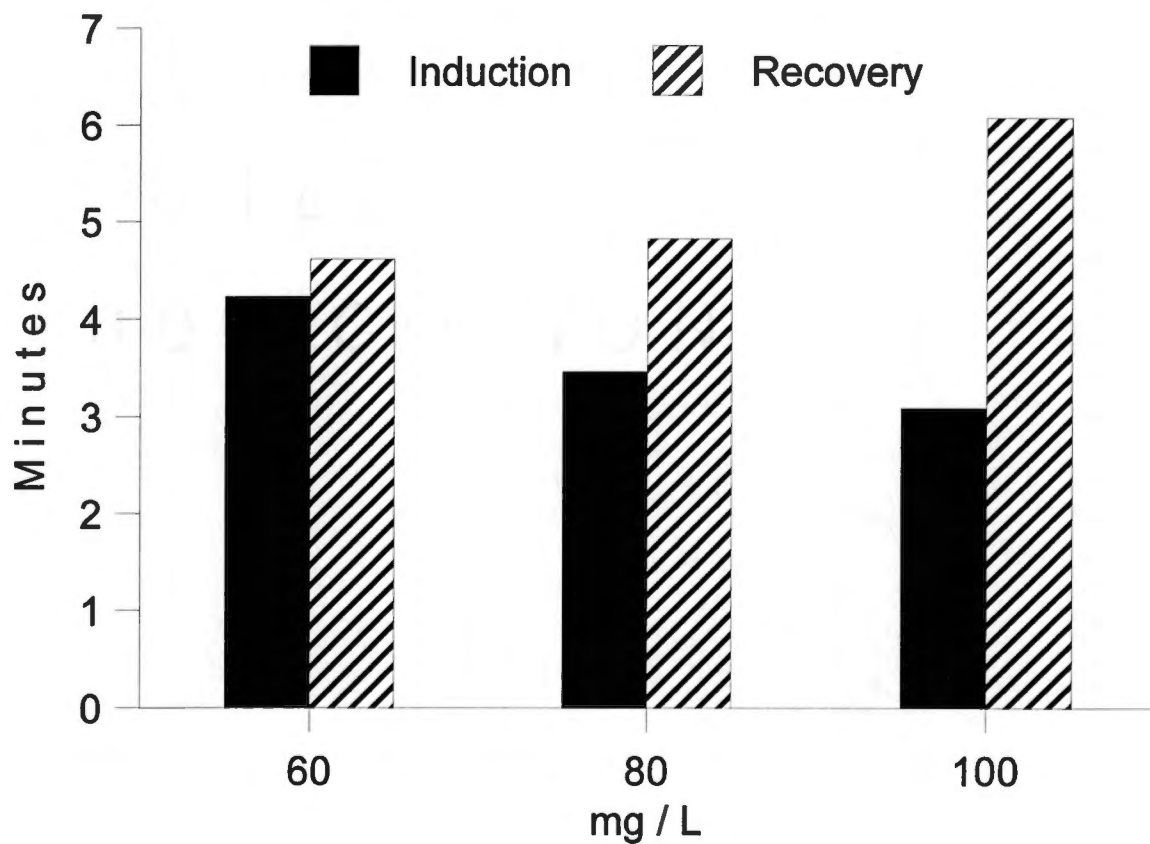


Figure 10. Experiment BC-1: Mean induction and recovery times for black crappie (20 fish per concentration) exposed to clove oil at concentrations of 60, 80, and 100 mg/L at 18.9° C.

Table 18. Experiment BC-1: Mean ($\pm$ SE) induction and recovery times (min) for black crappie (20 fish per concentration) exposed to clove oil at concentrations of 40, 60, and 80 mg/L at 18.9° C. Means followed by the same letter within a column were not significantly different ($P \geq 0.05$).

Concentration	Induction time		Recovery time	
	Mean	Standard error	Mean	Standard error
60	4.23 a	0.28	4.61 a	0.30
80	3.46 b	0.17	4.82 ab	0.42
100	3.09 b	0.16	6.07 b	0.44

Table 19. Experiment BC-2: Percent mortality of black crappie exposed to clove oil at 80 mg/L at 18.9° C for various exposure times; N = 10 fish for each treatment.

Treatment	Number of survivors	Percent mortality
control	10	0
10 min	10	0
20 min	7	30
30 min	5	50
40 min	5	50
50 min	3	70
60 min	0	100

Table 20. Experiment BC-2: Probability of mortality of black crappie exposed to clove oil at 80 mg/L at 18.9° C.

Probability	Time	95% Fiducial Limits	
0.10	14.10	-1.31	21.88
0.20	21.36	9.98	27.91
0.30	26.60	17.66	32.70
0.40	31.07	23.73	37.29
0.50	35.25	28.83	42.16
0.60	39.43	33.35	47.61
0.70	43.90	37.65	53.96
0.80	49.14	42.22	61.87
0.90	56.40	48.09	73.30

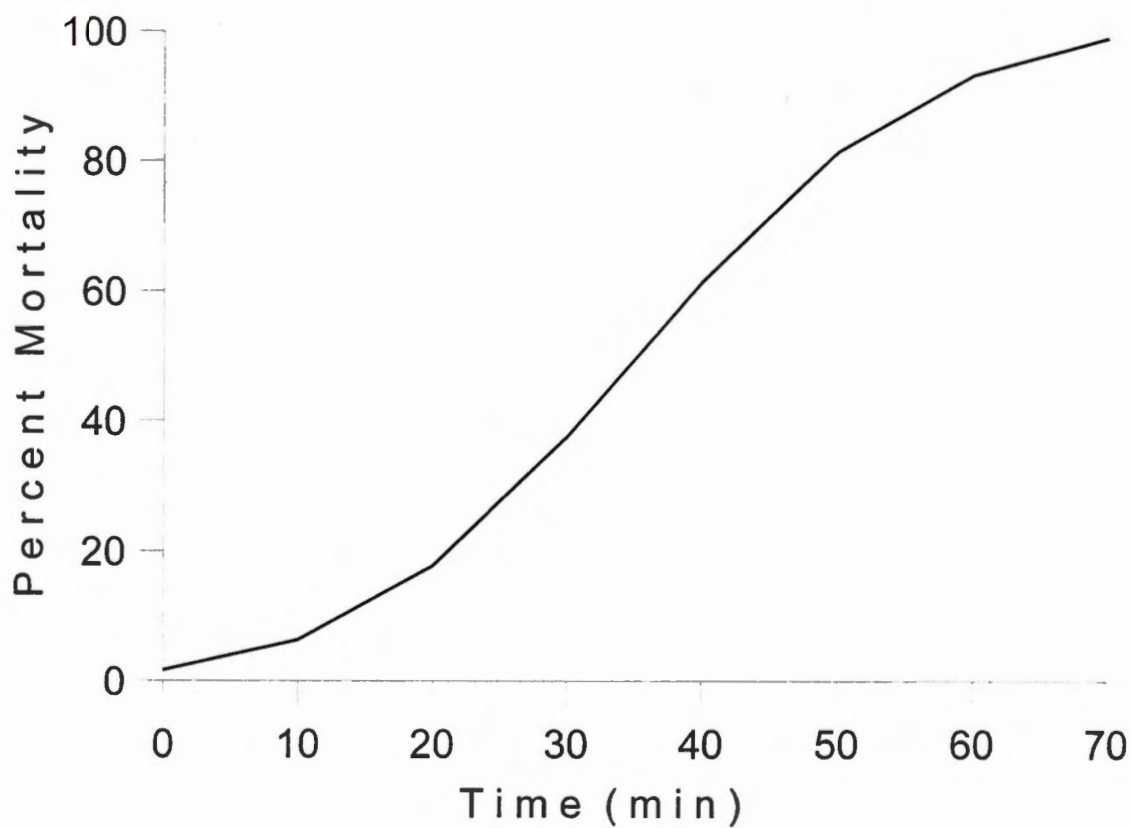


Figure 11. Experiment BC-2: Mortality curve for black crappie exposed to 80 mg/L of clove oil at 18.9° C.

CHAPTER V

DISCUSSION

Clove oil appeared to act as an anesthetic in the five species of fish studied. Increased concentrations of clove oil produced faster times to loss of equilibrium and longer times until recovery. This fact, along with the observance of fish progressing through the stages of anesthesia outlined by Plumb et al. (1983) suggested clove oil was indeed acting as an anesthetic (Keene et al. 1998).

Effective concentrations of clove oil may vary with different objectives. Therefore, results from all concentrations tested are reported to allow the users to select the concentration best suited for their needs. However, for the purposes of this study, effective concentration was determined by the guidelines presented in Marking and Meyer (1985). All concentrations meeting the criteria were then evaluated to choose the lowest (i.e., least expensive) concentration to control costs. Furthermore, the criteria for age-0 fish were made even more stringent due to the positive correlation of size and concentration found by Gilderhus (1989). Standards were not always met in every case, so the concentration closest to these standards were chosen. However, the third criterion, no mortality after a 15 min exposure, cannot realistically be compromised in aquaculture. Failing to meet this criterion should be an automatic rejection of that particular concentration.

The lowest effective concentrations of clove oil determined by this study ranged from 40-80 mg/L. These results were similar to those found in other nonsalmonid fish by Peake (1998) for walleye, smallmouth bass, northern pike, and lake sturgeon, and

Waterstrat (1999) for channel catfish. A comparison of brands of clove oils revealed no significant difference in induction, which was similar to the results produced by Taylor and Roberts (1999); however, there were significant differences in recovery times. Great care should be taken when using different brands of clove oil and relying on the results in this study.

Temperature was found to have a significant effect on recovery, but not on induction times. This result was similar to the findings of Lemm (1993) for striped bass exposed to MS-222, benzocaine, quinaldine, quinaldine sulfate, and metomidate. Length did not have a significant effect on induction or recovery times within any size range, except for recovery times of black crappie. Length was tested in yearling and brood grass carp, and not found to have an effect. However, there may have not been enough size differential in those two groups of fish to demonstrate a difference. Unfortunately, size comparisons of a larger difference were not able to be completed due to differences in temperature of tests with age-0 and yearling or brood stocks, but length could possibly be found to have a significant effect in that range of lengths.

Length did have an observable effect on survival. Taylor and Roberts (1999) determined that subadult white sturgeon had a greater LC50 (1000 mg/L) than juvenile white sturgeon (526 mg/L). This was also evidenced in this study, where age-0 grass carp had an LCT of 114.05 min at 20.0° C, while yearling grass carp suffered no mortalities for up to three hours at 60 mg/L.

Induction times for clove oil compared favorably well to induction times of other chemicals investigated in other studies (Booke et al. 1978; Lemm 1993; Keene et al.

1998). In some cases, recovery times of clove oil have been found to be significantly longer than for those of other chemicals. Munday and Wilson (1997) found that, on average, recovery time for *Pomacentrus amboinensis* after anesthesia with clove oil was two to three times longer than recovery time from other chemicals. Keene et al. (1998) reported that recovery times of rainbow trout from clove oil was significantly longer (6-10 times longer) than recovery times of MS-222. Similar results were reported by Peake (1998) for recovery from clove oil and sodium bicarbonate in walleye, lake sturgeon, smallmouth bass, and northern pike.

In contrast to these earlier studies, Waterstrat (1999) found that channel catfish exposed to 100 mg/L of clove oil did not exhibit the prolonged recovery time observed in rainbow trout (16 min), and there was no difference in recovery time between channel catfish exposed to clove oil (3.77 min) and those exposed to MS-222 (2.90 min). In that study, clove oil was not mixed with ethanol, but instead added directly to water and aerated. Longer recovery times may not prove to be a significant factor in some cases, since all fish that had longer recovery times in the present study survived.

The present study focused on concentrations of clove oil used for anesthetizing doses, while sedative doses were not taken into account. Sedative doses, such as those used in transporting fish, would obviously be lower than the anesthetizing doses. All mean values for induction and recovery times were determined from data using fish individually exposed to clove oil. However, while doing the prolonged exposure times, multiple fish were exposed at one time. During these experiments, it was noticed that fish in groups were reaching Stage 4 anesthesia much more quickly than they were

individually. This could possibly have been due to a higher concentration of CO₂ brought on by the respiration of the fish. This could be a factor in determining sedative or anesthetizing doses when using more than one fish at a time.

CHAPTER VI

SUMMARY

There is a recognized difference of anesthetic effects on different species. Fish react differently to anesthetic agents for unknown reasons. Therefore, mean induction and recovery times for all species are presented in Figure 12. The following is a breakdown of the effects of clove oil on each of the species:

1. *Grass carp*: Three size groups of grass carp were studied. Mean induction and recovery times for all sizes, concentrations, brands, and temperatures ranged from 1.08 (± 0.06) to 3.73 (± 0.20) and 1.84 (± 0.08) to 8.60 (± 0.66) min, respectively. It was observed that the larger brood and yearling fish took much longer to induce and recover from anesthesia than the smaller age-0 fish. Despite this, 60 mg/L appeared to be an effective dose across all size groups. Further tests could be run, however, to determine if a lower concentration could decrease the recovery time without increasing induction time significantly in the larger fish. It would appear from the results that any concentration above 80 mg/L would not have any effect on induction, but would increase recovery times. Grass carp can tolerate prolonged exposure to clove oil, especially when bigger, and they do not appear unreasonably stressed from the anesthetic. Therefore, grass carp would be an ideal candidate for clove oil use at any temperature.
2. *Striped Bass*: Induction and recovery times for striped bass ranged from 0.62 (± 0.03) to 1.52 (± 0.06) and 1.01 (± 0.04) to 1.66 (0.13) min, respectively, across

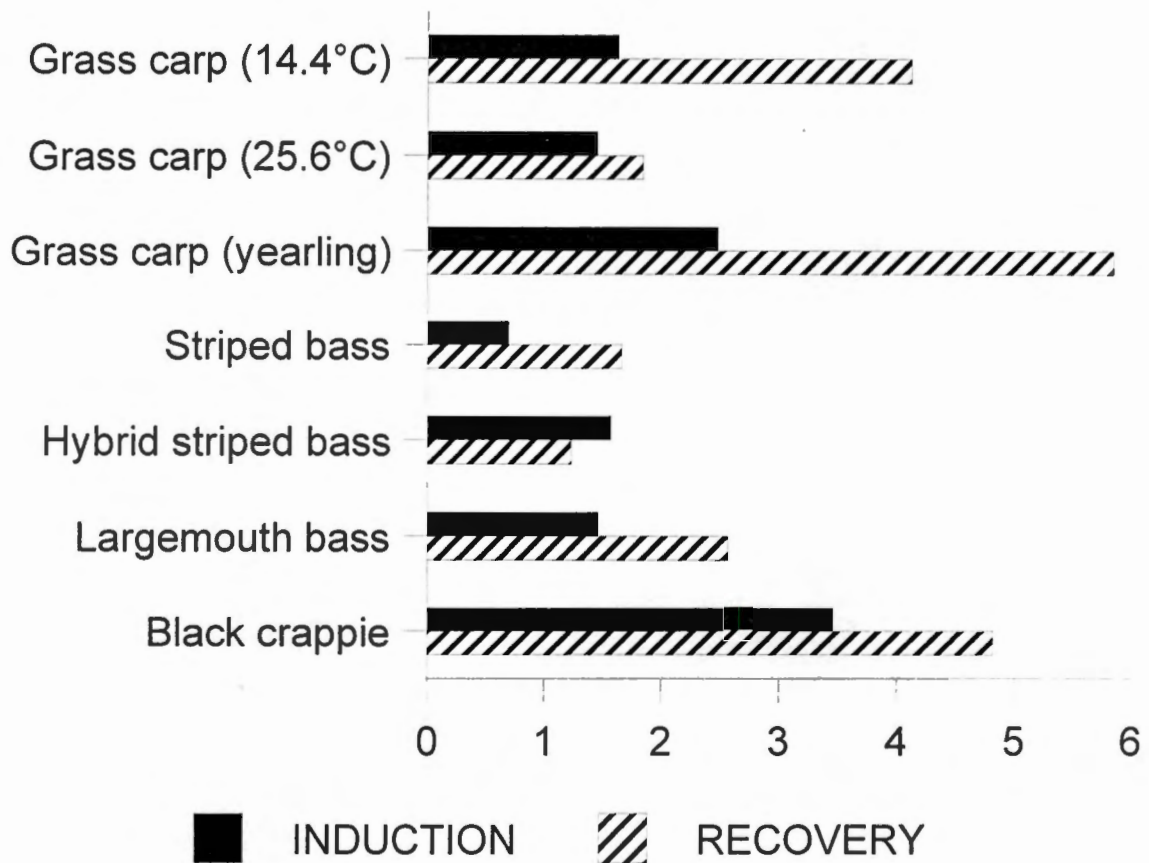


Figure 12. Mean induction and recovery times for all species at their respective effective concentrations and temperatures. Concentrations are: 40 mg/L for hybrid striped bass, 60 mg/L for grass carp and largemouth bass, and 80 mg/L for striped bass and black crappie.

all concentrations. Clove oil produced exceptionally quick induction and recovery times in striped bass. Unfortunately, delayed mortality (within 24 hours) occurred in this species. Eight fish died after recovering at 40 mg/L, and 2 fish died after recovering at 60 mg/L. Striped bass had the most agitated and violent reaction of all fish tested. They are known to be very intolerant of lactic acid buildup (Mike Smith, TWRA, personal communication), which could have been a contributing factor to mortality. The lower the clove oil concentration, the longer it took until induction, and the more time fish spent violently thrashing around in the tank. That could explain why mortality was greatest at 40 mg/L, since induction took significantly longer at that level. As a result, 80 mg/L was used in survival tests, and survival was still very low at that concentration. Based on results of this study, the use of clove oil would not be effective as an anesthetic at this temperature for striped bass.

3. *Largemouth Bass*: Induction and recovery for largemouth bass ranged from 1.07 (± 0.03) to 2.51 (± 0.13) and 2.42 (± 0.22) to 2.85 (± 0.20) min, respectively, across all concentrations. There were no significant differences found in any of the concentrations for recovery time, so fish could be given higher doses for quicker induction, with no substantially longer recovery times expected, if needed. Although no LCT was determined, no mortalities were found in exposures up to 40 min, which exceeds most requirements for anesthesia. Therefore, 60 mg/L of clove oil would be an effective concentration as an anesthetic for largemouth bass.
4. *Hybrid Striped Bass*: Induction and recovery for hybrid striped bass ranged from

0.81 (± 0.03) to 2.38 (± 0.18) and 0.95 (± 0.05) to 1.80 (± 0.13) min, respectively, across all concentrations. This species had the lowest effective concentration, 40 mg/L, with anything above 60 mg/L not producing a significantly faster induction time while at the same time increasing recovery times significantly. The overall times for hybrid striped bass were remarkably similar to those of striped bass, except that the hybrids were better able to survive prolonged exposure to the anesthetic. Therefore, 40 mg/L of clove oil would be an effective concentration as an anesthetic for hybrid striped bass.

5. *Black Crappie*: Induction and recovery for black crappie ranged from 3.09 (± 0.16) to 4.23 (± 0.28) and 4.61 (± 0.30) to 6.07 (± 0.44) min, respectively, across all concentrations. Although these times exceeded the criteria, they were not found to be too excessive due to the calm reaction of fish when being placed in the solution, and their survival times for prolonged exposure. Significant increases in induction time seemed to stop once the concentration reached 80 mg/L. These times far exceeded the times for largemouth bass at a similar temperature, highlighting the different species reactions to clove oil. Nonetheless, 80 mg/L of clove oil would be an effective concentration as an anesthetic for black crappie.
6. The low cost of clove oil is a big advantage in its use over other chemicals. In this study the cost of treating 1 L of water with clove oil at 50 mg/L was \$0.01. The cost of treating 1 L of water with MS-222 at 50 mg/L could reach as high as \$0.12 (both are costs for Sigma brands). A similar treatment with the NOW brand of clove oil would have been \$0.0083. This economic benefit, along with no need

for a withdrawal period for anesthetized fish, provides solid justification for clove oil as an ideal candidate for a fish anesthetic.

7. A final obstacle to overcome is to determine the physiological effects clove oil has on fish. In a study comparing the anesthetic effects of clove oil and MS-222 on physiology, Cho and Heath (2000) concluded that there appeared to be no adverse physiological repercussions in choosing clove oil over MS-222 to immobilize juvenile chinook salmon and possibly other salmonids. However, more work needs to be done with a wider variety of species to determine if clove oil does indeed cause any significant physiological changes in fish that would make it detrimental to use.

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LITERATURE CITED

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VITA

Randall T. Cooper was born 28 November 1972 in Albany, GA, where he attended both elementary and high school. Between those times, he attended middle school in Jeddah, Saudi Arabia. After graduating high school in 1991, he attended Mercer University for two years on a baseball scholarship before coming to the University of Tennessee, Knoxville, in 1993, where he graduated Magna Cum Laude in 1997 with a B.S. in Wildlife and Fisheries Science. In May of 2001, he received his Master of Science degree in Wildlife and Fisheries Science with a minor in Statistics from the University of Tennessee, Knoxville.

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