Determining and testing the optimal pure-tone frequency for use in acoustic conditioning of free-ranging common carp (*Cyprinus carpio*)

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By

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Abstract

Pure-tone frequencies of 400, 700, and 1000 Hz were tested to determine which achieved the strongest response when used in conditioning experiments on captive common carp (*Cyprinus carpio*) for the purpose of determining the most effective frequency to use in conditioning experiments on free-ranging common carp. Captive common carp were shown to have the strongest response to the 400 Hz frequency after training that associated sound with food. Response was quantified as moving into a feeding arena when the sound was broadcast, and all frequencies achieved conditioning within five days. Secondly, the attenuation of the three frequencies were compared in a Waikato lake (Lake Kaituna) containing common carp. The ability to maintain signal strength over distance is necessary for frequencies used in the acoustic conditioning of free-ranging common carp due to the potentially large distances involved. Attenuation of the 400 Hz and 700 Hz pure tone frequencies were not significantly different but were both lower than that of the 1000 Hz frequency. The 400 Hz pure tone frequency was used in subsequent experiments on the acoustic conditioning of free-ranging common carp in Lake Kaituna because of superior performance in acoustic conditioning of captive common carp and low attenuation in a natural water body containing free-ranging common carp. Thirdly, the 400 Hz pure tone was evaluated for its ability to be associated with food by free-ranging common carp. Capture rates at treatment sites where 400 Hz pure tone was broadcast compared to control sites where no sound was broadcast following a five-day training phase and 24 h of no sound or food, were 2.1 times greater during a three-day capture phase. This study demonstrates the trainability of common carp, a trait that can be used to improve control of wild common carp populations.

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1 Introduction

1.1 Animal training

The ability of animals to associate a stimulus with a learnt response provides the basis for humans to influence animals' behaviour (Thorndike, 1998). Examples of deliberate training of animals are numerous: for transport (horses, *Equus caballus*), work (oxen, *Bos primigenius* etc.) and hunting (dogs, *Canis lupus*) are a few of the areas in which the ability to train animals has been of benefit to humans (Pearson and Dijkman, 1994, Wen, 1993). Influencing the behaviour of animals to encourage them to congregate has proven useful in the harvesting of non-domesticated animal stocks (Solarin and Udolisa, 1993, Samples and Sproul, 1985). Fisheries, in particular, can benefit due to the difficulty of locating free-ranging populations. The attraction of wild fish into large aggregations has been achieved for certain species such as yellow perch (*Perca flavescens*), using visual cues (light); this method is also successfully used in commercial fishing operations (Manci et al., 1983). Floating objects (e.g. flat plastic sheets) have also been shown to attract fish; mature bluestriped chub (*Sectator ocyurus)* appeared at the structures within 20 days and tagged individuals remained at objects for up to a month (Hunter and Mitchell, 1968). Efforts to influence the behaviour of fish species by means other than triggering an innate response to a stimulus have been concentrated largely to studies on captive subjects (Zion et al., 2007, Zion et al., 2010, Levin and Levin, 1994, Chase, 2001, Willis et al., 2002). More recent efforts have been focused on training captive subjects to respond to a stimulus with the intention of achieving recall post release (Wahlberg, 1999, Zion et al., 2011a, Willis et al., 2002). For example, tilapia *(Oreochromis mossambicus)*, blue acara *(Aequidens pilcher)* and mojarra *(Caquetaia kraussii)* have all been successfully trained in an artificial ponds to congregate at the source of an audio broadcast (Levin and Levin, 1994); this training resulted in 35% of the population congregating at the sound source within 12 days. These examples show that fish species are receptive to sound, and respond to appropriate stimuli.

1.1.1 Audio capability of the species *Cyprinus carpio*

Sound has been identified as a possible lure for fish due to their wide-spread auditory abilities. In addition, audio lures may be more effective in flowing or turbid waters where olfactory and visual cues may not reach subjects (Kojima et al., 2005, Dingler et al., 1977). Common carp (*Cyprinus carpio*) is an international pest fish species that, due to their environmental impact and ability to withstand removal efforts, present a valid candidate for the development of an audio lure. Furthermore, this species' auditory abilities also support its role in audio lure development research. The common carp is a teleost fish which is able to hear due to otoliths in its head that move slower than the rest of the fish's body in response to sound waves; the difference in movement is detected by cilia and interpreted by the brain as sound (Wahlberg, 1999). Common carp are classified as otophysans, a group of fish that has enhanced hearing due to the inner ear and swim bladder being physically coupled by Weberian ossicles, giving this group the greatest sensitivity to the widest range of sounds of any fish (Chase, 2001). Common carp have been shown to be most sensitive to frequencies surrounding 505 Hz by auditory brainstem response (ABR) monitoring, and 1000 Hz by electrocardiogram (ECG) monitoring of fish after conditioning with electric shock (Kojima et al., 2005). common carp have the ability to discriminate between sounds of different frequency, and have been able to discriminate between recordings of blues and classical music (Chase, 2001).

1.2 Domestication of the common carp

Common carp (*Cyprinus carpio*: Cyprinidae) originate from the surrounds of the Black, Caspian and Aral seas, and has a large natural range spreading from Europe through to South East Asia (Mabuchi et al., 2005, Balon, 1995). The species has a long history of domestication for use as a food source and for aesthetics, with evidence suggesting that ancient Rome was the first culture to keep the species in captivity (Balon, 1995). Today the species is one of the most commonly cultured fish worldwide (Mabuchi et al., 2005), despite the fact that they are also a major pest species. The natural colour of common carp in their European range is olive grey.

The time at which other colour variations in the species first appeared, with various combinations of orange, white, and black, is uncertain, with possible occurrences in early European, Chinese and Japanese history (Mabuchi et al., 2005, Wang and Li, 2004, Balon, 1995). Bright and multi-coloured specimens of common carp were intentionally developed in Japan through artificial selection from the 19th century (Wang and Li, 2004), with rapid development of the strain in the last 100 years (Mabuchi et al., 2005, Balon, 1995). It is widely believed that the coloured variants were bred from semi-domesticated and pond populations, with genetic input from imported strains of the species (Wang and Li, 2004, Mabuchi et al., 2005). Other researchers, however, suggest that they were bred directly from wild colour mutants found in central Asia (Hulata, 1995). The brightly coloured strain was bred for aesthetic purposes as opposed to food, and is commonly referred to as “koi carp”, or in Japan, simply koi. The popularity of this new strain of common carp spread from Japan and led to the species becoming more financially significant as an ornamental fish, rather than as a food source (Balon, 1995).

1.3 Biology and management of carp

1.3.1 Biology and ecology of common carp

Common carp exhibit schooling behaviour and have an omnivorous diet (Koehn, 2004, Wright and Eastcott, 1982). The species is long lived, with individuals living greater than 20 years (Tlusty et al., 2008). A study of a large wild population of common carp in Australia found the median fork length and weight at maturity to be 307 mm, 584 g for males, and 328 mm, 688 g for females (Thorp, 1967). Populations of common carp can reach biomasses up to 3,144 kg ha^{-1} and densities up to 1,000 fish ha^{-1} (Koehn, 2004). The species can tolerate temperatures from 2 to 41^{o}C, salinities as high as 14‰, and survive a pH range of 5.0 to 10.5 (Koehn, 2004, Wright and Eastcott, 1982, Zion et al., 2011b). Common carp have a high tolerance to waters with low oxygen, surviving levels as low as 7% (Zion et al., 2011b).

Sexual maturity is reached early, with males and females reaching maturity at one and two years respectively in Australia (Koehn, 2004). The sex ratio of males to females varies amongst populations (Wright and Eastcott, 1982, Yan and Popper,

1991) and has been recorded at as high as 1.8:1 (males to females) (Yan and Popper, 1991). Spawning generally occurs in spring and summer but can also occur in autumn and winter (Chapman and Johnstone, 1974, Yan and Popper, 1991). Spawning in common carp in America did not commence until temperatures reached 17^{o}C, was optimal from 19 to 23^{o}C, and stopped ≥28^{o}C (Yan and Popper, 1991). The species has high fecundity; with females estimated to produce 163,000 eggs kg^{-1} of body weight (Chapman and Johnstone, 1974) and individual egg counts recorded of up to two million (Koehn, 2004). In New Zealand, koi have a fecundity of mean of 97 200 (±35 000 SD) oocytes kg^{-1} (Tempero et al. 2006). The fecundity of females and the size of eggs produced is strongly correlated with fish length and weight, but not age (Chapman and Johnstone, 1974). Reproductive success is further enhanced because females are capable of spawning repeatedly in a spawning season, over multiple seasons, and eggs are able to hatch after two days in ideal conditions (Chapman and Johnstone, 1974, Koehn, 2004).

1.3.2 Common carp in New Zealand

In New Zealand, the main variety of common carp present is commonly referred to as koi carp. This variant of the species is brightly coloured and is similar to others found globally, referred to by a variety of names (e.g. common carp, carp, European carp). Common carp were introduced into New Zealand in the 1960s. This occurred despite of concerns over the potential environmental consequences of their introduction, dating back as far as the 1850s in the United States (McCrimmon, 1968). New Zealand lakes and rivers lacked in predation pressure for the introduced koi, as large piscivorous fish species were not present (Rowe, 2007), and native predators, eels (*Anguilla* spp.), do not prevent establishment, possibly due to the high fecundity of common carp (Koehn, 2004, Rowe, 2007). Common carp grow quickly in New Zealand, reaching 165-280 mm in 190 days (McDowall, 1996), and have high fecundity, with some females capable of spawning multiple times per season as well as over multiple seasons (Tempero et al., 2006).

Common carp populations exist throughout the lower Auckland and Waikato regions of the North Island of New Zealand, as well as in smaller populations outside these regions, and have the potential for further spread in the North Island, as many water ways present habitable conditions (McDowall, 1996). The species has been spread intentionally and illegally in New Zealand to establish angling fisheries, and has been able to invade new water bodies through connecting wetlands and tributaries (Koehn, 2004, Rowe, 2007). A pest fish survey carried out by DOC in the South Island between September 2000 and March 2001 found that koi were present in the Nelson/Malborough region (Dean, 2003). This illustrates that the range of the species has potential for further spread in New Zealand and that the mechanisms exist to facilitate such spread.

1.3.3 Effects of common carp on the environment

Common carp have been linked to the degradation of water quality when they exist at high densities (Breukelaar et al., 1994, Bajer et al., 2009). A significant positive relationship has been found between common carp biomass and the level of suspended solids in experimental ponds (Breukelaar et al., 1994, Roberts et al., 1995), with increased sediment resuspension resulting from their benthic feeding behaviour. Their benthivorous feeding behaviour also affects nutrient levels in the water column as nutrients from the benthic zone are moved into the pelagic (Breukelaar et al., 1994, Roberts et al., 1995). As a result of their feeding behaviour, common carp can directly increase nutrient levels in lake waters by sediment resuspension and by excretion (Roberts et al., 1995, Bajer et al., 2009).

Although common carp sometimes directly forage on plant species, the main impact they have on vegetation is dislodgment during feeding (Rowe, 2007, Roberts et al., 1995). Such feeding behaviour by koi can reduce macrophyte cover, exposing sediments to higher levels of disturbance by common carp, other fish species, and wind disturbance (Rowe, 2007). Macrophyte damage has been recorded when common carp densities have exceeded 100 kg ha^{-1} (Bajer et al., 2009). The resulting increased sediment resuspension allows for nutrients previously stored in sediment to become available in the water column and increases turbidity (Breukelaar et al., 1994, Roberts et al., 1995). The increase in turbidity in the water column lowers light penetration and affects temperature as particles absorb radiation further degrading habitat for macrophytes (Roberts et al., 1995). This increase in pelagic nutrients coupled with macrophyte reduction can promote algal growth, which is further aided by predation on algal grazing cladocerans by juvenile common carp (Pinto et al., 2005). This results in the promotion of algal blooms and the eutrophication of water ways (Rowe, 2007, Breukelaar et al., 1994, Miller and Crowl, 2006). Common carp may also have direct effects on other species through predation on native species and their eggs and larvae, and through competition (Breukelaar et al., 1994, Harris, 1996).

1.3.4 Management of common carp populations

Reducing common carp populations is logistically difficult and costly, and there are currently a limited number of viable removal methods available to wildlife managers. Current removal methods, such as netting or applying rotenone to the water body, are time consuming, expensive, and involve losses to non-target species (Miller and Crowl, 2006). More recent methods involving electrofishing reduce non-target by catch but are still labour intensive and may not have a significant impact on the abundance of the pest fish population e.g., goldfish (*Carassius auratus*) (Hicks and Brijs, 2009). Common carp are able to evade capture by existing methods due to their behaviour, and as a result gill netting operations need to be performed at night to maximise catch rates. Common carp are able to take refuge in areas inaccessible to electrofishing (Pinto et al., 2005). Large areas of habitat remain as refuge to seine netting because logs, vegetation and other debris prevent the method (Zion et al., 2011a). Capture methods that involve manipulating or exploiting the behaviour of common carp are desirable if they can be used to attract common carp from areas of refuge.

Despite the difficulties encountered during removal programmes, increases in water transparency and decreases in cyanobacterial blooms have been recorded over the course of common carp removal operations in south east Australia (Pinto et al., 2005). Environmental improvement as a result of common carp removal is achievable, although methods and techniques that reduce the effort needed and increase catch rates and species accuracy are required.

1.3.5 The use of audio lures as a management tool

A method of luring common carp into high density aggregations for subsequent removal or poisoning would reduce the need to actively pursue the target species and limit non-target by-catch. Furthermore, the use of audio lures could potentially attract common carp from areas that are inaccessible using current methods. Captive common carp have previously been trained to move to a feeding area in response to auditory cues (Zion et al., 2007). This study used classical conditioning to train common carp to associate food with a 400 Hz pure-pulsed sound, although comparisons of the effectiveness of different frequencies were not investigated. Recorded feeding sounds of common carp have also been successfully used as an audio attractant and free-ranging subjects responded quickly by moving to within metres of the broadcasting speaker when these sounds were played (Hashimoto and Maniwa, 1966). Classical conditioning studies, however, have not been performed on free-ranging common carp. Recent research has shown, however, that common carp become acoustically conditioned during reinforcement feeding of captive trained fish released into a reservoir, and respond well to capture achieved through acoustic recall (Zion et al., 2011a). In addition, the ability of free-ranging common carp to expand their home range in order to exploit novel food sources, as well as their ability to remember where food rewards were delivered, has been demonstrated (Bajer and Sorensen, 2010). The attenuation rates of possible lure frequencies in natural water bodies containing common carp is currently unknown, as it is likely that sediment boundaries, as well as temperature and depth variations may affect how different frequencies travel and degrade over distance. The success of classical conditioning on captive common carp, coupled the learning capabilities of free-ranging specimens, supports the feasibility of using classical conditioning to train free-ranging subjects to respond to a broadcast sound by gathering in large aggregations at, or visiting areas where, the sound is broadcast.

1.4 Experiments into the response of carp to sound

1.4.1 Response of common carp to pure-tone frequencies

In order to increase attraction rates of common carp in removal operations, an audio lure is being developed which common carp will be conditioned to associate with a food reward. Common carp have specialised hearing (Chase, 2001), and classical conditioning experiments performed on captive common carp have had success using sound frequencies of 400 Hz (Zion et al., 2007). Studies have also shown that common carp are most sensitive to frequencies surrounding 505 Hz by auditory brainstem response (ABR) monitoring, and 1000 Hz by electrocardiogram (ECG) monitoring of fish after conditioning with electric shock (Kojima et al., 2005).

Previous classical conditioning work on common carp and other fish species in captivity has shown positive results to the conditioning in fewer than ten days (Willis et al., 2002, Zion et al., 2007). More complex conditioning based on the differentiation of two acoustic signals has also been achieved with common carp, with positive results achieved after 14 days (Zion et al., 2010). The use of previously trained teacher fish to aid in the conditioning of naive common carp to an acoustic signal was also studied by Zion et al. (2007) yielding positive results. An experiment performed by Hashimoto & Maniwa (1966) found that recorded common carp feeding sounds broadcast in a pond caused naive common carp to responded instinctively by moving to the point of broadcast. A more recent study found no advantage to the use of recorded feeding sounds when broadcast to grass carp however, with frequencies between 600 Hz and 1000 Hz achieving better results in conditioning experiments (Willis et al., 2002).

1.4.2 Attenuation of pure-tone frequencies in a Waikato lake

Although common carp have specialised hearing (Chase, 2001), any audio lure used will experience attenuation (reduction in dB level over distance). The ability to maintain signal strength over distance is a quality that is desired in an audio lure in order to be effective over as much of a water body as possible. Initial studies on the attenuation rates of low frequency sounds in freshwater were measured over several hundreds of kilometres in lakes over 100 m deep, and only attempted differentiation down to 800 Hz (Browning et al., 1972, Browning et al., 1968). The aim of this early work was to compare sound attenuation between marine and freshwater bodies in order to determine the effect that different ions have on sound transmission. Attenuations for some frequencies were found to differ by a factor of 10 between sea water and freshwater; the difference believed to be due to large concentrations of ions such as magnesium and boron in sea water compared to freshwater (Vadov, 2000, Browning et al., 1972).

More recent work concentrating on frequencies from 30 Hz to 1000 Hz in freshwater as shallow as 0.5 m was aimed at discovering the communication limitations of individual species (Crawford et al., 1997, Johnston and Johnston, 2000, Lugli and Fine, 2003, Mann and Lobel, 1997). Although the sounds studied dissipated within 0.5-10 m of their source due to a low initial SPL, the role of the frequency cut-off phenomena was explored, which explained unexpectedly high attenuation in low frequencies in shallow water. The phenomena is defined in waters with a rigid bottom, where frequencies that have a wavelength longer than or equal to four times the water depth experience increased attenuation or will not propagate (Mann and Lobel, 1997, Lugli and Fine, 2003). This phenomena also occurs in waters where a soft bottom is present, as the water surface and the soft bottom still provide two parallel barriers to sound propagation that limit the size of sound waves (Forrest, 1994). The limitation to propagation imposed by the soft sediment boundary is not as complete as with rigid bottoms however, allowing differing levels of propagation beyond expected cut-off frequencies depending on the level of absorption, as opposed to reflection, provided by the boundary (Lugli and Fine, 2003).

The attenuation rates of possible lure frequencies in natural water bodies containing common carp is currently unknown, as it is likely that sediment boundaries, as well as temperature and depth variations will affect how different frequencies travel and degrade over distance. If the lake water were homogenous and the effects of lake depth and sediment boundaries were not influential, an attenuation coefficient could be calculated for each frequency (Thorp, 1967, Culjat et al., 2010). Because the lake's water and sediment boundaries are heterogeneous and depth and temperature vary, the attenuation of each frequency through the lake water is best determined by standardised broadcast and measurement of each frequency over distance.

1.4.3 Acoustic conditioning of free-ranging common carp

To increase attraction rates of common carp in removal operations we are developing an audio lure; common carp will be conditioned to associate sound with a food reward. The ability to train captive common carp through classical conditioning to associate a food reward with an acoustic signal has been established (Zion et al., 2007, Zion et al., 2010). Apart from inadvertent training however (during reinforcement feedings of released captive trained fish) (Zion et al., 2011a), the ability to acoustically condition free-ranging common carp has not been demonstrated. The limitations imposed on captive subjects include free movement, patchy food availability, interaction with other fish, and inadvertent exposure to audio signals do not apply to free-ranging specimens. Consequently the ability to acoustically condition free-ranging common carp requires investigation.

The ability to train free-ranging common carp to associate a sound with a food reward could improve removal operations because sound to travels long distances through water with low visibility and where olfactory signals may not be received. Once a conditioned response to the broadcast sound has been developed, the sound could be broadcast in one or more locations in the water body where removal methods could be concentrated. The time and effort required for removal operations could be reduced, with no impact on non-target species. The ability of common carp to evade capture and to take refuge in areas inaccessible to fishing effort identified in Pinto et al. (2005) and Zion et al. (2011a) could be diminished if common carp were drawn to more suitable removal areas by broadcast sound. The 400 Hz pure-tone frequency was identified as having the fastest response time by captive trained common carp (captive common carp experiments) and had the lowest attenuation in lakes likely to contain common carp in the Waikato (equal to that of the 700 Hz frequency) (pure-tone attenuation experiment). As a result, 400 Hz was determined to be the most suitable pure-tone frequency to be used for acoustic conditioning free-ranging common carp.

1.4.4 Overview and objectives

Determining which pure-tone frequency is most suitable for use in the acoustic conditioning of free-ranging common carp in regards to response by captive common carp, and attenuation in shallow natural water bodies commonly containing common carp, forms the basis for the first two sections of this thesis. The third section tested the effectiveness of the frequency that best met these criteria through its use in acoustic conditioning experiments on free-ranging common carp. It is intended that the knowledge gained therein will assist in the removal and management of common carp populations.

The objectives of this research were to determine:

1) Whether captive common carp have a stronger response to 400, 700 or 1000 Hz pure-tone frequencies in regard to being trained to associate that frequency with food by moving into a feeding arena when that sound is broadcast, and the time taken for the conditioning to be achieved.

2) Which pure-tone sound frequency out of 400, 700 and 1000 Hz has the lowest attenuation when broadcast in shallow lakes likely to contain common carp in New Zealand.

3) If free-ranging common carp can learn to associate a sound broadcast with a food reward and subsequently be captured at a location where the sound alone is played.

2 Methods

2.1 Study site

Lake Kaituna is a 15-ha peat lake located in the Horsham Downs Wildlife Management Reserve, Waikato, New Zealand (McDonald, 2007, Fergie, 2003); Figure 1. Lake Kaituna was chosen to test potential acoustic conditioning frequencies for use on common carp as it has many of the characteristics commonly seen in lakes containing common carp in the Waikato. For example, it is a shallow (< 3 m), eutrophic peat lake with a catchment land use primarily dedicated to dairy farming. The lake contains numerous resident pest fish populations: common carp (*Cyprinus carpio*), gambusia (*Gambusia affinis*), rudd (*Scardinius erythrophthalmus*), catfish (*Ameiurus nebulosus*), goldfish (*Carassius auratus*), common bully (*Gobiomorphus cotidianus*) and koi-goldfish hybrids (Morgan and Hicks, 2010).

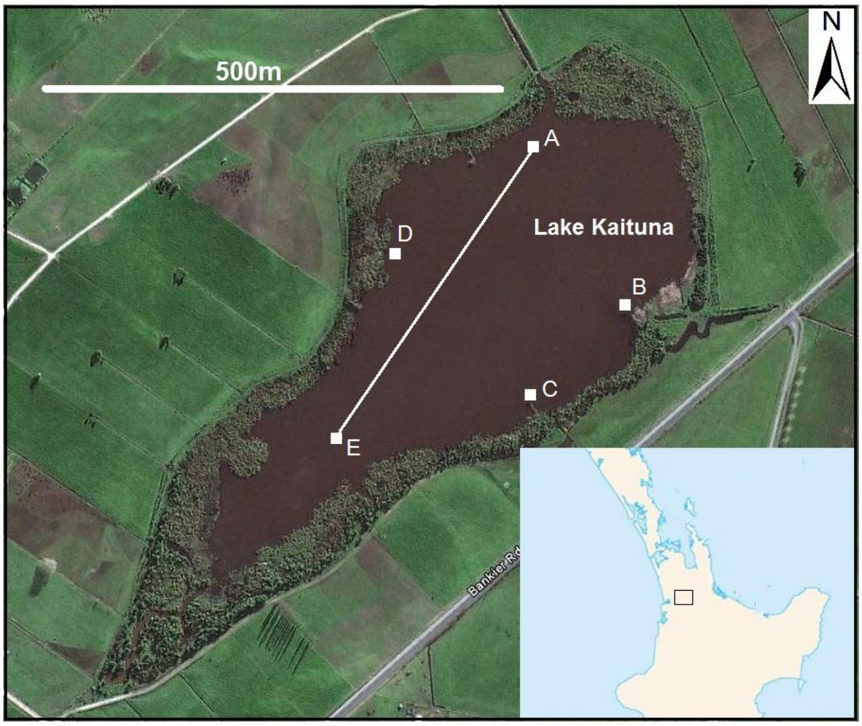

Figure 1. Location of Lake Kaituna showing experiment sites A,B,C,D and sound propagation track A-E (Source: Google Earth)

The Lake Kaituna Care Group, formed in 1999, has successfully fenced the lake from cattle and removed the introduced grey willow (*Salix cinerea*) which was the previously dominant riparian plant (Fergie, 2003, McDonald, 2007). Successful restorative planting by the group has resulted in the lake margins being dominated by the native wetland plants *Cordyline australis*, *Polygonum* sp., *Carex* sp., *Muehlenbeckia australis*, *Baumea* sp., *and Juncus* sp. (Fergie, 2003, McDonald, 2007).

2.2 Captive common carp experiments

Experiments were performed in six 500-L concrete tanks, with each pure-tone frequency (400, 700 and 1000 Hz) being tested in two of the tanks. Three trials were performed in which the six tanks were used, resulting in each frequency being tested six times on naive fish. Tanks were located at the University Waikato's Aquatic Research Centre and were continuously aerated, flushed with fresh water at a rate of 0.75 litres per minute, and were cleaned as required. The tanks were located outside and water temperature was not controlled. Water temperature ranged from 12.5 to 18^{o}C and dissolved oxygen ranged from 10.2-11.5 mg L^{-1} over the course of the experiments. A feeding arena was set up in each tank which took up one quarter of the tank area, and was divided from the rest of the tank apart from a 20 cm X 20 cm opening for fish passage. Each tank was equipped with a speaker enclosed in a pipe, with the pipes opening positioned 15 cm below the water's surface inside the feeding arena (Figure 2). Test subjects were wild common carp, caught by boat electrofishing or large fyke nets. Subjects were held individually in the test tanks for two weeks pre-trial, to acclimatise to test conditions and fed the trout pellets to be used in the experiment. Naive fish were used in each tank and weighed 300-900 g with a fork length of 250-380 mm. One fish was used per tank during the experiments. Food was delivered into the feeding arena of each tank through a tube extending behind black screening (Figure 2). The black screening prevented fish from seeing the person delivering food so that an association with people and food would not develop. The daily food delivery per tank was 1% of fish biomass (as used in captive koi conditioning experiments by Zion et al. (2007) and was split evenly over two feedings. Each tank was subject to one treatment and control feeding per day. Treatment feedings had sound broadcast for 10 s, 30 s before food was delivered. Control feedings were not accompanied by sound. The time and order that the control and treatment feedings occurred, and the order in which tanks were fed, was random. Trial feedings occurred between the hours of 8am to 4pm. The sound frequencies tested were 400 Hz, 700 Hz and 1000 Hz pure-tone sounds, with broadcasts of all frequencies ranging from 129 to 131 dB referenced to one micropascal (re. 1 μPa) throughout tanks, determined by recording with a calibrated hydrophone (Sonatech model 8185) and analysis with Adobe Audition Software Version 3.0. Broadcasts at sound pressure levels up to 145 dB (re. 1 μPa) at 1 m have been successfully used in acoustic conditioning experiments on common carp (Zion et al., 2007) with levels down to 80 dB (re. 1 μPa) also showing response by common carp (Kojima et al., 2005). Pure tone sounds were used as these have proven successful in similar experiments (Zion et al., 2007, Zion et al., 2010, Willis et al., 2002). Video cameras positioned above each tank (Figure 2) recorded the location of fish for 2.5 minutes before and after each feeding. Recording the time taken for fish to enter the feeding arena during each feeding allowed for comparison between treatment and control responses in each tank, as well as response times between frequencies. The tank broadcast experiments ran for ten working days over a two week period.

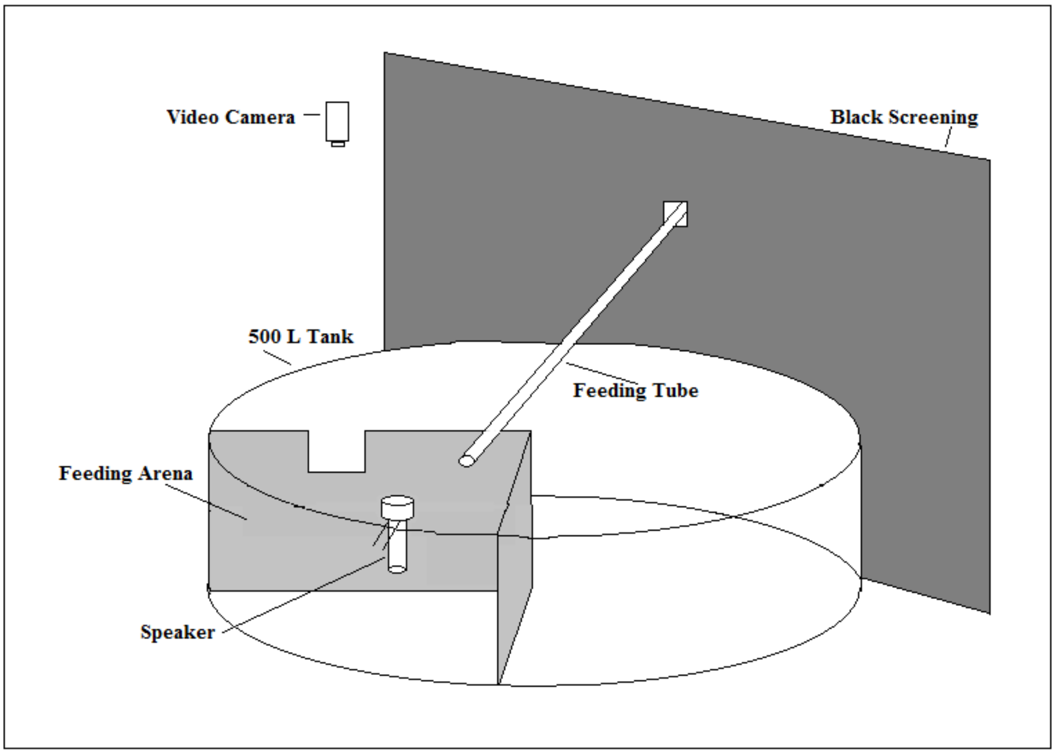

Figure 2. Tank setup used in acoustic conditioning experiments on captive common carp with 400, 700 and 1000 Hz pure-tone frequencies tested.

2.3 Determining attenuation of pure-tone frequencies in Lake Kaituna

The sound attenuation experiment was performed on a day of fine weather with no wind, between 0900 h and 1300 h. An underwater speaker setup (Lubell Labs LL916 Speaker, Lubell Labs AC205B Audio Isolating transformer, TOA Corporation 20 Watt Amp Model CA-200, iPod nano 3rd generation mp3 player) was used to broadcast test sounds from a fixed position attached to a pole near the shore of Lake Kaituna (marked A on Figure 1). The speaker was positioned 0.6 m below the water's surface and continuously broadcast a track consisting of 5 s segments of three different pure-tone frequencies (400, 700 and 1000 Hz), separated by 5 s intervals of silence. A hydrophone (Sonatech model 8185) was used to record the broadcasts from a boat at distances of: 1, 50, 150, 200, 250, 300, 350 and 400 m (measured with global positioning system (GPS) Garmin gps60cx), at a depth of 0.5 m in a straight line shown in Figure 1 as points A to E. At the broadcast point and at each recording interval, water depth and temperature were measured. Recordings were later analysed using Adobe Audition Software Version 3.0 to measure the attenuation of each frequency.

In order to determine if effects from the frequency cut-off phenomena were influencing attenuation in any of the frequencies, the wave length of each was calculated and compared to the cut-off limit of the sound propagation track used.

2.4 Acoustic conditioning of free-ranging common carp

To develop an association with the broadcast sound, an initial training phase was performed in which sound was broadcast with a food reward at treatment sites. Concurrently, food deliveries of the same quantity and timing were made at a control sites without sound. Following this training period, the sound was broadcast without food delivery at both treatment and control sites to test for a conditioned response to sound at the treatment site.

During the training phase of each experiment, two automated bait feeders were installed at Lake Kaituna (one at a treatment and one at a control site). Both control and treatment sites were setup to dispense 400 g of sinking bait pellets (Peck ’n’ Lay chicken pellets: moisture 12%, crude protein 16.5%, calcium 4.2%) over an 8 s period; 4 times a day at 12am, 6am, 12pm, and 6pm. Sound was not broadcast at control sites. One minute before each feeding at the treatment site, a broadcast of 400 Hz pure-tone was played at a level of 130 dB (re. 1 μPa) at 1 m, determined by recording with a calibrated hydrophone (Sonatech model 8185) and analysis with Adobe Audition Software Version 3.0, for 5 minutes.

Training was conducted for 5 days at which point the bait dispensers at the treatment and control sites were removed, and sound broadcasts at the treatment site was stopped. After 24 h without feeding or sound broadcast, a 10-m nylon gill net (115-mm mesh size) with a floating top line and weighted bottom line was set at each site surrounding the previous point of food delivery, and held in place by four metal stakes (Figure 3). The 400 Hz broadcasts were then resumed at the treatment site without food delivery. Nets at both sites were cleared after 24 h and reset between 9 and 11am daily for each three-day capture phase before being removed.

The ability for fish to evade gill nets is well known (Hamley, 1975, Shireman and Maceina, 1981, Collins, 1979). Visual and touch detection of a net allows for fish to attempt to manoeuvre around, under, or over the net, or to avoid the area (Collins, 1979, Shireman and Maceina, 1981). Size bias of fish caught in gill nets also exists (Collins, 1979, Johnsen and Hasler, 1977). Gill nets were chosen as the capture method despite their disadvantages due to their ability to be set easily in varying water depths and their ability to conform to uneven and very soft sediments. They also provided a means of capturing responsive common carp that did not move through any localised openings (as in fyke net type traps) and was highly selective to the target species (no native eel species were captured).

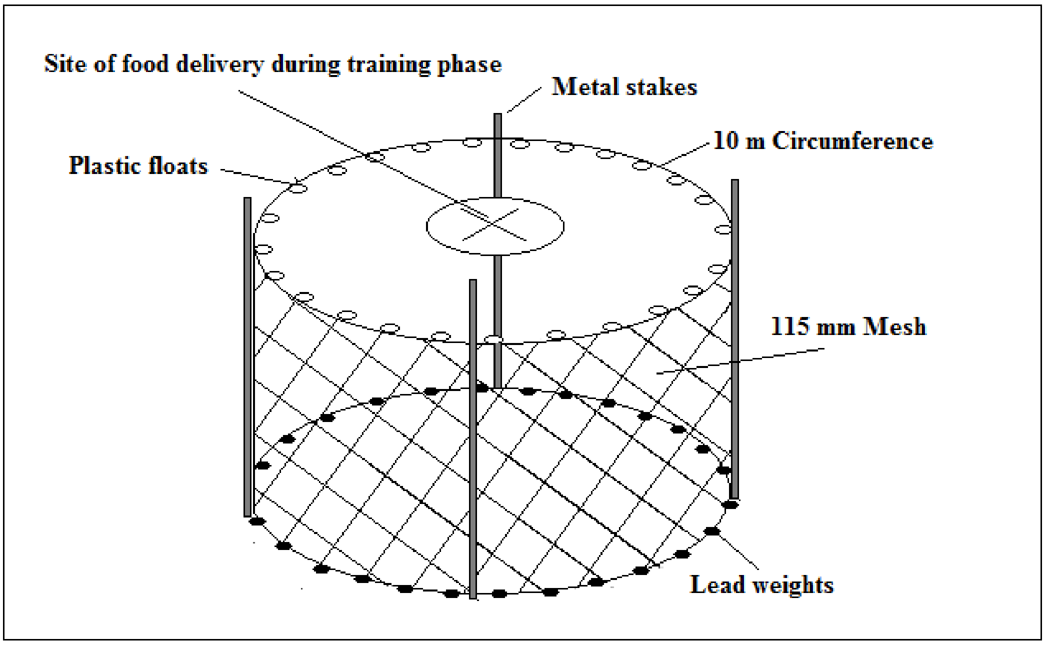

Figure 3. Diagram of net design used at treatment and control sites in acoustic conditioning experiments performed on common carp in Lake Kaituna.

The experiment was repeated four times, with four to six days separating experiments. Sites used are shown in Figure 1 as points A, B, C, and D, the order in which they were used is shown in Table 1.

Table 1. Treatment and control sites used in free-ranging common carp acoustic conditioning experiments

Experiment number	Site used for Control	Site used for Treatment
1	C	A
2	A	C
3	D	B
4	B	D

A pilot study using floating bait pellets (50% spent brewers grain, 30% rice flour, 20% wheat gluten) in a lake with clear water was performed to explore the possibility of using visual surveillance as a method of recording response to feedings. Free-ranging common carp failed to respond to the floating baits over the course of the trial (two 30-minute feedings of 40 g day^{-1} for 6 days). Sinking baits were then chosen because of their previous success in attracting free-ranging common carp to traps in pest fish removal operations. The use of sinking baits also removes the need for surface containment of floating baits and does not require common carp to alter their mostly benthivorous feeding behaviour (Breukelaar et al., 1994, Roberts et al., 1995).

2.5 Data analysis

Dependent, paired, and two sample *t*-tests, Friedman ANOVA and Wilcoxon Matched Pairs tests were performed using STATISTICA 9 software. Statistical relationships were considered significant at $P \leq 0.05$.

2.5.1 Captive common carp experiments

Control and treatment response data was tested for equality of variance and for normal distribution of differences between pairs in order to meet the assumptions of the paired *t*-test and the two sample *t*-test. For each fish, the response times to treatment and control feedings from a given day over the duration of the experiment were analysed for difference by a paired *t*-test. Paired *t*-tests were used to test for difference between treatment and control response for each frequency individually. Two sample *t*-tests were used to test for a significant difference between control and treatment difference, between the first and second five days for each frequency.

2.5.2 Determining attenuation of pure-tone frequencies in Lake Kaituna

Comparison of the decibel levels for the three frequencies was performed by Friedman ANOVA. This test was used as only one data point was recorded for each frequency at each distance and the data was not normally distributed, even after transformations. If the Friedman ANOVA showed that there were significant differences between attenuation rates of frequencies, Wilcoxon Matched Pairs tests were used to determine where these differences occurred.

Effect from frequency cut-off was tested for using Equation 1 ($\lambda \geq 4 \times W$), in which wave length (λ) and minimum water depth (W) are expressed in m. Wave length for each frequency was determined using Equation 2 ($\lambda = c / f$), in which the speed of sound in water (c) is expressed in ms^{-1} and frequency (f) is expressed in Hz. The speed of sound in water was determined using Equation 3, Marczak's equation for sound speed in pure water as a function of temperature ($c = 1.402385 \times 10^{3} + 5.038813\,T - 5.799136 \times 10^{-2}\,T^{2} + 3.287156 \times 10^{-4}\,T^{3} - 1.398845 \times 10^{-6}\,T^{4} + 2.787860 \times 10^{-9}\,T^{5}$), in which water temperature (T) is expressed in ^{o}C. The speed of sound in water at the average temperature of the sound propagation track (15.0^{o}C) was found to be 1466 m s^{-1} using Equation 3. The minimum depth along the sound propagation track (Figure 5) was 0.67 m, therefore 4W = 2.68. Equation 1, if true, verifies that the frequency cut-off phenomena could affect propagation at the frequency corresponding to the λ value used.

2.5.3 Acoustic conditioning of free-ranging common carp

Capture data from control and treatment sites were tested for normality and homogeneity of variance, and were compared using a dependent *t*-test. The length and weight of fish caught at control and treatment sites was tested for normality and homogeneity of variance, and was compared using a dependent *t*-test.

3 Results

3.1 Captive common carp experiments

3.1.1 Common carp learning period

Two sample *t*-tests comparing control and treatment response times between the first and second five trial days for: 400, 700 and 1000 Hz, resulted in $p \leq 0.001$. The tests show that the control and treatment difference is significantly different between the first and second five trial days (Table 2 and Table 3).

Table 2. Mean control and treatment response times (± 1 standard deviation) to feedings for the first five trial days of captive common carp acoustic conditioning experiments

Response time in the first five trial days (s)			
Frequency (Hz)	Mean control response	Mean treatment response	Difference
400	136 ± 16	130 ± 15	6
700	123 ± 40	137 ± 29	-14
1000	120 ± 11	133 ± 12	-13

Table 3. Mean control and treatment response times (± 1 standard deviation) to feedings for the second five trial days of captive common carp acoustic conditioning experiments

Response time in the second five trial days (s)			
Frequency (Hz)	Mean control response	Mean treatment response	Difference
400	118 ± 25	47 ± 34	71
700	142 ± 8	62 ± 47	80
1000	149 ± 1	97 ± 21	52

3.1.2 Control and treatment feeding response times

Response times decreased as the trials progressed, showing that training occurred. The average control and treatment response times to feedings for each frequency are shown in Figure 4. Faster response times to treatment feedings were seen for

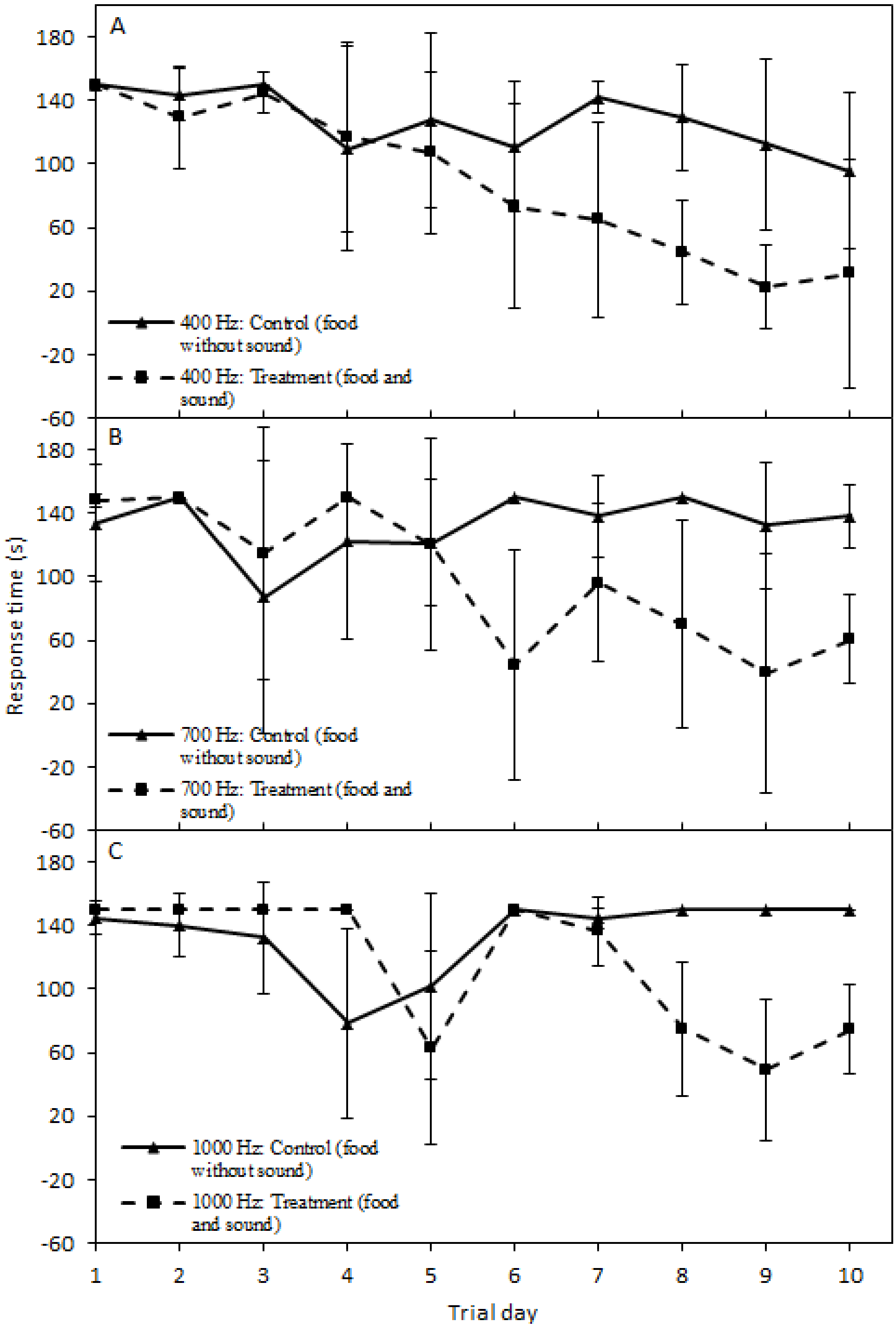

Figure 4. Mean control and treatment response times (± 1 standard deviation) for captive common carp to feedings for 400, 700 and 1000 Hz over ten trial days

400 Hz and 700 Hz after day five, while no clear difference is seen in the 1000 Hz trials until day 8. Paired *t*-tests comparing control and treatment response times were significantly different for 400 Hz ($p < 0.001$) and 700 Hz ($p = 0.003$), but not for 1000 Hz ($p = 0.058$).

Fish that did not respond to any feedings during the trials were excluded from results. One fish from each of the 700 Hz and 1000 Hz trials failed to respond at all and were excluded.

3.2 Determining attenuation of pure-tone frequencies in Lake Kaituna

Temperature and depth data for the sound propagation track is shown in Figure 5. The maximum and minimum temperatures of the sound propagation track, 15.9 and 14.4^{o}C, result in sound speeds of 1469 and 1,464 m s^{-1} using Marczak's equation. The change in sound speed due to temperature variation is 0.34% and is non-linear. Water temperature is therefore not responsible for the observed attenuation.

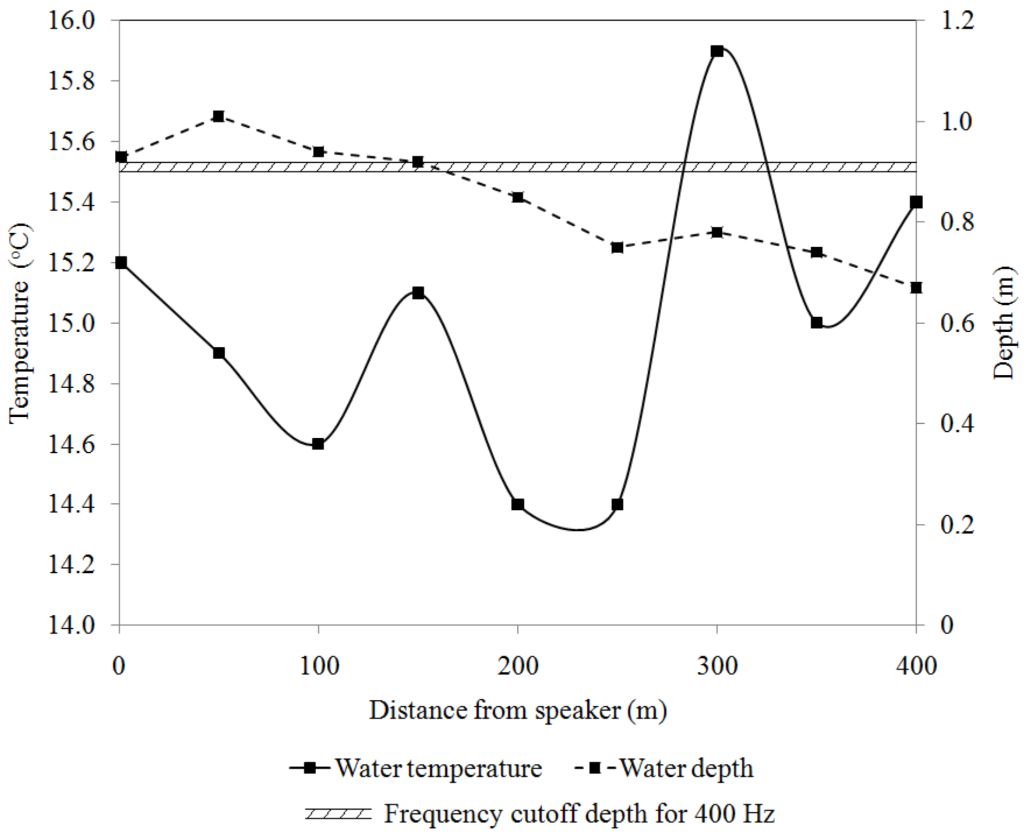

Figure 5. Water temperature and depth at each interval of the sound propagation track in Lake Kaituna and the cut-off depth for 400 Hz

The results in Table 4 show that the frequency cut-off phenomena (as defined in waters with a rigid bottom) is affecting attenuation in the 400 Hz frequency only, as the wave length for 400 Hz is the only wave length larger than four times the minimum water depth. Depth corresponding to frequency cut-off begins for the 400 Hz frequency from distances greater than 150 m from the speaker (Figure 5).

Table 4. Calculated wave lengths (λ) and cut-off depths for each frequency and the cut-off limit (4W) for the sound propagation track used

f (Hz)	λ (m)	4W (m)	λ ≥ 4W (True/False)	Theoretical cut-off depth (m)
1000	1.47	2.68	False	0.37
700	2.09	2.68	False	0.52
400	3.67	2.68	True	0.92

Comparison of the decibel levels for the three frequencies revealed difference (p = 0.008). The levels for the three frequencies are shown in Figure 6. Greatest attenuation occurred in the first 50 m for all frequencies and appeared to plateau after 150 m.

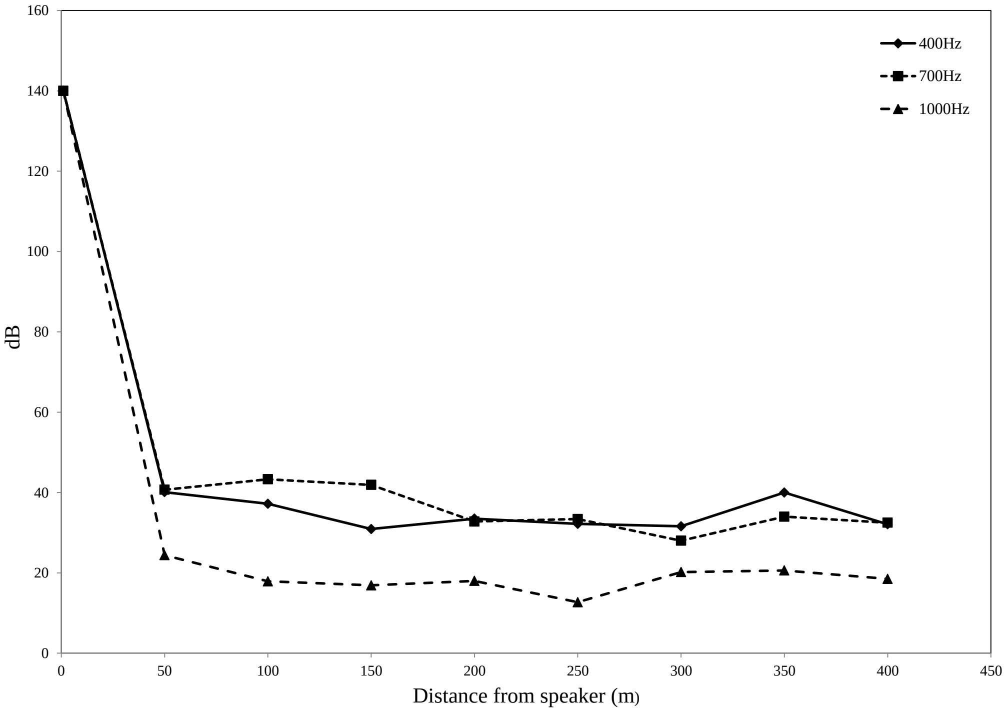

Figure 6. Decibel levels for each frequency at each interval over the sound propagation track used at Lake Kaituna

Testing on each pair of frequencies identified the 1000 Hz frequency as being significantly different from the 400 Hz and 700 Hz frequencies. The p - values for each comparison are shown in Table 5.

Table 5. Results of post-hoc analysis to identify differing attenuation of 400, 700, and 1000 Hz frequencies

	p value		
	400Hz	700Hz	1000Hz
400Hz	-	0.594	**0.012**
700Hz	0.594	-	**0.011**
1000Hz	**0.012**	**0.011**	-

Attenuation of the 1000 Hz frequency was significantly different from the attenuation of the 400 Hz and 700 Hz frequencies. The attenuation of the 400 Hz and 700 Hz frequencies were not significantly different.

3.3 Acoustic conditioning of free-ranging common carp

Catch rates were higher for treatment compared to control sites each day, and catch rates at both sites decreased over the three trial days (Figure 7). Control and treatment data were significantly different ($p < 0.001$).

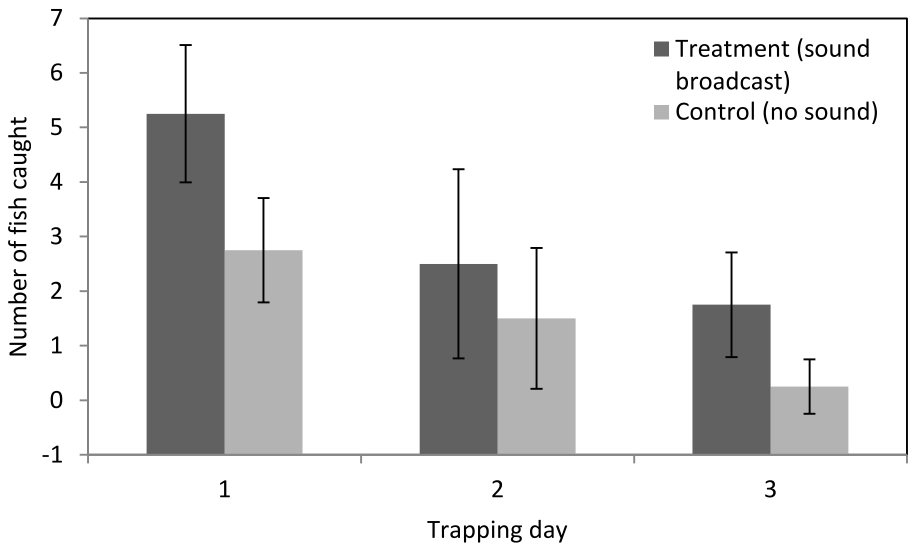

Figure 7. Mean daily free-ranging common carp catch rates (± 1 standard deviation) at treatment and control sites for the acoustic conditioning experiments

The mean catch was higher at treatment than at control sites for each of the four experiments and was on average 2.1 times greater at the treatment site (Table 6).

Table 6. Mean daily free-ranging common carp catch (± 1 standard deviation) at control and treatment sites for each experiment and all experiments combined during the acoustic conditioning experiments

Experiment number	Mean catch rate (fish net^{-1} 24 h^{-1})		
	Treatment	Control	Treatment/Control
1	2.33 ± 2.08	1.00 ± 1.00	2.33
2	3.33 ± 1.53	1.33 ± 1.15	2.50
3	3.67 ± 3.06	1.67 ± 2.08	2.20
4	3.33 ± 2.08	2.00 ± 1.73	1.67
Combined	3.17 ± 1.99	1.50 ± 1.38	2.11

Table 7 shows the average weight and length of fish caught at treatment and control sites. No significant difference was found between the average weight (p = 0.239) and length (p = 0.183) of fish caught at treatment and control sites. Probable size bias of fish caught in the gill nets is shown by the limited length and weight range (305 mm and 720 g to 440 mm and 1400 g).

Table 7. Mean lengths and weights (± 1 standard deviation) of free-ranging common carp caught at control and treatment sites during the acoustic conditioning experiments

	Mean fish weight (g)	Mean fish fork length (mm)
Control	1042 ± 21	376 ± 38
Treatment	960 ± 17	361 ± 35

4 Discussion

4.1 Captive common carp experiments

Common carp response times to control and treatment feedings differed significantly over the ten day tank trials for the 400 Hz and 700 Hz trials. Treatment data from the 1000 Hz trials was close to significant (p = 0.058). The average response times to treatment feedings in the second five trial days for the 400 Hz, 700 Hz and 1000 Hz trials were 47, 62 and 97 s respectively.

Common carp have been shown to be most sensitive to frequencies surrounding 505 Hz by auditory brainstem response (ABR) monitoring, and 1000 Hz by electrocardiogram (ECG) monitoring (Kojima et al., 2005). The differing results between ABR and ECG monitoring are hypothesised to arise from the lack of lateral line influence on ABR monitoring compared to ECG, and potential stress due to electric shock conditioning in ECG trials (Kojima et al., 2005). The primary role the lateral line plays in predator avoidance, schooling, and intraspecific communication (Nedwell et al., 2004) may detract from its use in conditioning situations and further explain why ECG trials do not reflect results found in ABR and this current study.

Laboratory trials by Willis et al. (2002) designed to determine the most effective sound for classical conditioning of triploid grass carp (C*tenopharyngodon idella*) tested frequencies of 600 Hz, 800 Hz and 1000 Hz as well as grass carp feeding sounds. The study found that subjects conditioned with 600 Hz and 800 Hz displayed the most effective recall response, supporting the current findings of greater success at lower frequencies. We did not assess frequencies below 400 Hz in this study. Recent studies on salmonids, a group of fish considered to be hearing non-specialists (Amoser and Ladich, 2005), found equal success was achieved on the acoustic conditioning of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar)* with frequencies from 50-400 Hz and that equal recall success could be achieved by broadcasting sounds that differed by as much as 20 dB and 200 Hz when a 250 Hz sound was used in training (Tlusty et al., 2008). Acoustic conditioning of rainbow trout showed that the use of a 150 Hz sound during training resulted in equal recall success when either the 150 Hz sound was used or when a novel 300 Hz sound was used (Abbott, 1972).

The sound pressure level (SPL) of the frequencies used in the test tanks ranged from 129 to 131 dB (re. 1 μPa) throughout tanks. Common carp have been shown to be receptive to sound pressure levels as low as 80 dB (re. 1 μPa) (Kojima et al., 2005), while broadcasts of sound pressure levels up to 145 dB (re. 1 μPa) at 1 m have been successfully used in acoustic conditioning experiments on common carp (Zion et al., 2007), due to the degradation of SPL over distance. As all frequencies were broadcast at sound pressure levels well within the hearing range of common carp, and similar sensitivities to SPL across the frequencies tested exists(Kojima et al., 2005), the differing responses to frequencies by common carp are not believed to be related to the SPL of the broadcasts.

Differences between treatment and control response times in the first and second five day periods of the trials resulted from response times to treatment feedings reducing at a greater rate than response times to control feedings. This indicates that the response to treatment feedings was learnt and not inherent, and that the subjects learned to associate the acoustic signal with a food reward within 5 trial days.

The average response time to control feedings in the first and second five days decreased for the 400 Hz experiments from 136 s to 118 s, and increased in the 700 Hz and 1000 Hz experiments (123 s to 142 s and 120 s to 149 s respectively). The increased time taken to respond to control feedings in two of the three frequencies may suggest that subjects were not as active in seeking food or as responsive to olfactory stimuli once the association between acoustic signal and food delivery had been established.

The time taken for subjects to associate the broadcast sound with a food reward is consistent with earlier work on acoustic conditioning of fish in captivity (Zion et al., 2007, Willis et al., 2002), despite differences in study design. For example, in Zion (2007) two feeding pads where used in each tank and the active feeding pad alternated, while the current study had one consistent feeding arena, which once entered recorded a positive response by the subject. Studies by Wright & Eastcott (1982) on the association of an acoustic signal with an existing trained feeding behaviour in common carp and thicklipped mullet *(Crenimugil labrosus)* recorded a positive response to the acoustic signal within 48 h. The speed at which the conditioning occurred with common carp in this study suggests that the association between acoustic signal and food reward in naive fish may be developing earlier than results would suggest, as the action required to consistently receive the food reward may take longer to master than the association takes to develop.

The length of time that the conditioned behaviour lasts in common carp was not determined in the current study but has been investigated for other fish species (Zion et al., 2011b, Tlusty et al., 2008). Evidence that conditioned behaviour to acoustic recall existed in St Peter's fish (*Sarotherodon galilaeus*) remained up to 55 days after training ceased (Zion et al., 2011b). The time taken to retrain fish after this point was significantly shorter than for naive fish for up to 6 months; suggesting that dormant memory may also exist. Acoustic conditioning was found to last for up to 7 months in rainbow trout and Atlantic salmon (Tlusty et al., 2008), both studies indicating that the conditioning in common carp may also last for a significant amount of time.

Comparable studies on acoustic conditioning of fish in captivity have differed in the number of fish used in each tank (Zion et al., 2007, Willis et al., 2002) with only one fish used in each tank during the current trial due to observed intraspecific competition in preliminary trials which resulted in the exclusion of some fish to the feeding arena by others. Tank size and temperature, food type and method of delivery, as well as fish size and level of domestication also varied amongst previous experiments on acoustic conditioning of captive fish with food reward (Levin and Levin, 1994, Willis et al., 2002, Zion et al., 2007, Tlusty et al., 2008, Abbott, 1972, Wright and Eastcott, 1982, Zion et al., 2011b). The similarity in response time despite the differences in study design suggest that common carp and other studied teleost fish are receptive to associating appropriate auditory signals with food reward in a relatively short amount of time, in this case 5 days.

4.2 Determining attenuation of pure-tone frequencies in Lake Kaituna

No relationship was found between water temperature and sound attenuation. Sound travels faster in water with increasing temperature and depth (Mann and Lobel, 1997) and temperature and depth varied over the sound propagation track. However, these variations proved insignificant over the relatively short distance of 400 m used. It is possible, however, that if the frequencies tested were broadcast in waters where there was a consistent temperature or depth gradient, the effect of the positive relationship between sound speed and increasing water depth and temperature could influence attenuation.

The absorption of sound by water is greater as the sound frequency increases (Ainslie and McColm, 1998, Francois and Garrison, 1982). This trend was seen in the increased reduction in the 1000 Hz recordings over the first 50 m compared to that of the 400 Hz and 700 Hz, but was not detected between the lower two frequencies. The length of the sound propagation track used (400 m) and the water depth (0.67-1.01 m) was suitable in regards to distances needed for attracting common carp, but may have been lacking in its ability to detect differences in attenuation rates at frequencies as low as 400 Hz and 700 Hz. This was unavoidable, as the depth and length of Lake Kaituna was similar to many lakes in the Waikato likely to be subject to common carp removal programmes.

The frequency cut-off effect, as defined in waters with a rigid bottom, was shown to theoretically be influencing attenuation for the 400 Hz frequency for distances over 150 m from the speaker. The ability of soft sediments to allow partial propagation at frequencies below the cut-off threshold may explain the lack of attenuation for 400 Hz from 150-400 m. Determining the exact role that frequency cut-off plays in waters with soft bottoms is logistically difficult and results will vary between and within lakes, due to the need to determine sound speed within the sediment and the variability of sediments (Mann and Lobel, 1997). A study by Rogers & Cox (1988) determined that the cut-off frequency in the same channel ranged from 116 Hz where a fine sand bottom was present, to 1070 Hz where a sand-silt-clay bottom was present at the same depth. The exact composition of the bottom sediments in Lake Kaituna is unknown. The expected variability of sediment within the lake and in other lakes where the frequencies tested may be used indicates that sediment analysis to determine frequency cut-off effects is likely to be similar to Lake Kaituna.

4.3 Acoustic conditioning of free-ranging common carp

The average three-day capture period following training resulted in 2.11 times more common carp being caught at treatment compared to control sites. The ability for fish to evade gill nets means that higher numbers of common carp may have been responding at sites than were captured, and that the association of the audio signal with food may have been developed but may not have been strong enough to cause all responsive subjects to become entangled in the net. As no difference was found between the length and weight of fish caught at control and treatment sites it appears unlikely that disproportionate numbers of undetected fish below the capture size of the nets used would respond at treatment or control sites. The same reasoning applies when considering the possibility of fish larger than the capture size of the nets used evading detection.

The differences between catch rates at control and treatment sites in the four experiments carried out in the current study do not increase as expected. As the difference is not increasing it appears that the common carp population in Lake Kaituna did not experience training overlap between experiments as was expected. Such overlap was expected as it was assumed that subjects could develop an association between the audio broadcast and the food reward but fail to be captured, only to cause stronger response when caught more readily in subsequent experiments. The fact that this did not occur means that either the trapping efficiency of the gill nets used is higher than expected or that common carp were not drawn from other areas of the lake to the trapping sites during the pre-feeding phase. As the efficiency of the gill nets used is unlikely to be 100% and the audio broadcasts were at sufficient levels to be heard over large portions of the lake, it seems likely that the food reward was insufficient to cause common carp to move to the pre-feeding area.

The length of time that the conditioned behaviour lasts in free-ranging common carp was not determined in the current study but has been investigated for other fish species in captivity (Zion et al., 2011b, Tlusty et al., 2008). Evidence that conditioned behaviour to acoustic recall existed in St Peter's fish (*Sarotherodon galilaeus*) remained up to 55 days after training ceased (Zion et al., 2011b). The time taken to retrain fish after this point was significantly shorter than for naive fish for up to 6 months; suggesting that dormant memory may also exist. Acoustic conditioning was found to last for up to 7 months in rainbow trout and Atlantic salmon (Tlusty et al., 2008), both studies indicating that the conditioning in common carp may also last for a significant amount of time.

The current study design did not allow for repeated sampling of fish visitation at control and treatment sites as the sampling technique was fatal. Repeated sampling of fish visitation at sites would have increased the ability to detect an association with the broadcast sound, as fish caught on day one or two of each trial would have then had the option to return on subsequent days. Studies by Zion et al. (2011a) and Levin & Levin (1994) on acoustic recall of fish species used recordings from video cameras above and below the water to determine response to broadcast. Although low water visibility in Lake Kaituna prevented the use of cameras, other non-lethal sampling techniques should be considered in future studies to increase the ability to detect response. Non-lethal sampling during an acoustic recall experiment was performed by Zion et al.(2011a) where a floating fish trap automatically closed after sound broadcast, allowing for fish to be measured and released. Other studies on free ranging common carp have monitored their movement using radio and acoustic tags (Shin et al., 2003, Stuart and Jones, 2006, Johnsen and Hasler, 1977), a non-lethal method which could be used in acoustic recall experiments. Although each fish to be included in the study would need to be tagged, radio or acoustic tagging would allow for monitoring of subjects in regard to sound broadcast, with no disturbance to subjects and in waters irrespective of visibility. Despite the disadvantages of the fatal sampling technique used, the study still produced significant results when the control and treatment data were compared.

Capture rates recorded in the current study cannot be directly compared with previous work which used the percent of population responding to acoustic recall as a measure of success on free-ranging and captive fish species (Willis et al., 2002, Levin and Levin, 1994, Zion et al., 2011a), as the population of common carp present in Lake Kaituna was not known and fish are able to leave and enter the lake. Results from Zion et al. (2011a) comparing control and treatment capture rates from an automated fishing machine using audio conditioning provide some basis for comparison. During treatment capture events 2.5 times more fish were captured, the majority being common carp, despite the added attractant effect of the fishing machine as a structure during control capture events. Free-ranging common carp were exposed to a longer pre-feeding period (1.5 months) than the current trial and more food (1 kg) was delivered during each training session, but there were longer quiet periods between training (Zion et al., 2011a). The average three-day capture period following training resulted in 2.11 times more fish being caught at treatment compared to control sites, similar to Zion et al. (2011a). The higher capture difference between treatment and control events may be due to the greater length of the training period fish were exposed to and the increased food volumes delivered.

The ability for captive trained common carp to aid in the training of free-ranging common carp to acoustic conditioning following release was not investigated in the current study. Results from captive studies found that the use of such “teacher fish" significantly reduced the time needed for naive subjects to respond to audio signals (Zion et al., 2007) suggesting that they may also benefit work on free-ranging subjects.

5 Conclusion

5.1 Captive common carp experiments

Individual captive common carp were successfully trained through classical conditioning to move into a feeding arena when pure-tone sound frequencies of 400, 700 and 1000 Hz, were broadcast at 129 to 131 dB (re. 1 μPa) within tanks. The association between the pure-tone sound broadcast and food delivery was achieved for all frequencies. The average time taken for common carp to enter the feeding arena following sound broadcast during the second five trial days was 47, 62 and 97 s for the 400 Hz, 700 Hz and 1000 Hz trials respectively. The experimental design required common carp to enter a feeding arena through a 0.2 m by 0.2 m opening, demonstrating a more exploitable response to the training in regard to trapping applications than previous studies where fish were trained to move to a location in an open tank.

Support for lower frequencies being more effective in acoustic conditioning of captive fish was also found in earlier studies on grass by Willis et al.(2002), and the 400 Hz frequency has proven successful in auditory conditioning of common carp by Zion et al.(2007). Comparisons of the effectiveness of frequencies below 400 Hz on common carp have yet to be studied, with results from studies performed on other species suggesting that differentiation below this level may not be significant (Tlusty et al., 2008).

Comparison of response times between the first and second five days showed that the response to treatment feedings was learnt and not instinctual. Previous work on common carp has suggested that instinctual responses to broadcast feeding sounds may exist (Hashimoto and Maniwa, 1966), although more recent work on captive grass carp found no evidence of an instinctual response to broadcast feeding sounds (Willis et al., 2002). The time frame of five days was supported by similar studies and there appears to be flexibility in training methods used as demonstrated by similar results from studies of varying methods. The length of time that the conditioned response is retained in trained common carp was not tested, although studies on other fish species have shown retention times of several months.

Response of conditioned captive common carp to pure tone broadcasts was shown to be fastest in those conditioned to the 400 Hz frequency. The 400 Hz pure-tone frequency was therefore determined to be the most appropriate frequency for use in the conditioning of free-ranging common carp, and a training period of five days was recommended for the conditioning to develop.

5.2 Determining attenuation of pure-tone frequencies in Lake Kaituna

Water temperature and depth variations, as well as unknown acoustic properties of sediments, meant that the attenuation of the 400 Hz, 700 Hz, and 1000 Hz frequencies in natural shallow water bodies containing common carp could not be determined by established methods. The ability to maintain signal strength over distance is a necessary quality for an audio signal to possess in order to be effective in large bodies of water containing free-ranging common carp. Standardised broadcast and recording of the frequencies over distance was performed in Lake Kaituna to determine attenuation. No relationship between either water temperature or depth with attenuation was found. There was no difference between the attenuation of the 400 Hz and 700 Hz frequencies; the 1000 Hz frequency however, was shown to have the highest attenuation. Equal attenuation was found for the 400 Hz and 700 Hz frequencies beyond the point where frequency cut-off for 400 Hz was expected. This suggests that frequency cut-off limits calculated for water bodies with hard bottoms do not apply in soft bottom lakes such as Lake Kaituna. The use of the frequencies tested in natural waters should, however, take into account possible effects of lower water depths on attenuation in reference to frequency cut-off. This should also be considered if frequencies below 400 Hz are to be used in similar water depths.

Further work is needed to determine if there is a difference in attenuation in the 400 Hz and 700 Hz frequencies, requiring a longer sound propagation track in deeper water. It would, however, be important to understand acoustic behaviour in lake water only, but not important in the context of developing audio lures for common carp. This is because the current experimental conditions, although limited, represent the environment in which carp need to be caught. The attenuation results support the use of 400 Hz and 700 Hz frequencies over the 1000 Hz frequency for the purpose of audio conditioning of free-ranging common carp, on the basis that these frequencies will maintain higher signal strength over distance.

5.3 Acoustic conditioning of free-ranging common carp

Due to the superior performance of the 400 Hz pure-tone frequency in audio conditioning experiments on captive common carp and the low attenuation rates of the 400 Hz and 700 Hz frequencies; 400 Hz was chosen for use in audio conditioning experiments on free-ranging common carp. The experiments were performed at Lake Kaituna, where automated bait feeders were used with and without sound broadcasts at treatment and control sites during an initial training phase, followed by a capture phase without bait delivery to test for a conditioned response. The study found a significant difference between the number of fish caught at control and treatment sites, with 2.1 times more fish caught at treatment sites during the three-day capture phase on average. The difference in catch rates was similar to that found in previous studies on free-ranging species including common carp.

No significant difference was found between the length and weight data of common carp caught at control and treatment sites, leading to the assumption that fish below or above the capture size of the nets used would not have been more numerous at either site. Other issues with the capture rate of the gill nets used were discussed, but should not present a catch bias between sites as the same type of nets were used at all sites. A suspected failure of the baits used to attract common carp to the feeding sites from other areas of the lake was suggested as an explanation for why the catch difference between control and treatment sites did not increase over the four experiments performed. An increased amount of bait used during the training phase or the use of stronger smelling bait is suggested for future studies, as the ability for an audio lure to be effective over distance is limited by the distance over which the food reward used during training can be detected. The ability for common carp to detect baits over distance was not addressed in the current study and should be considered in future studies.

The use of radio or acoustic trackers in future studies is highly recommended due to the issues identified with the fatal sampling method used. Such electronic monitoring of test subjects would also determine if fish were or were not responding to multiple experiments and if size bias existed amongst responsive subjects at control and treatment sites. The effectiveness of control and treatment sites to attract fish over distance during the training phase could also be compared by radio or acoustic tracking.

Although further investigation into the amount and type of food reward used is required, the ability to train free-ranging common carp to associate a 400Hz pure tone sound with a food reward and be recalled by sound broadcast alone has been demonstrated by the current study. This could prove beneficial in removal operations due to the ability of sound to travel large distances through water with low visibility, and when fish that have been exposed to auditory conditioning move out of range of olfactory signals but remain within the range of sound broadcast for recall. Once a conditioned response to the broadcast sound has been developed, the sound could be broadcast in one or more locations in the water body where removal methods could be concentrated. The deployment of automated bait feeders and sound broadcasters in a lake simultaneously should also be considered, as this would allow for the conditioning effect of the training phase to potentially reach the entire population of a lake, followed by fewer broadcasts during the capture phase at ideal capture or poisoning locations. The time and effort required for removal operations could be reduced, as could the impact on non-target species. The ability of common carp to evade capture and to take refuge in areas inaccessible to fishing effort could be diminished if common carp were attracted to areas more suitable for removal with 400 Hz pure tone broadcasts.

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